

The prognostic value of the P53 protein and the Ki67 marker in breast cancer patients

Rahim Golmohammadi,¹ Akbar Pejhan²

Anatomy Department,¹ Physiology Department,² School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.

Corresponding Author: Akbar Pejhan. E-mail: a.pejhan@yahoo.com

Abstract

Objective: To investigate and compare the prognostic value of P53 and Ki67 markers in patients with breast cancer in Sabzevar, north east of Iran.

Methods: A descriptive analytical study was conducted on 80 patients with breast cancer who were admitted to the hospitals in Sabzevar in 2006 and they were followed up to 2010. The expression of ki67 and stability of p53 genes were determined by immunohistochemistry. To assess the disease prognosis, patients were followed up to 48 months. Data were analyzed using SPSS software version 11.5. Chi-square, Fisher's exact test, Kaplan-Miere and Log Rank tests were used for statistical purposes.

Results: Eighty cancerous tissue samples were examined. The Ki67 marker was present in 37 (46.3%) cases and the P53 protein stability in 39 (48.8%) cases were observed. There was a significant relationship between ki67 gene expression and tumour stage ($p = 0.001$) or tumour type ($P = 0.02$). There was also a significant relationship between the survival rate and the tumor stage ($P = 0.008$). The Ki67 marker had significant relationship with the survival rate ($P = 0.031$), but over expression of P53 protein did not show such significance ($P = 0.385$).

Conclusion: The results showed that the Ki67 marker was more important than P53 protein in prognosis of the breast cancer patients.

Keywords: Breast cancer, P53 Protein, Ki67 marker, Prognosis. (JPMA 62: 871; 2012)

Introduction

Breast cancer is one of the most important malignant tumours in the world. It is the second most frequent cause of death in west.¹ In recent years the prevalence of breast cancer in Asia, including Iran, has increased and it is the third major cause of death in Iran.² Breast cancer is associated with a number of environmental factors and genetic disorders.³

Genes related to breast cancer are divided into two groups: (a) Tumour suppressor genes which hamper tumour growth e.g. p53 gene whose product is a phosphoprotein composed of 393 amino acids with a role in various cell functions such as apoptosis, transcription and senescence.⁴ (b) Genes which lead to the progress of the tumour such as ki67 gene that codes a proliferating cell nuclear antigen (PCNA) which is a non histonic protein with a life time of 60 to 90 minutes.⁵ The Ki67

marker can be detected by immunohistochemistry method in dividing cells because PCNA is active throughout all cell cycle stages including Gap1, S, Gap2 and mitosis.⁶

Though some researchers have suggested that Ki67 marker and the PCNA measurement would be valuable markers in prognosis of breast cancer,^{7,8} there is no consensus in this regard as some other authors did not consider this marker as a predictive factor.⁹ Therefore, the diagnosis, management and treatment of patients with breast cancer issue has remained a controversial dilemma and needs further investigations.¹⁰ Taken together, the value of P53 and Ki67 markers have not been yet established as a constant factor in determination of the prognosis of breast cancer. There is also not sufficient information regarding the survival rate of these patients and its relationship with these biomarkers in Sabzevar, north east of Iran. The present study was therefore, conducted to investigate the expression and prognostic value of these markers and their relationship with survival rate in breast cancer patients in this part of Iran.

Patients and Methods

A descriptive analytical study was conducted on 80 patients with breast cancer who were admitted to the hospitals in Sabzevar in 2006 and followed up to 2010. All positive breast cancer specimens in this year were selected for the study. The diagnosis was made by two expert pathologists. This study was approved by the committee of ethics guidelines in Sabzevar University of Medical Sciences. Samples were collected before the patients received any radiotherapy and/or chemotherapy. Samples were fixed in 10% formalin immediately after the surgery, and tissue passage was done after 24 hours. Four-micron thick series sections were stained then by Haematoxylin and Eosin (H&E) method. After diagnosis and confirmation of malignancy by two pathologists who blindly examined the specimens, immunohistochemical examination was conducted on the samples. Normal breast tissues of patients were prepared as above and used as controls.

