Diagnostic and clinicopathological significance of Ki67 mRNA expression in cervical cancer tissue

Dawn Chung

Department of Medicine

The Graduate School, Yonsei University

Diagnostic and clinicopathological significance of Ki67 mRNA expression in cervical cancer tissue

Directed by Professor Eun Ji Nam

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Dawn Chung

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YONSEI UNIVERSITY

This certifies that the Master's Thesis of
Dawn Chung is approved.

Thesis Supervisor : Eun Ji Nam

Thesis Committee Member#1 : Jae Myun Lee

Thesis Committee Member#2 : Kwang Hwa Park

The Graduate School
Yonsei University

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ABSTRACT

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Ki67 is a key biomarker associated with cancer cell proliferation and poor prognosis. We previously evaluated the diagnostic potential of quantitatively measured Ki67 mRNA levels in formalin-fixed paraffin-embedded (FFPE) cervical cancer tissue samples. In the present study, we continued this avenue of research using quantitative reverse transcription PCR (RT-qPCR) to measure Ki67 mRNA levels in FFPE cervical tissues and performed an assessment with each clinical prognostic factor of patients. A total 190 FFPE cervical tissue samples comprised of 80 squamous cell carcinoma (SCC), 10 adenocarcinoma (ADC), 30 HSIL, 30 LSIL, and 40 normal cervical tissue samples were obtained. Using this RT-qPCR assay, the predictive value of Ki67 in cases with low-grade squamous intraepithelial lesions (LSIL) and those with high-grade squamous intraepithelial lesions (HSIL) were measured. As a result, Ki67 mRNA levels were increased in SCC and ADC cervical cancer tissues (n = 90) compared to those in normal cervical tissues (n = 40) ($P < 0.001$). The diagnostic validity of the Ki67 mRNA assay was evaluated and demonstrated a sensitivity of 93.3% (95% confidence interval (CI) = 86.1 to 97.5) and a specificity of 97.5% (95%

CI = 86.8 to 99.9). Ki67 mRNA expression level was 93.3% for cervical cancer, 40.0% for HSIL, 13.3% for LSIL, and 2.5% for normal tissue samples. Furthermore, high levels of Ki67 mRNA expression in cervical cancer were associated with lymph node status (P = 0.02). In conclusion, Ki67 mRNA assay can provide an additional accurate approach for molecular diagnosing cervical cancer, and also predict prognosis of cervical cancer depending on LSIL and/or HSIL status.

Key words: Cervical cancer, Ki67 mRNA, RT-qPCR, molecular diagnosing

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I. INTRODUCTION

Cervical cancer is the third most common malignancy in women globally and one of the leading causes of morbidity and mortality in women worldwide. The World Health Organization estimates that approximately 527,600 women are newly diagnosed and there are 265,700 deaths from cervical cancer every year.[1] Human papillomavirus (HPV) is a major cause of cervical cancer and is the most common sexually transmitted pathogen among women and men.[2] So the detection of HPV is routinely performed in exfoliated infected cervical cells or tissues of patients with cervical cancer or patients with precancerous lesion.[3-7] But most high-risk HPV infections resolve spontaneously within 1 to 2 years.

Several studies have tried to investigate the molecular mechanisms underlying cervical cancer carcinogenesis to identify potential diagnostic or prognostic biomarkers for cervical cancer.[8-11] For example, putative molecular markers such as p16, p53, and Ki67 have been identified in cervical carcinogenesis. Their respective coding genes and proteins have been characterized, and their roles in the process have been studied with the aim of improving diagnosis and treatment of cervical cancer. Some studies found that among these different markers, immunohistochemical (IHC) staining of Ki67 was an effective method for the

prognosis of different tumor types.[12-15] Ki67 is associated with cell cycle activity and is expressed at varying levels during G1, S, G2, and M phases, but is not expressed in G0.[10-16] In the previous research, the potential diagnostic value of Ki67 in cervical cancer was demonstrated by quantitative measuring of Ki67 mRNA levels in formalin-fixed paraffin-embedded (FFPE) samples.[17]

