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Ki-67이 Luminal B형 유방암 환자의 예후에 미치는 영향

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Ki-67 index, Luminal B Type, and Prognosis of Patients with Breast Cancer

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Abstract

Background: Ki-67 expression has been considered to be a reliable marker for assessing tumor cell proliferation. The aims of the ourstudy were to assess the correlationbetween Ki-67 expression and clinocopathologic factors and to analyze the effect of Ki-67 expression on survival rate. Methods:The study subjects, 679 women with breast cancer, were a subset of patients operated at OOO hospital from Jun 2001 to Dec 2005.Patients are grouped into 3 categories according to Ki-67 determined in surgical specimens. Clincopathologic factors were compared with 3 categories of Ki-67. Chi-squared tests were used for statistical analysis. Kaplan–Meier estimates are presented for thesurvival function, and differences in survival were analyzed usingthe log rank test. Results: The median age was 46yrs, and median tumor size was 3.2cm. The strong correlation was observed between tumor staging and Ki-67 staining(p=0.007) and Group III(more than 20% staing) showed more advanced N-staging(p=0.003). The number of patients with estrogen receptor negative was higher in Group III(p=0.001) and that with HER-2 receptor positive was also higher in Group III(p =0.001) comparing with other groups. The univariate analysis for prognostic factors associated with DFS revealed that both the tumor groupas T1, T2, T3 or T4 and lymph node as N0, N1, N2 or N3 were statistically significant(p=0.0003 and p=0.0015 respectively) but the Ki-67 staining as <10%, 10~20%, >20% was statistically not significant(p=0.6112). The univariate analysis for prognostic factors associated with OS revealed that Tumor staging and N-staging was statically significant(p=0.003 and p=0.0015 respectively). Conclusions: According to our study, Ki-67 positive groups was associated with more advanced staging, ER-negative, PR-negative and HER-positive respectively. But there is no significant association between Ki-67 staining and 5-year disease free survival rate including overall survival rate.

Key words : Ki67, breast cancer, survival rate, T-stage, N-stage, Luminal B

Introduction

The nuclear proliferation antigen, Ki-67, is found in all phases of the cell cycle except G0 and early G1. Its expression has been considered to be a reliable marker for assessing tumor cell proliferation.[1-4] Proliferationis an important prognostic factor that forms part of the gradingclassification and is associated with many other prognosticbiomarkers. With regard to steroid hormone receptor status, most studieshave found an inverse correlation between Ki-67 and both theestrogen receptor and progesterone receptor.[5-7] The associationbetween HER2/neu status and Ki-67 is controversial, as somestudies have identified a positive correlation, while others havenot.Recently the association between Luminal B type(ERorPR positive and HER-2positive) and Ki-67 was reported.[2] The aims of the our study were to assess the correlationbetween Ki-67 expression and clinocopathologic factors. And we wanted to assess the effect of Ki-67 expression on survival rate. Also we wanted to assess the effect of Ki-67 expression on survival rate in patients with Luminal B type breast cancer.

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Methods

Patients and Specimens

The study subjects, 679 women with breast cancer, were the subset of patients operated at OOO hospital from Jun 2001 to Dec 2005. In order to obtain an etiologically homogenous group of study subjects which should help in elucidating the phenomenon of tumorogenesis, we restricted our study to histologically confirmed primary invasive ductal carcinoma. No patient had a familyhistory of breast cancer in their first-degree relativesfamilies, so these tumors were most likely sporadic. Institutional review board approved informed consent was obtained from each patient prior to tissue collection.

