

Correlation of Ki67-Positivity in Tumoral Cells' Percentage with Effective Factors on Prognosis in Primary Breast Cancer

¹Mohammad Reza Jalali Nadoushan, ²Elham Neisani and ³Mitra Karbassi

¹Department of Pathology, University of Shahed, Iran

²Department of Gynecology, Shahid Beheshti University of Medical Sciences, Iran

³General Practitioner, Qazvin University of Medical Sciences, Iran

Abstract: Breast cancer is the most common cancers in women in the world. Molecular markers are one of the most important prognosis factors. In this study, correlation of proliferative factor, Ki67, has been evaluated by grade, stage and axillary involvement in primary breast cancer. This study has been done by cross-sectional study on 69 paraffin blocks of patients with breast cancer which obtained from pathology department of Shahid Mostafa Khomeini hospital, since 2000-2004. From each, two samples with 3 micrometer thickness were provided. One from tumor and one from lymphnode(s) which were stained by hematoxylin and eosin. Then we determined number of lymphnode(s) and grade according to Nottingham Modification of Bloom-Richardson criteria. Another section for Ki67 was evaluated by immunohistochemical staining. The results have been analyzed statistically according to grading, lymphnode(s) involvement and ki67-positivity. Our study showed that 100% of samples of ki67 were positive. The most samples were grade III, 43.5% and the least were grade I, 17.4%. 69.6% of the patients had axillary lymphnode(s) involvement. In this study, there was correlation between ki67 and axillary lymphnode(s) involvement. The expression of ki67 in patients with breast cancer should be noticed. It has to be paid attention to this marker as effective factor in prognosis of breast cancer in Iran. It can't be concluded definitely by this study in its role in prognosis and it needs more study with more samples and longer following up.

Key words: Breast cancer, prognosis, ki67, tumoral cells, correlation

INTRODUCTION

Breast cancer is the most common cancer among women (Kumar and Cotran, 1997). It is the most current malignancy in the whole world (Kumar and Cotran, 1997; Clark and Harris, 2000; Stenberg, 1999) and the most common reason for death between cancers among women, at the age of 40-55 (Clark and Harris, 2000).

Breast cancer is the second reason for death between cancers after lung cancer at any age of women (Kumar and Cotran, 1997; Clark and Harris, 2000; Stenberg, 1999). In advanced countries, between 8-12 persons, one person is affected by breast cancer (Stenberg, 1999). In America it has seen about 21200 new cases of invasive breast cancer and 56000 new cases of insitu breast cancer (Speroff *et al.*, 2005).

Because primary breast cancer's clinical progress is different from patient to patient then final determination of each patient is not distinguished. Some patients have disease-free survival, whereas some other groups die

because of recurrence. Some of these differences are defined by changing of tumor growth, invasion and metastasis.

Knowing factors which can determine final prognosis of patients directly or indirectly are useful for clinical deciding and choosing of treatment (Clark and Harris, 2000).

Ki67 is a nuclear protein which is binding to cell cycle tightly. It is a proliferative marker which uses in good or bad prognosis groups in invasive breast cancer (Mahjoob, 1998).

Ki67 is a protein antigen which is related to cell nucleus and is expressed by the cells in G1, G2, M and S phases (not G0 phase) from breast cancer to malignant lymphomas. The more number of stained cells by ki67 antibody in a tumor, the higher grading of tumor and the worse prognosis (Namita and Hall, 1992).

It is about two decades that ki67 antibody has been used as a proliferative marker. A number of studies have shown that there is a very tight association between

ki67 immunoreactivity and the cell cycle, with expression appearing in mid to late G1, rising through S phase and G2 to reach a maximum in mitosis. After mitosis the antigen is rapidly degraded (or the epitope lost), ki67 antibody has been very widely used as clinical prognosis value (Namita and Hall, 1992).

The goal of this study is correlation of proliferative factor between ki67 and tumor size, axillary lymphnode(s) involvement and grading on primary breast cancer.

MATERIALS AND METHODS

This study has been done on 69 samples of patients with primary and invasive breast cancer who had been mastectomy from 2000-2004 in Shahid Mostafa Khomeini hospital in Tehran.