The concept of precancerous disease of the cervix was first presented in 1947, when it was noticed that epithelial changes would be identified that had appearances of invasive cancer but were confined to the epithelium.[18] Comparing to aggressive treatments of carcinoma in situ (CIS), the dysplasias were believed to be less significant and were not treated or were treated only by colposcopic biopsy and cryosurgery. In 1968, the concept of cervical intraepithelial neoplasia (CIN) was presented by Richart, and he suggested these dysplasias have potential for progression.[19] Most untreated low-grade CIN lesions regress spontaneously, whereas untreated high-grade CIN refers to a lesion that may progress to invasive carcinoma.[20] Without mitotic activity, proliferating metaplasia could not be called dysplasia or CIN because of very rare progression to invasive cancer. The criteria for diagnosing intraepithelial lesion may be various according to pathologist. However, the key features are cellular immaturity, disorganization, and nuclear abnormality including increased mitotic activity. The extent of immature cellular proliferation, nuclear atypia and mitotic activity identifies the degree of intraepithelial neoplasia.[21] The lesion is specified as CIN grade 1, if the extent of mitosis and immature cells is limited to the lower third of the epithelium. Involvement of the middle and upper thirds is designated to CIN grade 2 and 3, respectively. Two-tier system including low-grade and high-grade squamous intraepithelial lesion was introduced as an alternative cytopathologic classification in the late 1980s, and has been translated into histopathological purpose, especially in North America.[22-24]

Recently, by the endorsement of World Health Organization classification of

tumor of female reproductive organs, two-tier system is preferred again. This SIL system was conceptually founded on the presence of two forms of HPV infection, with productive infection causing low-grade squamous intraepithelial lesion (LSIL) and transforming infection leading to high-grade squamous intraepithelial lesion (HSIL), whereas the CIN system focused on identification of CIN lesions and determination of their grades.[25] These LSILs and HSILs are important precancerous courses in the development of cervical cancer, but their exact carcinogenesis has had divergent opinions.[26, 27] Cox et al performed a meta-analysis and concluded that the likelihood of progression from LSIL to HSIL was approximately 10% within 2 years.[28] Nevertheless, there is no accurate approach to identify which LSILs will progress to HSILs. Therefore, a better understanding of cervical cancer development is necessary to improve the management of either LSILs or HSILs.

A purpose of the current study is to investigate the relationship between Ki67 mRNA level and clinicopathological measures of patients with cervical cancer. In this study, the discriminatory power of Ki67 mRNA assay in cervical lesions using quantitative reverse transcription PCR (RT-qPCR) was tested. Then clinical predictive relevance of Ki67 mRNA in each histologic grade of cervix were evaluated by selecting a diagnostic cutoff value using one hundred and ninety FFPE cervical tissue samples.

II. MATERIALS AND METHODS

Patients and samples

One hundred and ninety FFPE cervical tissue samples were collected which were finished pathological diagnosis at the Department of Pathology, Yonsei University Wonju Severance Christian Hospital between January 2010 and

December 2014. The study was approved by the Institutional Ethics Committee of Yonsei University Wonju College of Medicine (approval no. CR315052), and all subjects were provided written informed consent. Of the 190 FFPE tissue samples collected, 40 (21.1%) were normal, 30 (15.8%) were LSIL, 30 (15.8%) were HSIL, 10 (5.3%) were adenocarcinoma (ADC), and 80 (42.1%) were squamous cell carcinoma (SCC) (Table 1). FFPE normal tissue samples included 40 chronic cervicitis specimens obtained from patients who underwent a hysterectomy for other benign gynecological diseases such as leiomyoma and adenomyosis. To analyze clinicopathological prognostic parameters of 90 FFPE samples of cervical cancer, electrical medical records of the patients were retrospectively reviewed and 11 FFPE cervical cancer samples had missing medical information because of referral to other institutes.

Deparaffinization of FFPE tissue and total RNA extraction

Three 10-μm sections from each paraffin block of cervical tissue were used for total RNA extraction. Extractions were performed using the Qiagen RNeasy FFPE mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA purity and concentration were determined by measuring absorbance at 260 nm and 280 nm using a spectrophotometer (Infinite 200, Tecan, Salzburg, Austria). All RNA preparation and handling was performed in a laminar flow hood under RNase-free conditions. Isolated RNA was stored at -70℃.

cDNA synthesis

Complementary DNA (cDNA) was synthesized using the M-MLV Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, USA) and random hexamers (Invitrogen) according to the manufacturer's recommendations. Briefly, 10 μL of total RNA was added to a master mix containing 10 mM dNTPs at neutral pH, 0.25 μg random hexamers, and 5-μL DEPC-treated water. Reactions were

incubated at 65℃ for 5 min and chilled on ice. A mixture of 4 μL 5× First-Strand Buffer, 2 μL 0.1 M dithiothreitol, and 1 μL M-MLV reverse transcriptase (RT) was added, and cDNA synthesis was synthesized at 25℃ for 10 min, followed by 37℃ for 50 min, and 70℃ for 15 min.

