RESEARCH ARTICLE

Ki67 Frequency in Breast Cancers without Axillary Lymph Node Involvement and its Relation with Disease-free Survival

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Abstract

Background: Breast cancer prognosis is influenced by several histopathology and clinical factors including expression of Ki67 which may have a predictive role in lymph node negative breast cancer patients. The aim of this study was to assess Ki67 expression in breast cancers without axillary lymph node involvement and to evaluate its prognostic value with regard to disease-free survival. Materials and Methods: Subjects were selected from non-metastatic invasive breast cancer patients who were referred to the oncology department of Ghaem hospital during 1 April 2001 to 1 April 2008. Ki67 levels were measured using immunohistochemistry (IHC) and compared with clinicopathological features. The relation of Ki67 expression with disease-free survival was also analysed. Results: A total of 106 women with a mean age of 49 were examined. Some 94.3% were classified as having invasive ductal carcinomas and the mean tumour diameter at the time of diagnosis was 2.8 cm. Some 50.9% of cases were ER positive and 47.2% were PR positive. P53 expression was positive in 48.1% of the cases. According to the IHC results, only 8.5% of the patients were Her2/neu positive. Ki67 was positive in 66 (62.3%) with a significant relation to lower age (p=0.0229) and P53 positivity (p=0.005). After an average of 40-months follow up, 13 (12.3%) demonstrated recurrence, most commonly systemic. Of 13 cases with relapse, 10 patients (77%) were Ki67 positive. Conclusions: In our population Ki67 appeared to be an independent prognostic factor for three-year survival. However, we stress that a survival study with a bigger sample size would help to support this conclusion.

Keywords: Breast cancer - Ki67 - negative axillary lymph node - disease-free survival

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Introduction

Breast cancer is the most common cancer in women globally and the second cause of cancer-related death in Iranian women (Mousavi et al., 2009). Although there has been no routine screening program in Iran until recently, some breast cancer cases can be diagnosed in the early stage of breast cancer. Therefore, it is essential to evaluate prognostic and especially predictive factors to choose the optimal adjuvant therapy for an individual patient based on established predictive factors.

The prognosis of breast cancer is influenced by several histopathology and clinical factors (Tanriverdi et al., 2014). Axiliary lymph node involvement is one of the most important factors for prognosis and selection of the treatment method (Wexler, 2003). In patients without lymph node involvement, several factors such as tumour size, histopathology grade, patient's age, oestrogen receptor, progesterone receptor, human epidermal growth factor 2 (HER2) and invasion to lymph and blood vessels have prognostic and predictive values for treatment (Mirza et al., 2002). Ki67 as an immunologic proliferation marker, has probably a predictive effect in lymph node negative breast cancer patients (Rossi et al., 2015).

There are several genetic tests to help with selecting treatment method such as Oncotype diagnostic test (21-genes tests) which is commercially available (Kittaneh et al., 2013). Unfortunately these tests are expensive and

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are not validated and also available in some countries . However, assessment of of some of these markers such as nuclear antigen Ki67, as a cell proliferating marker, is possible using immunohistochemistry. In this study, we assessed the value of Ki67 prognostic factor in 3 years of disease-free survival in breast cancer patients and its relation with other prognostic factors such as age, size of tumour, pathologic subclass, ER, PR, P53 and HER-2/neu status to see if it can be used as a prognostic factor in decision making for the most appropriate treatment.

Materials and Methods

In a retrospective cohort study, 106 cases of non-metastatic invasive breast cancer who were refered to the oncology department of Ghaem hospital from 1 April 2001 to 1 April 2008 were selected according to the following criteria: All of them, had no lymph node involvement where at least 6 axillary lymph node dissections were negative. Ki67 assesment was done by the assigned pathologist for all samples using immunohistochemistry (IHC) method. All patients received adjuvant therapy according to the routine guidelines. Clinicopathological data including sex, age, time of the diagnosis and surgery, pathologic subtype, tumour size and staging, ER, PR, P53, HER2/neu status were also collected. All patients were followed up until 1 April 2011; follow up duration, time of surgery and the last referral time to the oncology center were recorded on a monthly time scale. Local recurrence in the same side, regional recurrence in the regional lymph node involving axillary, supra and infra clavicular and systemic recurrence as distant metastasis to the other organs were defined. If there were any recurrence during the follow up period, the interval between surgery and recurrence would be calculated in month as disease-free survival.

According to the previous studies, ER and PR more than 1% (2010), p53 more than 10% (Stal et al., 1995), HER2/neu 3+ (Wolff et al., 2014) and Ki67 more than 10% by IHC were considered positive (Moriya et al., 2000). With respect to the fact that there is no agreement about certain Ki67 proliferative factor levels, the results were examined separately using two different cut-off points; Ki67>10% and Ki67>20%, however, in this study Ki67 >20% was considered as positive. Tumour staging (T) was performed using the available tumour size in the pathology report.