Histological grading was performed based on the three following parameters, mitotic activity, nuclear pleomorphism and the extent of tubule formation by microscopy of the slides. Grades were therefore proposed in three groups: I, II and III.¹¹ Tumour stages in breast cancer were designated numbers as: in situ carcinoma, (0), and four possible stages including stage I, II, III, and IV.¹²

For antigen retrieval, the sections were placed in a coupling containing buffer citrate solutions (PH=6,

molarity 0.01), and were kept in a microwave oven at 95°C for 20 minutes. After tissue peroxidase inhibition, the sections were then washed by phosphate buffer saline (PBS) 3 times. The primary monoclonal antibodies including the P53 (DAKO company) and the Ki67 mouse monoclonal antibodies (Novocostra, UK) were added at room temperature for at a 1/50 dilution for one hour. After being washed thrice with PBS the slides were incubated with biotinylated secondary antibody for 15 minutes. The slides were then washed three times with PBS and thereafter sections were stained with 3, 3'-Diamino benzidine (DAB) substrate solution. The streptavidin linked to Horse Radish Peroxidase (HRP) is capable of oxidizing DAB. Cells associated with the P53 and ki67 genes expression would make up a brown colour non-soluble precipitation.

The stained tissue backgrounds with haematoxylin were dehydrated and mounted. These slides were studied by a light microscopy. When the stained cells were less than 10% of total they were regarded as negative, between 10% to 25% the cells considered 1 positive (+); between 26% to 50% they were considered 2 positive (+ +) and when over 50% of the cells were stained they were considered as 3 positive (+ + +).¹³

All patients were followed up to 4 years. We contacted the family of all patients and checked their current status.

Statistical Analysis:

Data analyzing was performed using SPSS software 11.5 and chi-square and Fisher's exact tests were performed when required. By using Kaplan-Meier method and Log Rank test, the relationship between the survival rates of those patients with biomarkers and tumour stage were also evaluated. Significant differences were considered when p value was less than 0.05 in all experiments.

Results

A total of eighty cases with breast cancer were involved of which 79 cases were females and one case was a male (35-years age). Their age ranged between 20 and 86 years with a mean of 48.78±14.82. In 17 (21.3%) cases the carcinoma was in situ or at 0 stage, in 30 (37.5%) cases at the first stage and in 25 (31.3%) cases at the second stage, in 6 (7.5%) cases at the third stage and in 2 (2.5%) cases at the fourth stage. One of them had metastasis into the lung and another one into the chest. According to the grades, out of 80 cases, 59 were at the grade 1, 18 cases at grade 2, and the rest 3 cases were at grade of 3.

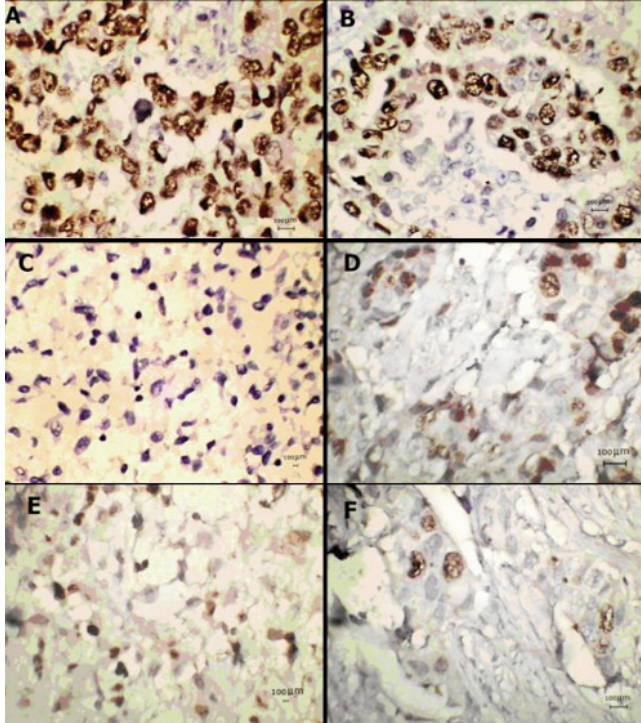


Figure-1: Four-micron cross section of cancerous breast tissue, showing the A (+++), B (++) P53 protein stability, C (P53 protein instability), D (+++), E (++) , F (+) Ki67 gene expression. Cancerous cells have been stained brown by immunohistochemistry. (magnification 400x).

P53 Protein Stability and The Ki67 Marker:

In the present study the P53 protein stability was observed in 39 (48.8%) cases. The P53 protein stability was designated (+) in 21 cases, (++) in 10 cases and (+++) in 8 cases (Figure-1 A and B). The stained cells were considered as cancerous because of the presence of the P53 protein stability. Such stability was not observed in 41 (51.3%) cases, and the cancerous cells were not stained brown by immunohistochemical methods (Figure-1 C). No significant relationship was observed between tumour stage and P53 protein stability in the cancerous cells in the breast tissue ($p = 0.16$).