Ki67 mRNA RT-qPCR assay

Quantitative real-time PCR amplification of the OPTIMYGENE Ki67 mRNA assay (Optipharm, Osong, Korea) was performed in 10 μL 2× Thunderbird probe qPCR mix (Toyobo, Osaka, Japan), 3 μL primer and TaqMan probe mixture, 2 μL template cDNA, and distilled water (DW) to a final volume of 20 μL per sample. Positive and negative controls were included. No-template controls were included in each run and consisted of sterile DW instead of template DNA. PCR cycling was 95℃ for 3 min, followed by 40 cycles of 95℃ for 3 seconds, and 55℃ for 30 seconds. mRNA levels were quantified by determining the cycle threshold (CT), which is defined as the number of PCR cycles required for fluorescence to exceed a value significantly higher than that of the background fluorescence. To avoid false negatives because of mRNA degradation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The amount of Ki67 mRNA was determined using the comparative CT method (ΔΔCT method), [29] measuring mRNA relative to a reference gene using CFX Manager Software v1.6 (Bio-Rad, Hercules, CA, USA). The amount of Ki67 mRNA was normalized to the internal housekeeping gene GAPDH using the following equation:

$$\Delta\Delta CT = (\Delta CT\ [\text{target sample}] - \Delta CT\ [\text{normal sample}])$$

Statistical analysis

Statistical analyses were performed using GraphPad Prism v5.02 (GraphPad, La

Jolla, CA, USA) and SPSS (Statistical Package for the Social Sciences) v23.0 (SPSS Inc., Chicago, IL, USA). The Wilcoxon matched-pairs test was used to compare nonparametric-matched samples, and the Student's t-test and 95% confidence interval (CI) were used to determine statistical significance. Receiver operating characteristic (ROC) curves were used to predict cutoff values of the marker. Sensitivity and specificity were calculated using MedCalc v12.5 (MedCalc software, Ostend, Belgium). The Pearson's chi-square test was used to analyze associations between the positivity of Ki67 mRNA expression and histologically diagnosed samples. For all tests, a P value < 0.05 was considered statistically significant.

Table 1. Clinical characteristics of patients

	Cancer (n = 90)	**HSIL** (n = 30)	**LSIL** (n = 30)	**Normal** (n = 40)
	n (%)	**n (%)**	**n (%)**	**n (%)**
Age				
≤ 50 years	41 (45.6)	23 (76.7)	20 (66.7)	24 (60.0)
> 50 years	49 (54.4)	7 (23.3)	10 (33.3)	16 (40.0)
HPV DNA chip				
Negative	12 (13.3)	1 (3.3)	11 (36.7)	
Positive	67 (74.5)	21 (70.0)	10 (33.3)	
Unknown*	11 (12.2)	8 (26.7)	9 (30.0)	
Histology				
SCC	80 (88.9)			
ADC	10 (11.1)			
FIGO stage				
< IIB	36 (40.0)			
≥ IIB	43 (47.8)			
Unknown*	11 (12.2)			
Lymph nodes				
Negative	40 (44.4)			
Positive	39 (43.4)			
Unknown*	11 (12.2)			
Tumor size				
≤ 4 cm	45 (50.0)			
> 4 cm	34 (37.8)			
Unknown*	11 (12.2)			

ADC, adenocarcinoma; LSIL, low-grade squamous intraepithelial lesion;
HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus;
SCC, squamous cell carcinoma.
*Unknown stands for cases referred to other institution after biopsy without any baseline study.

III. RESULTS

Patient characteristics

Patient characteristics are summarized in Table 1. One hundred and ninety FFPE tissue samples were used in this study of which 90 (47.3%) were cancer, 30 (15.8%) were HSIL, 30 (15.8%) were LSIL, and 40 (21.1%) were normal samples. For cervical cancer cases, data on histology, FIGO stage, tumor size, and lymph node metastasis were retrospectively reviewed from patient electrical medical records (EMR) (Table 1). Among 90 cases of cervical cancer, 80 (88.9%) cases were histologically diagnosed as SCC and 79 cases were analyzed their clinicopathological information archived from EMR. 43 (47.8%) cases showed FIGO stage IIB and more, 39 (43.4%) cases showed positive in lymph node metastasis, and 34 (37.8%) cases had more than 4 cm of tumor size.