At the first, the information including age, tumor size, number of lymphnode(s) involvement has gotten from patients documents in pathology department and then it had been cut in 2 sections from paraffin blocks of tumor. One of sections was stained with hematoxylin eosin. The kind of tumor and grading were determined by Nottingham modification of Bloom-Richardson criteria, in three grades 1, 2, 3 and staging was determined by TNM criteria. Another section was studied immunohistochemically with polyclonal antibody, according to Manufacturer Company's kit order (Dako) and ki67 positivity percentage in tumoral cells were observed by light microscope.

The results have been analyzed by statistical software SPSS, for grading, lymphnode(s) involvement, tumor staging and its relation with ki67% positivity.

RESULTS

This study has been done on 69 samples of primary ductal infiltrative breast cancer and all of the patients were female.

The average age was 53.35±12.80, the youngest 27 and the eldest 81 years old. The most samples were grade 3, 43.5% and the least were grade 1, 18.8 and 56.5% of samples were grade 2 and lower.

According to staging, the most group was stage 2, 58%.

The average age of patients were in grade I, 51.92 and in grade II, 54.23 and in grade III, 52.9. The average Age of patients were in stage I, 54.14, in stage II, 50.85 and in stage III, 53.88. The situation of ki67 in 69 patients showed that 100% were positive for ki67.

In Fig. 1, the situation of axillary lymphnode(s) involvement in 69 patients has been presented and shows 69.6% of patients had axillary lymphnode(s) involvement.

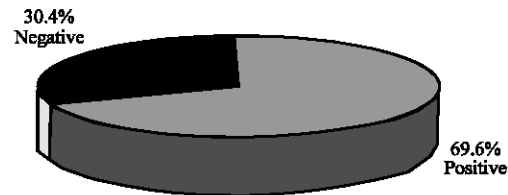


Fig. 1: Frequency distribution of patients with primary breast cancer with Lymphnode(s) involvement

Axillary lymphnode(s) have been arranged according to number of lymphnode(s) involvement, in three groups: group 1 (without involvement), group 2 (1-3 lymphnode(s) involvement), group 3 (4 lymphnode(s) and higher) that frequency distribution were in group 1, 30.4%, group 2, 36.2%, group 3 33.3%.

The scoring of positivity of ki67 (ki67d) was, degree 1 (mild), degree 2 (moderate) and degree 3 (severe). Degree 1 (0-12)%, degree 2 (12-27)% and degree 3 (28-100)%.

49.3% were degree 1, 33.3% degree 2, 17.4% degree 3. There was correlation between stage and ki67 by statistical test, chi-square ($p = 0.000$) which means the higher stage, the higher positivity of ki67. In the analysis, there was correlation between grade and ki67 too ($p < 0.018$) Which means the higher grade, the higher positivity of ki67. There was also correlation between lymphnode(s) and ki67 ($p < 0.001$) which means the more lymphnode(s) involvement, the higher positivity of ki67.

DISCUSSION

Our study showed, the expression of ki67 were 100% positive. In all patients' samples with primary breast cancer and also showed that ki67 is correlated with axillary lymphnode(s) involvement. There is no study about the expression of ki67 in the country, but in other countries like England in 2005, Dowsett and his colleagues confirmed, the value of ki67 as a molecular marker and provide information regarding the relationships between treatment-induced changes in ki67 and other important biomarkers (Dowsett *et al.*, 2005). Ackland and his colleagues study in 2005 showed that the reduction in cell proliferation was associated with decreased expression of nuclear proliferation antigen ki67. In research that Surowiak and his colleagues in Poland, in 2005, did, the relationship of the expression of proliferation-related antigens ki67 in the cells of ductal breast cancer was evaluated. This study aims at examining the relationship between intensity of expression of proliferation-related antigens-ki67 in cells of ductal breast cancer that has been performed in 60 paraffin Sections with immunohistochemical method. This study showed that ki67 correlates with morphologic features of proliferation