Immunohistochemistry (IHC)

All data were collected from the pathology reports. Histopathological features such as hormone receptor status and HER2/neu status on immunohistochemistry(Dako, Copenhagen, Denmark)were all analyzed at the Institute of Pathology at the University of OOO. Expressions of p53, ERa, Ki-67 and ErbB-2 were determined immunohistochemically with paraffin sections using antibodies against ERa(Dako, Copenhagen, Denmark),Ki67 (Dako, Copenhagen, Denmark), ErbB2(Dako, Copenhagen, Denmark) and p53(Dako, Copenhagen, Denmark). Histologic grading was performed using the criteria of Bloom and Richardson.[12,13]Lymphatic vascular invasion (LVI) was defined as the presence of tumor emboli in peritumoral lymphatic spaces, capillaries or postcapillary venules. ER status and PR status were taken as positive if more than 10% of tumor cells showed staining. Immunohistochemical score of 3+ or FISH + for HER2 was accepted as HER2 positivity.The patients are grouped into 3 categories according to Ki-67 determined in surgical specimens: Group I)165(24.3%) patients with Ki-67 in less than 10% of neoplastic cells; Group II) 216(31.8%) patients with Ki-67 present in 10~20% of neoplastic cells; Group III) 461(67.8%) patients with Ki-67 present in more than 20% of neoplastic cells.

Statistical Analysis

Statistical tests were performed using the SPSS 12.0statistical software package for Windows (SPSS Inc, Chicago, IL). The survival functionwas calculated from the time of the onset of disease to theoccurrence of death. Survival data were censored on December 31,2009, which was the date on which the survival data were correlatedwith the death registry for the last time or 5 years after theonset of the disease. Kaplan–Meier estimates are presented for thesurvival function, and differences in survival were analyzed usingthe log rank test. Associations between specific histopathological and clinical survival estimates and curves were established using the Kaplan–Meier method and differencesin observed survival distribution among patient subgroups were tested with two-sided log-rank test. All survival rates were presented with their standard errors. We used Pearson's correlation to determine the association of pairs of explanatory variables and differences in qualitative variables were evaluated by Chi-squared test, where necessary. All p-values were two-sided and a p-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

The median age and mean tumor size of patients was 46 yrs, and 3.2cm respectively. All patients were classified as Luminal A type tumor(ER or PR-positive and HER-2-negative, 148 patients, 21.8%), Luminal B type tumor(ER or PR positive and HER-2-positive, 242 patients, 35.7%), HER-2 type tumor(ER and PR negative and HER-2-positive, 183 patients, 26.9%) and a triple negative type(ER and PR and HER-2-negative, 106 patients, 15.6%)(Table1). No correlation was observed between age and Ki-67 staining(p=0.812).But the strongcorrelation was seen between tumor staging and Ki-67staining. T1, 2, 3 and 4 showed an average cell staining of 46.3%, 58.6%, 49.0% and 70.0%, respectively(p=0.116). The strong correlation was seen between N-staging and Ki-67 staining. N0, 1, 2 and 3 showed an average cell staining of 46.5%, 55.9%, 62.7% and 72.0% respectively(p=0.003). Tumors graded 1,

Table 1. Patients and tumor characteristics and association with Ki-67 staining

Characteristics		Ki-67 <10%	Ki-67 10%~20%	Ki-67 >20%	Total(n)	*p*-value
Age	≤35	12(26.0)	9(19.6)	25(54.3)	46	0.812
	>35	153(24.2)	150(23.7)	330(52.1)	633	
*pT	T1	80(28.8)	63(24.9)	118(46.3)	299	0.116
	T2	69(19.4)	78(21.9)	209(58.6)	356	
	T3	16(27.5)	13(23.5)	28(49.0)	57	
	T4	4(30.0)	0(0)	7(70)	11	
†pN	N0	111(66.5)	97(64.3)	181(50.1)	389	0.003
	N1	32(19.4)	28(18.6)	77(21.2)	137	
	N2	13(7.7)	14(9.3)	45(12.5)	72	
	N3	11(6.5)	12(7.9)	58(16.1)	81	
AJCC Stage	I	48(29.3)	48(29.3)	67(41.4)	163	0.007
	IIa	62(24.9)	61(24.4)	125(50.7)	248	
	IIb	19(17.8)	24(23.3)	62(58.9)	105	
	IIIa	16(23.0)	12(16.4)	43(60.7)	71	
	IIIb	3(33.3)	0(0)	7(66.7)	10	
	IIIc	12(14.3)	13(15.7)	57(70.0)	82	
Grade	G1	27(33.3)	27(33.3)	27(33.3)	81	0.0001
	G2	68(26.5)	71(27.6)	117(45.9)	256	
	G3	59(17.4)	69(20.2)	214(62.4)	342	
ER	Negative	41(12.8)	65(20.4)	212(66.8)	318	0.0001
	Positive	122(33.8)	97(26.8)	142(40.1)	361	
PR	Negative	60(16.7)	75(20.9)	224(62.4)	359	0.0001
	Positive	103(32.1)	85(26.7)	132(41.3)	320	
P53	Negative	127(31.7)	112(28.1)	161(40.3)	400	0.00001
	Positive	36(13.1)	51(18.3)	192(68.7)	279	
Microarrary	Luminal A	63(42.9)	37(25.2)	48(31.9)	148	0.001
	Luminal B	62(25.6)	65(26.7)	115(47.7)	242	
	HER-2	17(9.5)	41(22.4)	124(63.0)	183	
	Basal cell	15(14.1)	20(18.8)	71(67.1)	106	