Ki67 mRNA levels for matched FFPE non-cancerous and cancerous tissues

To evaluate the validity of the Ki67 mRNA assay, 16 matched-pairs of non-cancerous and cancerous lesion FFPE samples of the same cervical cancer patient from 16 cervical cancer patients were tested. Using ΔΔCT to determine mRNA levels in matched tissue samples, we found that levels of Ki67 mRNA in cancer tissues were higher than those in matched normal tissues ($P = 0.0005$). The Ki67 mRNA levels in normal tissues ranged from 0.01 to 3.43 compared to those in cancer tissues ranged from 0.22 to 131.60. The Ki67 mRNA levels in cervical cancer tissues were 1.84 to 1167.33-fold higher compared to levels found in normal tissues (Table 2).

Diagnostic value of Ki67 for cervical cancer

ROC curve analysis was performed to determine the optimal diagnostic cutoff value for the assay to discriminate normal tissues from those with cervical cancer.

Table 2. Ki67 mRNA expression in matched non-cancerous and cancerous lesions

	Age	BMI	Cytology	Histology	Ki67 mRNA expression			*P* value
					Non-cancerous lesion	Cancerous lesion	Fold change	
P001	62	33.3	ADC	ADC	0.06	0.22	3.67	
P002	39	22.1	ASCUS	SCC	0.26	22.32	85.85	
P003	41	25.8	SCC	SCC	0.04	7.01	175.25	
P004	49	20.5	SCC	SCC	0.04	5.90	162.10	
P005	36	22.8	ADC	ADC	2.62	38.85	14.82	
P006	30	21.6	HSIL	SCC	0.64	1.18	1.84	
P007	40	16.4	SCC	SCC	3.12	35.02	11.22	
P008	57	21.4	ADC	ADC	0.19	11.79	62.05	*P* =
P009	30	19.6	ADC	ADC	0.01	4.35	435.00	0.0005*
P010	57	22.7	SCC	SCC	0.19	53.34	280.74	
P011	46	20.7	SCC	SCC	3.43	131.60	38.37	
P012	35	27.6	HSIL	SCC	0.25	24.59	98.36	
P013	42	21.9	SCC	SCC	1.19	39.95	33.57	
P014	57	26.2	ADC	ADC	0.20	34.54	172.70	
P015	67	26.6	HSIL	SCC	0.03	35.02	1167.33	
P016	65	27.9	SCC	SCC	0.28	29.86	106.64	

*Ki67 mRNA levels were higher in cancerous tissue lesion than that found in matched Non-cancerous tissue lesion (Wilcoxon matched-pairs test). ADC, adenocarcinoma; ASCUS, Atypical cell of undetermined significance; BMI, body mass index; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma.

Ki67 mRNA levels were analyzed in 90 FFPE cancer tissues and 40 FFPE normal tissues, and found that the area under the ROC curve (AUC) was 0.97 (95% CI = 0.94 to 1.00, P < 0.001, Figure 1A). Besides, Ki67 mRNA levels in cervical cancer tissues were significantly increased compared to those in normal cervical tissues. Based on these findings, a diagnostic cutoff (threshold) was set of 5 (P < 0.001, Figure 1B). Using a threshold of 5, the assay had a sensitivity of 93.3% (95% CI = 86.1 to 97.5), a specificity of 97.5% (95% CI = 86.8 to 99.9), a positive predictive value of 98.8% (95% CI = 93.6 to 100.0), and a negative predictive value of 86.7% (95% CI = 73.2 to 95.0) (Table 3).