Statistical Analysis

All data were analyzed using SPSS software (version 13). Kaplan-Meier survival analysis was used to compare survival in two groups, Ki67 positive and negative. Chi-square was used to compare the others prognostic factors and Ki67 status. A p-value less than 0.05 was considered statistically significant.

Results

In this study, 106 non-metastatic invasive breast cancer patients without lymph node involvement were assessed. They were aged between 28 and 79 years with the mean age 49±5 and were followed up between 4 to 109 months (the mean follow up duration was 40 months). The clinicopathological features are shown in Table 1.

94.3% of patients were invasive ductal carcinoma and the mean size of the tumour at the time of diagnosis was 2.8 centimeter. 50.9% of patients were ER positive and 47.2% of them were PR positive and P53 was positive in 48.1% of the cases. According to the IHC results, only 8.5% of patients were Her2/neu positive.

The assessment results of Ki67 by IHC method according to the two different cut-off values are shown in Table 2.

In this study, Ki67≥20% was considered as positive, so 62.3% of patients were Ki67 positive. The mean age of the patients was 53±11 for negative Ki67 and 47±11 for positive ones. Results indicated a significant relation between Ki67 and age (p=0.0229). In both conditions the

Table 1. Clinicopathological Features in Breast Cancer Patients

clinicopathological features	Type	Number (percentage)
Tumour variant	Ductal	100 (94.3)
	Lobular	6 (5.7)
	Total	106 (100)
Tumour location	Right breast	45 (42.5)
	Left breast	61 (57.5)
	Total	106 (100)
Grade	Grade1	12 (10.9)
	Grade2&3	84 (79.1)
Staging (T)	T1≤2	29 (36.8)
	T2 (2-5)	67 (63.2)
	Total	106 (100)
Oestrogen receptor	Negative	52 (49.1)
	Positive	54 (50.9)
	Total	106 (100)
Progesterone receptor	Negative	56 (52.8)
	Positive	50 (47.2)
	Total	106 (100)
p53	Negative	55 (51.9)
	Positive	51 (48.1)
	Total	106 (100)
HER2/neu	Negative	97 (91.5)
	Positive	9 (8.5)
	Total	106 (100)

Table 2. Frequency of Ki67 Positive Patients Based on Threshould

Ki67 staus	Negative No. (%)	Positive No. (%)	Total No. (%)
Ki67≥20%:	40 (37.7)	66 (62.3)	106 (100)
Ki67≥10%:	22 (20.8)	84 (79.2)	106 (100)

Table 3. Frequency Distribution of Recurrence Region in Patients

Recurrence region:	Number (Percentage)
Local	4 (30.75)
Regional	0 (0.0)
Systemic	7 (53.84)
Local and regional	1 (7.7)
Local and systemic	1 (7.7)
Total	13 (100)

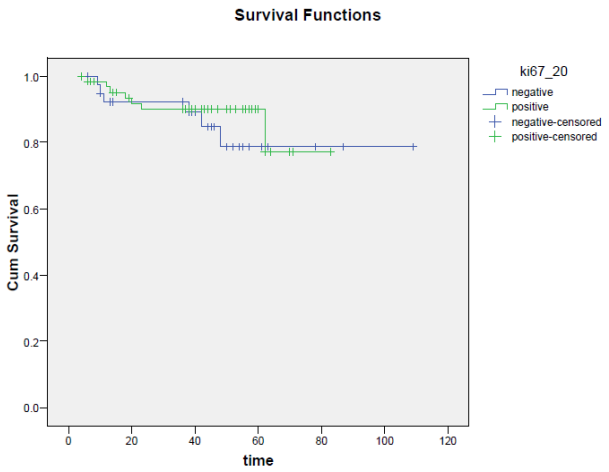

Figure 1. Disease-free Survival According to Ki67 Results in Patients

mean age of Ki67 positive ones was less than that of the Ki67 negative ones.

There was no significant difference between the tumour size in negative and positive Ki67 cases (p=0.237).

After an average of 40 month follow up, 13 patients (12.3%) had recurrence of which 10 patients (77%) were Ki67 positive (p=0.0235). The region of recurrences is shown in Table 3. The most common kind of recurrence was distant metastasis in the cases with recurrence.

According to the Kaplan–Meier test in patients with Ki67≤20%, the 3-year disease-free survival period was 89% and in patients with Ki67>20%, it was 85% (Figure 1).

Mean survival period in patients with Ki67≤20% was 92±6 months and 73/5±3 months for Ki67>20%. Logistic regression analysis showed no significant difference between the two groups (p=0.556).