(Surowiak *et al.*, 2005). In Ding and colleagues' study in Taiwan, in 2004, ki67 in relation to pathological and molecular features in early-onset infiltrating ductal carcinoma evaluated. These results showed that ki67 were the only important marker significantly and independently associated with tumor grade. Ki67 negative tumors frequently displayed a low tumor grade whereas ki67-positive tumors were more likely to exhibit a high tumor grade. These findings suggest that ki67 might be involved in distinct pathological and molecular features during breast cancer development (Ding *et al.*, 2004). A study has been done by Li and his colleagues in China, in 2004. The expression of ki67 in 151 cases of breast cancer were assessed by immunohistochemistry and their correlations with clinicopathological factors were statistically analyzed. The result of this study showed that the expression of ki67 correlated with tumor stages but didn't relate to age, tumor size and lymphnode status. From this study concluded that ki67 is a biological marker for estimating the occurrence and progression of breast cancer (Li *et al.*, 2004).

The method of the study of ki67, like the other studies, was immunohistochemistry. The advantage of this study, in comparison with previous studies is that the population has been chosen accidentally and by chance and it has been done in one center, with similar method at the same time. Consequently, intervention factors, like differences in stain kits and methods have been eliminated. Whereas axillary lymphnode(s) is known as an effective and independent factor in breast cancer prognosis, its relation with ki67, can help to determine prognosis of breast cancer. In this study, lymphnode(s) involvement have been seen in 69.6% and correlation found between ki67 and axillary lymphnode(s) and also correlation found between grade and ki67 and between stage and ki67 which shows ki67 can be used as an effective factor in determination of breast cancer prognosis.

CONCLUSION

It should be noticed to this marker (Ki67) and its relation with effective factors in prognosis in patients with breast cancer in Iran. Evaluation of ki67 in breast cancer's patients can be guidance in determination of treatment, how to follow-up and prognosticate disease-free survival and the whole survival as well.

REFERENCES

- Ackland, M.L., S. Van de Waarsenburg and R. Jones, 2005. Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. *In vivo*, 19: 69-76.
- Clark, G.M and J.R. Harris, 2000. Diseases of the Breast. (2nd Edn.), Philadelphia: Lippincott Williams and Wilkins, pp: 489-515.
- Ding, S.L., L.F. Sheu, J.C. Yu, T.L. Yang, B. Chen, F.J. Leu and C.Y. Shen, 2004. Expression of estrogen receptor- α and Ki67 in relation to pathological and molecular features in early-onset infiltrating ductal carcinoma. *J. Biomed. Sci.*, 11: 911-919.
- Dowsett, M., S.R. Ebbs, J.M. Dixon, A. Skene, C. Griffith, I. Boeddinghaus *et al.*, 2005. Biomarker Changes during neoadjuvant anastrozole, tamoxifen, or the combination: Influence of hormonal status and HER-2 in breast cancer-a study from the IMPACT Trialists. *J. Clin. Oncol.*, 23: 2477-2492.
- Li, B.J., Z.H. Zhu, J.Y. Wang, J.H. Hou, J.M. Zhao, P.Y. Zhang *et al.*, 2004. Expression correlation of Ki67 to p53, VEGF and C-erb B-2 genes in breast cancer and their clinical significances. *Ai Zheng*, 23: 1176-1179.
- Mahjoob, F., 1998. The new findings in Pathology, Immunohistochemistry: Generalities, Application, Technique. (1st Edn.), Tehran: Sherkat Sahami Enteshar, pp: 9-33.
- Namita Sawhney and P.A. Hall, 1992. Ki67-structure, function and new antibodies. *J. Pathol.*, 168: 161-162.
- Speroff, L., R.H. Glass and N.G. Kase, 2005. Clinical Gynecologic, Endocrinology and Infertility. (17th Edn.), Philadelphia: Williams and Wilkins, pp: 589.
- Sternberg, S.S., 1999. Diagnostic Surgical Pathology. (3rd Edn.), Philadelphia: Williams and Wilkins, pp: 319-385.
- Surowiak, P., Pudelko, A. Maciejczyk, P. Dziegiel, A. Wojnar and M. Zabel, 2005. The relationship of the expression of proliferator-related antigens Ki67 and PCNA in the cells of ductal breast cancer with the differentiation grade. *Ginekolog Pol.*, 76: 9-14.