Ki67 mRNA levels in histologically diagnosed FFPE cervical tissues

Ki67 mRNA levels were evaluated with histologically diagnosed cervical tissues to determine whether Ki67 mRNA levels could discriminate between normal to precancerous tissues and cancer tissues. Ki67 mRNA levels were ranged from 0.0 to 5.6 (median 0.8) in 40 normal tissues, from 1.1 to 7.2 (2.2) in 30 LSIL tissues, from 0.5 to 13.7 (4.5) in 30 HSIL tissues, and from 0.2 to 131.6 (26.2) in 90 cancer tissues. The highest Ki67 mRNA levels were expressed in cancer tissues with lowering in histological sequence but still elevated levels were checked in HSIL and LSIL tissues compared to the levels in normal tissues (P < 0.0001) (Figure 2 and Table 4). Using a cutoff value of 5, positivity for Ki67 was 2.5% (1/40 cases) for normal, 13.3% (4/30 cases) for LSIL, 40.0% (12/30 cases) for HSIL, and 93.3% (84/90 cases) for cancer. We found that this Ki67 mRNA assay using a diagnostic threshold of 5 discriminated between normal and abnormal cervical lesions (P < 0.001) (Table 4).

Table 3. Sensitivity, specificity, NPV, and PPV of Ki67 mRNA levels in cervical cancer and normal tissues

	Ki67 mRNA by RT-qPCR assay (n = 130)	95% CI
Sensitivity	93.30%	86.1-97.5
Specificity	97.50%	86.8-99.9
PPV	98.80%	93.6-100.0
NPV	86.70%	73.2-95.0

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

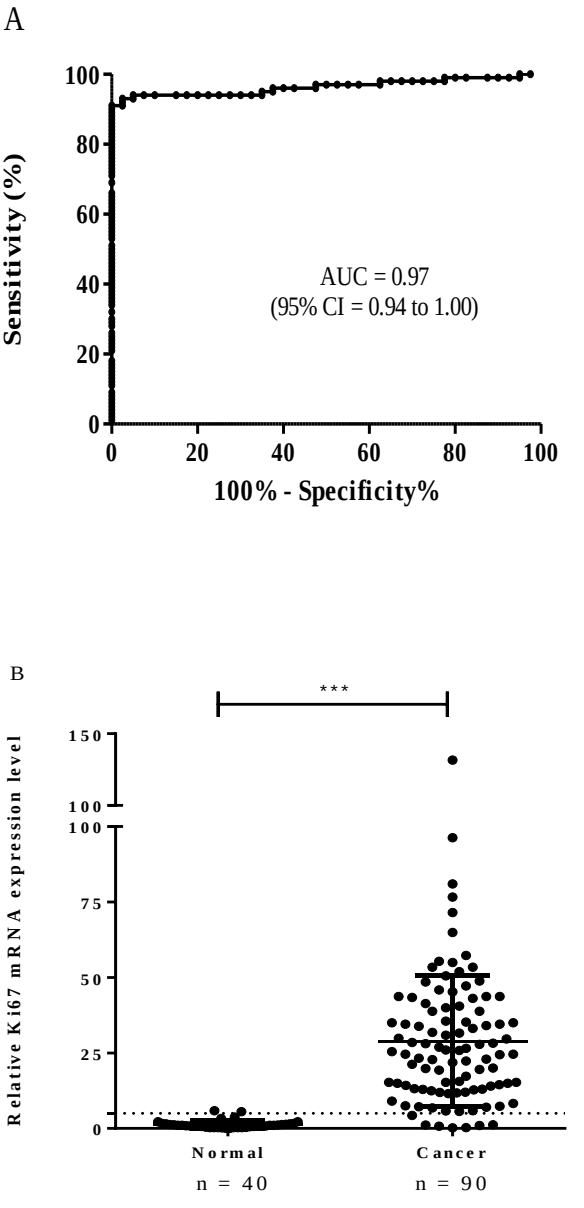

Figure 1. Receiver operating characteristics (ROC) curve analysis. **A.** The area under the ROC curve (AUC) was 0.97 (95% CI = 0.94 to 1.00, P < 0.001).
B. Ki67 mRNA levels were significantly higher in FFPE cancer tissues compared to that found in FFPE normal tissues (t-test, P < 0.001) using a diagnostic threshold of 5 (shown as a horizontal dotted line). ***P < 0.001.

Table 4. Association between Ki67 mRNA expression levels and histologically diagnosed cervical grades

Ki67 mRNA RT-qPCR assay			
	Ki67-Positive Cases	**Ki67 expression levels**	**Chi-square**
	n (%)	**Median (Min-Max)**	**(x^2, *P* value)**
Normal (n = 40)	1 (2.5)	0.8 (0.0-5.6)	Reference
LSIL (n = 30)	4 (13.3)	2.2 (1.1-7.2)	7.4, $P < 0.0001$
HSIL (n = 30)	12 (40.0)	4.5 (0.5-13.7)	17.9, $P < 0.0001$
Cancer (n = 90)	84 (93.3)	26.2 (0.2-131.6)	100.9, $P < 0.0001$

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion;
SD: Standard deviation.