The Ki67 proliferative marker was observed in 37 cases out of 80 cases with breast cancer. Their staining slides were (+) in 15 cases, (++) in 14 cases, and (+++) in 8 cases (Figure-1 D, E and F). The ki67 gene expression was negative in 43 cases.

The Table shows that there is a statistically significant ($p = 0.001$) relationship between the tumour stage and the Ki67 proliferative marker. However, there was no significant relationship ($p = 0.08$) between the tumour grade and the ki67 gene expression. Fisher's exact test showed a significant relationship ($p = 0.026$) between the tumour type and the ki67 gene expression.

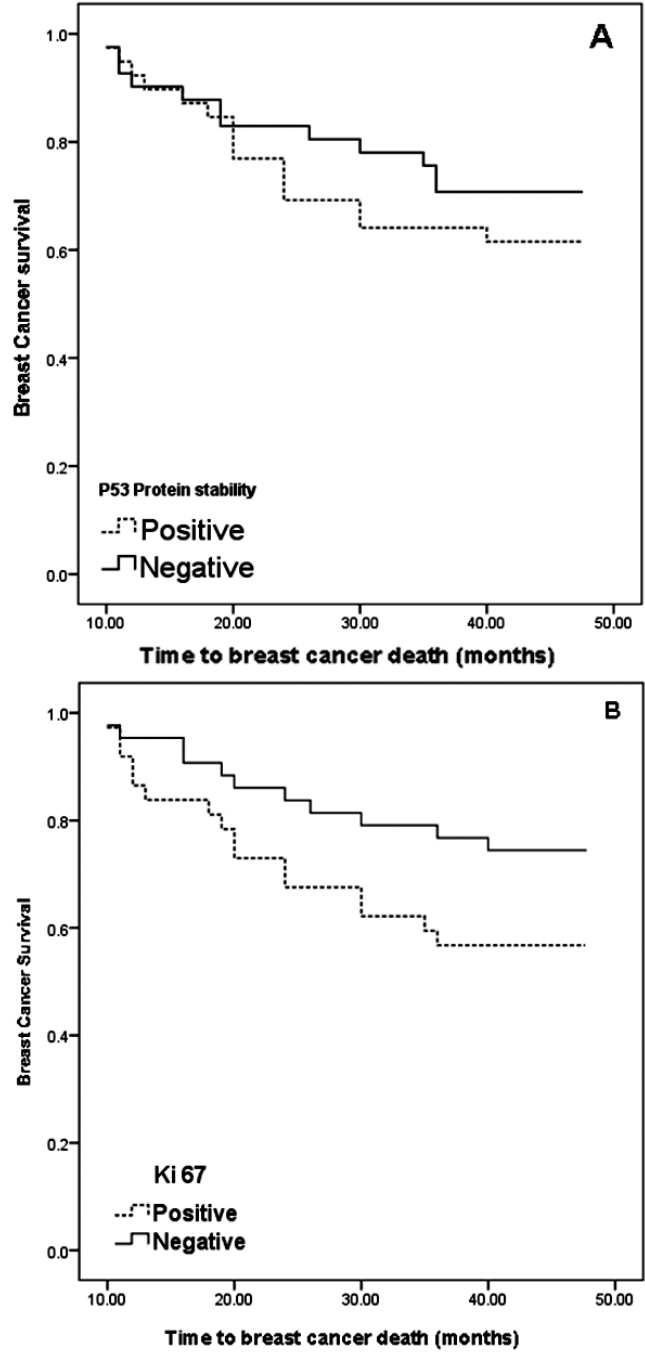


Figure-2: Kaplan-Meier survival curves for P53 protein stability (A) and Ki67 marker (B) in breast cancer patients.

Since the cells had expected count less than 5 in table 1, the chi-square test could not be applied, so according to statistical consultation, stage 2 and stage 3 frequencies were combined in table 1 to obtain a considerable reliability.

At the end of the four years follow up period 33

Table: The frequency of the ki67 gene expression in the different tumor stages in the cells of the breast cancer tissue (p < 0.001).

