Analysis of telomerase activity and human telomerase reverse transcriptase (hTERT) mRNA expression of the endometrium in patients with endometriosis

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저자 씀

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Analysis of telomerase activity and human telomerase reverse transcriptase (hTERT) mRNA expression of the endometrium in patients with endometriosis

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(Directed by Professor **Byung Seok Lee**)

Introduction: Endometriosis is considered a frequent, benign disease with the ability to undergo neoplastic processes. Although retrograde menstruation theory, multiple genetic and environmental factors seem to be important, the critical factors that affect the implantation and propagation of endometriotic lesions are still largely unclear. Recent studies demonstrated that some genes or gene products were aberrantly expressed in the endometrium of endometriosis. Human telomerase reverse transcriptase (hTERT) mRNA is core functional components of telomerase activity. They have been detected in various cancers and are related with the proliferative potential. The aim of this study is to evaluate the limitless replication potential of eutopic

endometrium in patients with endometriosis by examining quantitative human telomerase reverse transcriptase (hTERT) mRNA expression and telomerase activity.

Materials and Methods: Endometrial tissues from 30 endometriosis patients (endometriosis group) and 30 patients without endometriosis (control group) were obtained via endometrial biopsy. Each group was subdivided and analyzed according to the menstrual phase and clinical disease stages.

The expression of hTERT mRNA was determined by real-time RT-PCR assay based on Taq-Man fluorescence methodology. Telomerase activity was measured by telomerase repeat amplification protocol (TRAP) assay. The variables were analyzed by Student`s t-test, Kruskall-Wallis test and Pearson`s correlation coefficient.

Results: The expressions of hTERT mRNA were detected in 24 of 30 (80%) normal group and in 27 of 30 (90%) endometriosis group. The normalized hTERT mRNA level was significantly higher in the endometriosis group than the control group (p=0.004). The hTERT mRNA level during the secretory phase in the endometriosis group was significantly higher than that of control group (p=0.036), although the difference was not significant during the proliferative phase. Telomerase activities were noted 90% in both group. We found a prominent difference of telomerase activity between moderate-to-severe endometriosis group and the control group (p=0.048). The level of hTERT mRNA and telomerase activity increased as the disease became more severe (p=0.038, p=0.016).

Conclusion: This study showed the overexpression of hTERT mRNA and telomerase activity in the endometrium of endometriosis patients. This findings

suggest that endometrial replication potential may have an important role in the pathogenesis of endometriosis.

Key Words : endometriosis, pathogenesis, telomerase,
human telomerase reverse transcriptase (hTERT)

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I. INTRODUCTION

Endometriosis is defined as an implantation of endometrial tissues outside their normal location in the uterus. Overall, endometriosis may affect 7-15% of women of reproductive age and usually results in various symptoms such as chronic pelvic pain, dysmenorrhea, and infertility.[1] In infertile women, the prevalence may be as high as 30-40% and the incidence of this disease is increasing.

In 1927, pelvic implantation theory by retrograde menstruation of endometrial tissues was first described by Sampson.[2] It is the most widely accepted theory on the pathogenesis of endometriosis. However, no single mechanism can explain all cases of endometriosis and the pathogenesis is not yet fully understood. Recently, other familial predispositions, immunological

factors, cell adhesion factors, angiogenic factors, and hormonal factors have been introduced.[3] In fact, immunological factors may affect susceptibility to the implantation of endometrial cells which are shed from the endometrial cavity and there is much evidence supporting the hypothesis that endometriosis is inherited as a complex genetic trait correlated with susceptibility.[4,5]

Endometrium from endometriosis patients could have endogenous abnormalities that predispose these patients to the disease, and some genes or gene products were aberrantly expressed in these tissues.[6,7] Up-regulation of anti-apoptotic genes, such as *bcl-2*, was noted in the eutopic endometrium of women with endometriosis, as well.[8]

Although it would be inappropriate to define endometriosis as a premalignant lesion, endometriosis is clinicopathologically similar to neoplastic disease. Endometriosis undergoes malignant transformation such as clear cell or endometrioid type ovarian cancer. Also, this disease shares a predisposing factor and molecular similarity with cancer.[9] Angiogenesis, tissue invasion, and metastasis are all found in endometriosis patients. Similarly, endometriotic lesions have also displayed an overexpression of anti-apoptotic (*bcl-2*) genes and an underexpression of pro-apoptotic (*bax*) genes.[10]