*pT: pathologic T-stage
†pN: pathologic N-stage

2, and 3 showed an averagecell staining of 33.3%, 45.9%, and 62.4%, respectively. The percentage of Ki-67 positive cellswas remarkably high in patients with hormone receptor-negative tumors(p=0.001) and the percentage of Ki-67 positive cells was remarkably high in patients with HER-2 positive tumors(p=0.001).All of the clinical and histopathological data were tested fortheir prognostic value in a univariate analysis for disease-freesurvival (DFS) and overall survival (OS). The 5-year overall survivaland disease-free survival probabilities calculated by Kaplan-MeierEstimates (p-value for log rank test) are shown in Table 2.The univariate analysis for prognostic factors associated with DFS revealed that the tumor group as T1, T2, T3 or T4 was statistically significant(p=0.0003)and that revealed that nodal status as N0, N1, N2, N3 was statistically significant(p=0.0001)but that revealed thatKi-67 staining was not statically significant (p=0.6112)(Fig. 1). The univariate analysis for prognostic

Table 2. Association between patients' characteristics and the absolute percentage of positively stained cells

Characteristics		Absolute Ki-67 positive(%)	Total (n)	*p*-value
Age	≤35	54.8	46	0.2111
	>35	47.1	633	
*pT	1	41.9	299	0.067
	2	53.3	356	
	3	44.6	57	
	4	66.7	11	
†pN	0	42.6	389	0.0001
	1	52.3	137	
	2	57.1	72	
	3	65.7	81	
Grading	G1	27.6	81	0.001
	G2	43.7	256	
	G3	55.6	342	
ER status	Negative	56.1	318	0.0001
	Positive	39.6	361	
PR status	Negative	53.0	359	0.001
	Positive	40.4	320	
ER,PR and HER-2	ER(+)PR(+)HER(-)	26.4	148	0.0001
	ER(+)PR(+)HER(+)	48.5	242	
	ER(-)PR(-)HER(+)	61.4	183	
	ER(-)PR(-)HER(-)	53.9	106	

*pT: pathologic T-stage
†pN: pathologic N-stage

Table 3. Five years overall and disease-free survival probabilities as calculated by Kaplan-Meier estimates (P-value for log rank test)

Characteristic		N(events)	5-year DFS(%)	p-value	N(events)	5-year OS	p-value
Age	≤35	46(2)	95.24	0.1873	46(0)	100	0.1437
	>35	633(68)	89.32		633(28)	95.55	
*pT	1	299(15)	94.83	0.0001	299(6)	98.14	0.0003
ER , PR and HER-2 status	ER(+)PR(+) HER-2(-)	148(10)	90.83	0.8493	148(3)	98.11	0.5902
	ER(+)PR(+) HER-2(+)	242(21)	91.06		242(7)	96.95	
	ER(-)PR(-) HER-2(+)	183(20)	89.13		183(9)	95.12	
	Negative	400(39)	90.20	0.7240	400(15)	96.35	0.8196
P53	Positive	279(28)	90.05		279(14)	95.07	