Ki67 Stage	Positive		Negative		Total	
	N	Percent	N	Percent	N	Percent
Zero	1	1.3	16	20	17	21.3
One	12	15	18	22.5	30	37.5
Two and three	22	27.5	9	11.3	31	38.8
Four	2	2.5	0	0	2	2.5
Total	37	46.3	43	53.8	80	100

patients had died. One patient the lone male died within the first year after diagnosis while his Ki67 marker and P53 protein tests were negative. During four years follow up 32 women died. In 20 (62.5%) of those patients the Ki67 marker was positive and in 12 (37.5%) it was negative. The results showed that the over expression of P53 protein had no significant (P = 0.385) relationship with survival rate, but the presence of the Ki67 marker had significant (P = 0.031) relationship. There was also a significant relationship (P = 0.008) between the survival rate and the tumour stage (Figure 2). The prognosis in the patients with more advanced stages 3 and 4 of breast cancer had significantly worsened than that in those with stages 0, 1 and 2 (Log Rank test P value = 0.008). There was no significant relationship (P = 0.2) between the tumour grade and the survival rate in the patients.

Discussion

Of the 80 patients with breast cancer, the Ki67 marker was positive in 37 (46.3%) cases, whereas the P53 protein stability was observed in 39 (48.87%) cases. There was a significant relationship between the tumour stage and the ki67 gene expression but no significant relationship was observed between the tumour stage and P53 protein stability. Our findings were consistent with Tan et al who found that the P53 protein stability was present in 59 (49%) cases out of 102 breast cancer cases in Singapore.¹⁴ In a study in Japan, Yamashita et al investigated 73 breast cancer cases and observed p53 protein stability in 16 (21.9%) cases which were significantly resistant against hormone therapy compared with controls.¹⁵ In a study in France in which sera of 158 breast cancer cases at stages 3 and 4 were examined, the P53 specific antibody was detected in 30 (19%) cases.¹⁶ Although those results are different from our findings, the difference is probably due to the method of the study as we examined and detected the P53 monoclonal tissue specific antibody in the breast cancer cases. We have, therefore, proposed that factors stated above are involved in the various patterns in the expressions of p53 gene and its mutation. In this respect, it is shown that the higher expressions of this gene are created in the higher grades and in invasive tumours.¹⁷

Pinder et al in a study in the UK found a significant relationship between the tissue grade and the Ki67 proliferative marker.¹⁸ Similarly, we found, a significant relationship between the presence of Ki67 marker and P53 protein stability. Our results also demonstrated, as Kaplan-Meier test confirmed, that the survival rate in those who were positive for Ki67 marker was less compared to those who were negative. We also found that the relationship between tissue grade and the Ki67 factor was not significant which is similar to the results of the Tan et al who reported that increasing the ki67 gene expression was related to the higher histological grades and mitosis.¹⁹ However, Mylonas et al in Germany found a significant relationship between the Ki67 factor and ductal carcinoma. However that study did not show such a relationship between the tumour type and the P53 protein stability.²⁰ Ringberg et al in Sweden examined the presence of the Ki67 marker in 170 breast cancer cases. They observed that the Ki67 marker was present in 72 (42%) cases, but they did not find any significant relationship between the cancer and some biological factor such as the age (mean age was 58 years).²¹ The result was similar to our findings in terms of the ki67 gene expression rate but it was different in terms of the age as the mean age in our study was nearly 10 years less. This indicates that in Sabzevar, breast cancer may initiate in earlier ages compared with other areas such as Sweden. Therefore, this issue merits more investigations in terms of other possible influencing factors.

Earlier reports have shown that the prevalence of breast cancer is less than 1% in men.²² As expected, we detected the breast cancer in only one male who died in the first year after the diagnosis. However, in his collected sample both P53 protein and the Ki67 marker were negative. This case was at the stage 3 at the time of diagnosis. As there is not enough information about breast cancer among males, the cancer may be diagnosed at an advanced stage and with unfavourable prognosis. We also found that 32 women died within four years of our follow up. According to several studies, classical and novel prognostic factors are important in the prediction of the survival rate.²³

Conclusions

Results of the study suggested that the Ki67 marker is likely an important factor in the prognosis of breast cancer compared with the P53 protein in north eastern Iran. To the best of our knowledge it is the first report about the prognosis of the breast cancer in Sabzevar which is a thickly populated area in Iran.

Acknowledgments

We wish to thank Dr. Mohajeri, Dr. Ebrahimi, Mr. Borughani, Ms. Landarani and Ms Mahmoudi for assistance in pathological diagnosis, and Dr Namazi and Shomoossi for editorial assistance.

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