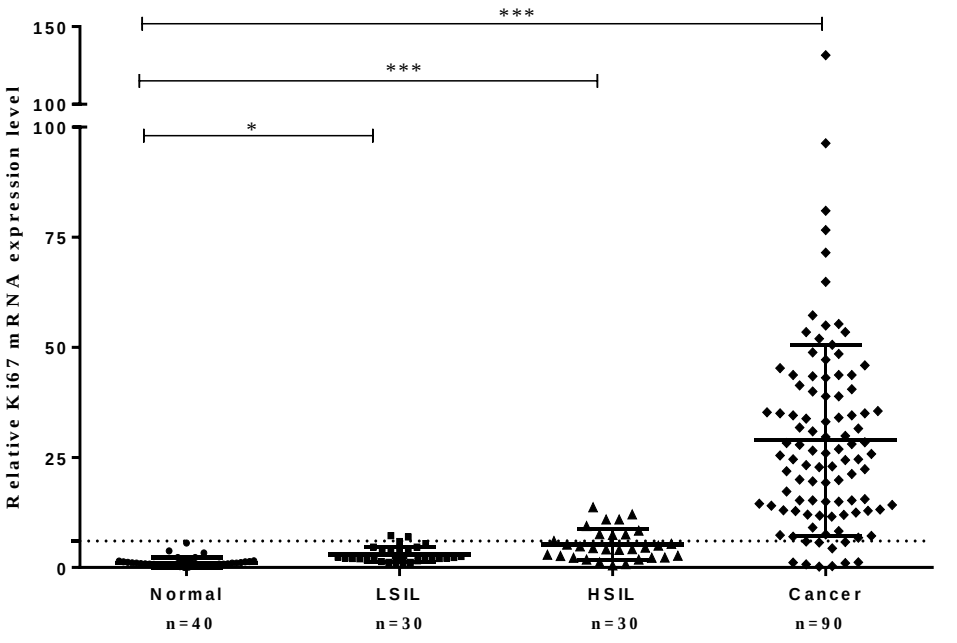

Figure 2. Dot plots of Ki67 mRNA levels in histologically diagnosed FFPE cervical tissues. Ki67 mRNA levels in FFPE normal tissues were significantly lower than that found in low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and cervical cancer tissues (t-test, P <0.0001).

***P < 0.001, *P < 0.05.

Ki67 mRNA levels and clinicopathological parameters in cervical cancer

The median value of Ki67 mRNA levels in cervical cancer tissues was 26.2. To inquire into whether there was an association between Ki67 mRNA expression levels in cervical cancer tissues and their clinicopathological parameters, such as age, HPV infection status, FIGO stage, lymph node metastasis, and tumor size, the Ki67 mRNA levels were divided into two groups: a low Ki67 (below the median Ki67 mRNA level) group and a high Ki67 (above the median Ki67 mRNA level) group. Among the parameters, lymph node metastasis showed statistically significant relation with a high Ki67 group (P = 0.02) (Table 5).

Table 5. Ki67 mRNA expression levels correlated with clinicopathological parameters in 79 cervical cancer patients

	Ki67 mRNA expression			
	Number of cases	Low (Ki67 < 26.2)	High (Ki67 ≥ 26.2)	*P* value
Age (years)				
≤ 50 years	36	18	18	0.46
> 50 years	43	21	22	
HPV DNA chip				
Negative	12	5	7	0.40
Positive	67	34	33	
FIGO stage				
< IIB	36	20	16	0.16
≥ IIB	43	19	24	
Lymph node				
Negative	40	24	16	0.02
Positive	39	15	24	
Tumor size				
< 4 cm	45	25	20	0.10
≥ 4 cm	34	14	20	