Moreover, Chi-square was used to compare the other prognostic factors with Ki67 status. With respect to the fact that age in both groups with Ki67 positive and negative had a significant difference, we entered this factor accompanied with Ki67>20%, into the Cox regression, and the result did not show any relation with the age effect, nor with the survival period.

Discussion

In this study, the value of Ki67 prognostic factor in a 3-year of disease-free survival in breast cancer patients and its relation with other prognostic factors were assessed.

With regard to the fact that there are no agreements about certain Ki67 proliferative factor levels, in the current study, we considered Ki67 > 20% as positive according to the proposed scale in the study done by Colleoni, M. et al. (Colleoni et al., 2004).

The mean age of Ki67 negative patients was 49±11. We found a significant relation between Ki67 and age (p=0.0229) where the mean age of Ki67 positive patients was less than that of Ki67 negatives'. Previously, it has been shown there was a strong relation between Ki67 and age (Sahin et al., 1991).

The mean tumour sizes in Ki67 negative and positive cases were 2.79 cm and 2.88 cm respectively, so the mean tumour size in Ki67 positive cases was more than that of the negative ones. However, there was no significant relation between Ki67 and tumour size (p=0.581). Previous studies indicated contravertial results on this issue. The results of some studies showed that Ki67 had a strong relation with tumour size (Gonzalez-Sistal et al., 2014). Furthermore, the results of other studies were different in which there were no relations between Ki67 and tumour size (Haroon et al., 2013; Knutsvik et al., 2014).

According to our results, there was not any correlation between Ki67 and oestrogen receptor (P value=0.029), which was similar to Haroon S et al. (Haroon et al., 2013). This is in contrast with two other studies where there was a reverse relation between ER and Ki67 (Li et al., 2014). In addition, no correlation was found between Ki67 and progesterone receptor in this study (P value=0.112) while previous studies showed an inverse correlation (Haroon et al., 2013; Wong et al., 2014).

In our study there was a significant relation between Ki67 and p53 expression (P value=0.005). It has been suggested that overexpression of p53 is due to frequent mutations and overexpression is associated with higher Ki67 (Sirvent et al., 1995). In this study, there was no relation between Ki67 and HER2/neu oncogene (Pvalue=0/47) which is similar to Osman I et al. (Osman et al., 2001).

The mean survival rate was 92±6 months in Ki67 negative cases and 73±3 in positive ones, respectively. However, this difference was not significant (p=0.556). Considering the fact that age had a significant relation with Ki67 in both groups, survival rate was assessed by removing the age from the equation to omit the age effect. However, some reports indicated that Ki67 did not have any prognostic value, and there was no relation between Ki67 and survival rate (Payandeh et al., 2014). In contrast, as mentioned before, in many other studies, the prognostic value of Ki67 has been frequently reported and this protein has been introduced as a predictive marker in recurrence (Kontzoglou et al., 2013; Inwald et al., 2013).

In this study, Ki67 was positive in 10 patients with recurrence (77%) and there was a significant correlation between recurrence and Ki67 status (p=0.0235). In a meta-analysis by Azambuja, it has been suggested that Ki67 is a recurrence marker in patients' survival with primary breast cancer (de Azambuja et al., 2007). In another study after assessing 669 cases during 53 months, researchers proposed that low rate of proliferation in invasive breast cancer without lymph node involvement is a good prognostic sign (Jones et al., 2001). Several studies have shown that Ki67 provides independent prognostic information especially in breast cancer without lymph node involvement (Mirza et al., 2002) and higher Ki67 was associated with a lower disease-free survival (Kilickap et al., 2014). Therefore, in our population Ki67 can be used as an independent prognostic factor.

One of the current limitations which may justify some differences in results of this study with other similar ones is the limited number of the studied patients. This was due to the low number of patients who met the criteria as we considered at least examination of six axillary lymph nodes. In most of the cases the number of the evaluated

lymph nodes was uncertain or less than six, so they were not assessable for the study. In addition, the follow up period in 18 cases was less than 36 months, therfore it makes the exact judgment about recurrence difficult in these cases as the recuurence might be likely after a longer period.

In conclusion, according to this study, although there is not a significant relation between Ki67 and 3-year disease-free survival, there is a significant relation between Ki67 and recurrence rate and also with some prognostic factors such as P53 status and age. We suggest if it would be available, a physician should use standard tests such as Oncotype for the assessment of the recurrence risk, otherwise prognostic factors such as Ki67 may be helpful.

Further assessment is needed to evaluate the relationship between Ki67 and the recurrence risk. More precise results can be achieved by analyzing more subjects, paying more attention to the lymph node involvement status and increasing the follow up duration. Performing a meta-analysis in this area is recommended as the new results are emerging.

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