*pT: pathologic T-stage
†pN: pathologic N-stage

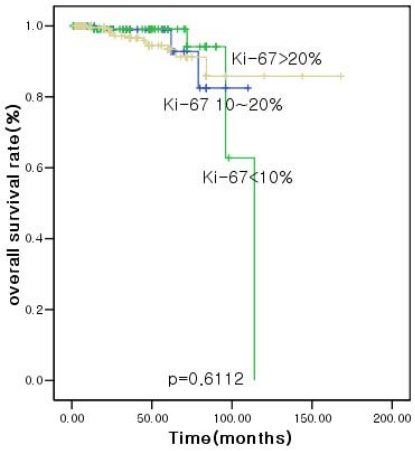

Fig 1. Overall survival rate curves according to the Ki-67 status.

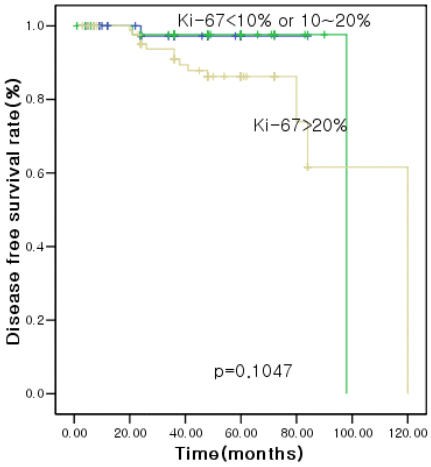

Fig 2. Disease free survival rate curves according to the Ki-67 status in Luminal B type

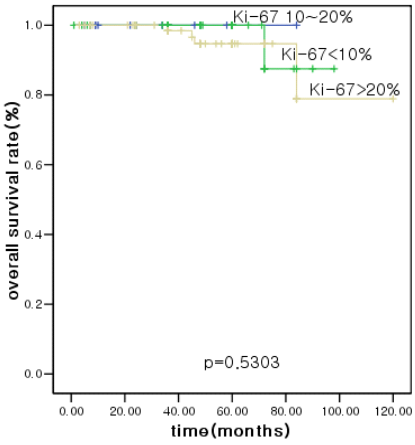

Fig 3. Overall survival rate curves according to the Ki-67 status in Luminal B type

factors associated with OS revealed that Tumor staging and N-staging was statistically significant.(p=0.0003, p=0.0015 respectively). Also All of the clinical and histopathological data in luminal B type were tested fortheir prognostic value in a univariate analysis for disease-freesurvival (DFS) and overall survival (OS)(Fig. 2). The 5-year overall survivaland disease-free survival probabilities calculated by Kaplan-MeierEstimates (p-value for log rank test) are shown in Table 3.The univariate analysis for prognostic factors associated with DFS revealed that the tumor group as T1, T2, T3 or T4 was statistically significant(p=0.0001).But that revealed that Ki-67 staining was not statically significant(Fig. 3)(p=0.5303). The univariate analysis for prognostic factors associated with OS

revealed that Tumor staging and N-staging was statically significant(p=0.0001, p=0.0309 respectively).