IV. DISCUSSION

Cervical cancer is a worldwide leading cause of cancer mortality in 35-55 year old women. To avoid unnecessary or invasive treatment such as ablation, conization and/or hysterectomy in benign precancerous lesions with transient HPV infection, the optimal screening strategy for cervical cancer should efficiently and accurately identify precursor lesions that will progress to invasive cancers.[30] Ki67, a nuclear protein, is expressed in proliferating vertebrate cells so that nuclear staining of this nuclear antigen is widely used as a surrogate marker, measuring proportions of dividing cells to grade tumors. But estimating of Ki67 expression level is not inconsistent and has some limitations in conventional histopathological fields.[31]

Therefore, the purpose of this article was to evaluate Ki67 mRNA expression levels with histological grades to identify and understand the relationship between the Ki67 mRNA assay and clinicopathological parameters of cervical cancer, and assess the performance of this assay as a diagnostic tool for cervical cancer.

First of all, Ki67 mRNA expression levels of matched non-cancerous and cancerous portion FFPE samples obtained from 16 cervical cancer patients were compared to validate the assay. There were statistically significant differences in Ki67 mRNA expression levels between matched non-cancerous and cancerous samples ($P = 0.0005$) (Figure 1). Comparing to Ki67 mRNA expression levels in normal tissues, those of cervical cancer tissues enabled to differentiate regardless of their cell types. There have been few other studies comparing Ki67 mRNA expression in normal and cervical cancer samples so far. Similar but earlier studies have widely proved the validation of Ki67 mRNA assay in breast cancer samples as a distinctive prognostic biomarker. Yamamoto et al reported that Ki67 mRNA from FFPE breast cancer samples were clinically relevant enough

to estimate prognosis, as well as conventional Ki67 labeling index of breast cancer.[8]

Using 90 cervical cancer and 40 normal FFPE tissue samples, this study revealed that the Ki67 mRNA assay was able to accurately discriminate between cervical cancer and normal tissues with a high sensitivity of 93.3% (95% CI 86.1 to 97.5) and a high specificity of 97.5% (95% CI 86.8 to 99.9) (Table 3). Several studies have demonstrated that Ki67 expression using immunoquantification can provide greater discrimination power not only between normal and cancer tissues but also between LSIL and HSIL. And the reported average positivity rates found in normal, LSIL, and HSIL were 7.9%, 49%, and 90%, respectively.[11, 32] In this research, the proportion of Ki67 mRNA expression cases were 2.5%, 13.3%, and 40.0% in normal, LSIL, and HSIL respectively. In cervical cancer the proportion was 93.3%, and these data were all statistically significant ($P < 0.001$) (Table 4).

Moreover, increased Ki67 mRNA expression in cervical cancer samples was significantly higher than those of normal, LSIL, and HSIL samples (Figure 2). Since less than 10% of LSIL cases progress to HSIL, cytological and/or histological follow-up are more frequently needed only in LSIL cases with tendency of progression. Chen et al and Zhou et al attempted to predict the progression using Ki67 immunocytochemistry and immunohistochemistry tests and showed similar results distinguishing LSIL and HSIL.[33, 34] In the aspect of burdening the national healthcare system, such molecular diagnosis accompanied with pathological diagnosis may reduce overtreatment for follow-up in LSIL patients, the majority of who do not progress to HSIL.

According to clinicopathological prognostic parameter analysis, separating patients into high Ki67 expression (median $\geq$ 26.2) and low Ki67 expression (median $<$ 26.2) groups, lymph node metastasis was associated with Ki67 mRNA levels (Table 5). Shokouh studied that Ki67 IHC was correlated to lymph

node status in breast cancer.[9] Yang et al described that Ki67 immunohistochemical marker was correlated for predicting lymph node metastasis in endometrial cancer.[35] Cervical cancer in this study as well as breast cancer in others also showed the interrelation of high Ki67 and lymph node metastasis, which reflected a poor prognosis of cancer.

Through many former studies, IHC staining of Ki67 can be applied to complementary test of HPV testing.[36, 37] Due to cervical cancer screening guidelines of PAP smear and HPV co-testing, HPV test is now widely utilized. But there are few additional predictors able to determine the risk amount of carcinogenesis by high-risk HPV infection. Even though the further study about direct comparison Ki67 mRNA assay with Ki67 IHC staining as a treatment responder is needed, it is meaningful for this research to demonstrate that Ki67 mRNA assay can provide additional quantitative information as a molecular diagnostic marker of cervical cancer, and also predict prognosis of each grade of precancerous lesions.

V. CONCLUSION

In summary, to identify the diagnostic and clinicopathological predictive value of Ki67 mRNA assay in uterine cervical cancer and precancerous lesions, RT-qPCR was utilized for 190 FFPE cervical specimens in the research. The validation which referred to discriminating power of Ki67 mRNA for cervical cancer diagnosis was verified and the assay showed high sensitivity, specificity and positive predict value by using a diagnostic cutoff value of 5. Among normal cervical tissue, LSIL, HSIL and cervical cancer tissue samples, Ki67 mRNA expression level of cervical cancer tissue samples was remarkably highest and Ki67 mRNA expression was lowered as tissue samples' grade went down.

Lymph node metastasis from among clinicopathological parameters of cervical cancer had statistically significant relation with high Ki67 mRNA expression

As a result, Ki67 mRNA expression analysis of FFPE cervical specimens using RT-qPCR was proved its clinicopathological performance as a discriminative biomarker in uterine cervical disease. Ki67 mRNA is also able to be a promising prognostic predictor which in cervical cancer and precancerous lesions as an important part of gene expression profiling. Based on collaboration with this Ki67 mRNA assay, further research for predicting a recurrence of HSIL and/or cervical cancer might be expected in the near future.

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ABSTRACT (IN KOREAN)

자궁경부암 조직에서 발현되는 Ki67 mRNA의 진단적, 임상적 의의

<지도교수 남 은 지>

연세대학교 대학원 의학과

정 다 은

Ki67은 암의 증식과 예후와 관련된 중요한 바이오마커로 널리 알려져 있다. 이전의 연구들을 통하여 포르말린고정 파라핀 포매된 자궁경부암 조직샘플로부터 Ki67 mRNA 발현을 정량적으로 측정할 수 있는 가능성을 평가하였다.

선행연구를 토대로, 본 연구에서는 실시간 중합효소연쇄반응 정량검사를 이용하여 포르말린고정 파라핀 포매된 자궁경부 조직샘플의 Ki67 mRNA 발현을 정량적으로 측정, 비교하였다. 정상 자궁경부 및 자궁경부 병변별 Ki67 mRNA 발현량을 비교하여 Ki67 mRNA의 예측적 가치를 확인하고자 하였다. 또한, 자궁경부암 샘플에서의 Ki67 mRNA 발현량과 해당 암환자들의 임상적 예후인자들 간의 관계를 분석하였다.

전체 190건의 자궁경부 검체가 연구에 사용되었으며 이 중에는 80건의 편평상피세포암, 10건의 선암, 30건의 고등급 상피내병변, 30건의 저등급 상피내병변, 40건의 정상 자궁경부 조직 (대조군)이 포함되었다. 실험 결과, 정상 자궁경부 조직 (N

= 40)에 비해 자궁경부암 조직 (N = 90)에서 Ki67 mRNA 발현이 높게 나타났고 이는 통계학적으로 유의하였다 (P < 0.001). Ki67 mRNA 분석법의 민감도는 93.3% (95% 신뢰구간 86.1-97.5), 특이도는 97.5% (95% 신뢰구간 86.8-99.9)를 보였다. 각 병변에 따른 Ki67 mRNA 발현량을 비교한 결과, 자궁경부암 조직에서 93.3%, 고등급 상피내병변에서 40.0%, 저등급 상피내병변에서 13.3%, 그리고 정상조직에서는 2.5%로 나타나, 고등급 병변으로 진행할수록 발현량이 유의하게 높아짐을 확인하였다. 특히, 자궁경부암 조직에서의 Ki67 mRNA 과발현 양상과 해당 암환자들의 임상적 예후 인자들간의 관계를 분석한 결과, 림프절 전이와 Ki67 mRNA 과발현 간의 통계학적으로 유의한 상관관계를 보였다 (P = 0.02).

이와 같이, Ki67 mRNA는 자궁경부 상피내병변이 암으로 진행될 가능성을 예측할 수 있으며 경부암 환자의 예후인자와도 관련있는 바이오마커임을 알게 되었다. 따라서 실시간 중합효소연쇄반응 정량검사를 이용한 Ki67 mRNA 분석법은 향후 자궁경부암에서 유용한 분자 진단법으로 자리매김할 수 있을 것으로 기대한다.

핵심되는 말: 자궁경부암, Ki67 mRNA, 실시간 중합효소연쇄반응 정량검사, 분자 진단