Discussion

Ki-67 antigen is the prototypic cell cycle related nuclear protein, expressed by proliferating cells in all phases of the active cell cycle (G1, S, G2 and M phase).[1-4]It is absent in resting (G0) cells. Ki-67 antibodies are useful in establishing the cell growing fraction in neoplasms (immunohistochemically quantified by determining the number of Ki-67 positive cells among the total number of resting cells = Ki67 index).[6-8]In neoplastic tissues the prognostic value is comparable to the tritiated thymidine labelling index. The correlation between low Ki-67 index and histologically low grade tumours is strong.[9-11]Ki-67 is routinely used as a neuronal marker of cell cycling and proliferation.Monoclonal Ki-67 is an antibody specific for a nuclear antigen expressed in proliferation cells(late G1, S, M, G2 phases of the cell cycle).[11,12]The function of the protein detected by the Ki-67 antibody remains unknown, although the gene(gene symbol MKI67) is now known tobe located on chromosome 10q25. The original antibody was only useful on fresh or frozen tissue.[13,14,22]Newer antibodies(MIB-1 and polyclonal Ki-67) have been developed that recognize peptide from recombinant fragments of the Ki-67 antigen and are effective in fixed, paraffin embedded archival tissue.[15-17]The distinction between low and high proliferation is subtle and assessment is generally quantitative rather than semiquantitative, requiring point counting to assess the percentage of cells with nuclear staining for Ki-67 or MIB-1. Ki67/MIB-1 has been evaluated as a prognostic factor in several studies, and high proliferation, as measured by this methodology, is correlated with poor clinical outcome in most studies on univariate analysis.[18-20]Brown et al. examined 673 patients with a median follow-up of 52 months and found on multivariate analysis that high Ki-67 was independent prognostic significance in predicting shorter DFS on multivariate analysis. High Ki-67 is also correlated with other adverse prognostic factors like tumor size, node involvement, histologic grade, and vascular invasion and is inversely correlated with good factors such as steroid receptors. The advantages of this assay are lower costs, easy handling of fixed slides, and relative specificity for cells in S, G2, and M phases of the cell cycle. However, additional studies, especially those with multivariate analysis, are required to more clearly define the role of Ki-67/MIB-1 as a clinically useful prognostic factor. In summary, proliferation indexes are useful prognostic markers. SPF on frozen material is the most validated technique and is recommended as a category .Also Ki-67 is a well-established cell proliferation marker in cancerand an excellent candidate biomarker for HER-2 positive tumors.[2]Tworecent meta-analyses have reported a statistically significant associationbetween high Ki-67 expression and increased risk of breastcancer relapse and death. However, assessment of Ki-67 hasbeen a matter of controversy because some studies have used 10% or 20% cut points, whereas others dichotomizedaround the mean or median value.

Conclusion :

According to our study, Ki-67 positive groups was associated with more advanced staging, ER-negative, PR-negative, HER-positive respectively. So Ki-67 positive groups could be thought of advanced breast cancer. Especilly Ki-67 positive groups was associated with Luminal B type tumor group and HER-2 type tumor group. But there is no significant association between Ki-67 positive and 5-year survival rate including overall survival rate. In our study, post operative AJCC stage should be the most important factor affecting survival rate. And there is no significant association between Ki-67 positive and 5-year survival rate including overall survival rate in Luminal B type tumor group. But because our study is small size study, more abundant patients' date will be needed to evaluate of the Ki-67 predictive role.

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국문초록

배경: Ki-67는 세포의 분열지수로서 특히 세포분열초기 나타나는 것으로 알려져 있다. 그래서 Ki-67의 분열지수가 높은 경우 조기 재발 및 예후와 관련되어 있다는 보고가 있다. 본 연구에서는 본원의 자료를 토대로 Ki-67의 분열지수가 예후에 영향을 미치는지를 알아보고자 한다
방법: 2001년 1월부터 2005년 12월까지 본원에서 유방암으로 수술을 받은 679명의 환자를 대상으로 후향적으로 조사를 하였다. 술후 조직병리학적 보고서를 토대로 조사하였다. Ki-67의 수치를 기준으로 10%이하군, 10%에서 20%군, 20%이상군으로 나누었다. 세군사이의 임상병리학적 요소의 분포를 비교하였으며 Kaplan-Meier를 이용하여서 생존율을 분석하였다.
결과: 환자들의 평균나이는 46세였으며, 평균종양의 크기는 3.2cm였다. 20%이상군에서 진행성 유방암환자의 비율이 높았다.(p=0.007) 특히 림프절의 전이정도와도 관련이 있었다.(p=0.003) 그러나 Ki-67과 생존율과는 의미있는 관련성을 없었다.(p=0.6112).
결론: 본원의 조사에 따르면 Ki-67은 진행성 유방암과 호르몬 수용체 음성과는 밀접한 관계를 가지고 있었으나 생존율에는 영향을 미치지 않았다. 그러나 본조사는 추적기간이 짧고 조사 규모가 크지 않아서 향후 계속적으로 추적 관찰한다면 더 의미있는 결과과 나올것이다.

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