Telomeres are repetitive DNA sequences (5`-TTAGGG-3`) at the ends of linear chromosomes which shorten along with cellular division in somatic cells. Telomerase is a ribonucleoprotein complex and plays an important role in telomere maintenance and cellular immortality.[11] Normal somatic cells do not usually express telomerase, except in hematopoietic stem cells and endometrial cells. However, telomerase activity has been detected in various cancers and is related to the limitless replication potential. Human telomerase reverse

transcriptase (hTERT) mRNA is a core functional component of telomerase activity. It is a catalytic protein subunit and a strong correlation has been observed between hTERT mRNA expression and telomerase activity in various tissues.[12,13] It was reported that endometrial telomerase activity is high during the proliferative phase but is suppressed during the secretory phase of the menstrual cycle. This finding reflects the relation between telomerase activity and features of the normal endometrium which is regularly regenerated and has proliferative potential.[14] Lehner et al[15] reported that telomerase activity and hTERT mRNA levels were significantly higher in endometrial cancer, compared to these in the normal cycling endometrium. Also, it was proven that hTERT mRNA is a critical determinant of telomerase activity.[16]

However, no study has been published concerning telomerase activity in endometriosis patients. Endometriosis is an estrogen- dependent disease with a process similar to that of a neoplastic disease, therefore we suppose that increased telomerase activity in the endometrium of endometriosis patients may have a role in the pathogenesis of endometriosis.

In present study, we evaluated the limitless replication potential of the endometrium in women with endometriosis by quantitative examination of hTERT mRNA expression and telomerase activity. In addition, the correlation between hTERT mRNA level and telomerase activity was analyzed.

II. MATERIALS AND METHODS

1. Patients and tissue selection

Fresh surgical specimens of 30 endometriosis patients and 30 normal control tissues were collected via endometrial biopsy. Biopsies were performed during explore-laparotomy or operative laparoscopy at the Department of Obstetrics and Gynecology, Yongdong Severance Hospital, Yonsei university college of medicine from September 2005 through March 2006. Each sample was immediately frozen and stored at -80℃ until use. Ectopic endometrial tissues in the endometriosis group were sent to the Pathology Department and endometriosis was histologically determined. Informed consent was obtained from all patients and the study was approved by the institutional review board.

Postmenopausal women, previous hormone or GnRH agonist users, and patients who had adenomyosis, endometrial cancer, endometrial hyperplasia or endometrial polyp were excluded.

The endometriosis and control groups were then divided and analyzed according to menstrual phases, and clinical disease stages established by the revised classification of The American Society for Reproductive Medicine.[17] Menstrual phase was determined by the histology of the endometrium, and then classified into proliferative or secretory phases. Thirteen of the 30 women with endometriosis were in the proliferative phase, and 17 were in the secretory phase. Thirteen of 30 the control women were in the proliferative phase and 17 were in the secretory phase. Four women were classified as endometriosis stage I, four as endometriosis stage II, eleven as endometriosis stage III, and eleven as endometriosis stage IV.

2. Quantification of human telomerase reverse transcriptase (hTERT) mRNA

A. Isolation of total RNA

Total RNA was isolated from biopsied tissue, using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) following the protocol suggested by the manufacturer. Briefly, the tissues were grinded and homogenized in buffer RLT. The tissue lysates were completely homogenized with QIAshredder spin column (QIAGEN, Valencia, CA, USA) and centrifuged at 13.000 rpm for 2 minutes. 590 ul of double-distilled water was added to the homogenated lysate. It was incubated at 55℃ for 10 minutes and centrifuged for 3 minutes at 12,000 rpm. The supernatant was transferred into a new tube and 0.5 volumes of absolute ethanol was added to the cleared lysate. The lysate was put into an RNeasy mini column and centrifuged for 15 seconds at 12,000 rpm. After that, we added 350 ul of buffer RW1 into the RNeasy mini column, and centrifuged for 15 seconds at 12,000 rpm to wash. 500 ul of buffer RPE was put into the RNeasy column and centrifuged for 15 second at 12,000 rpm for washing. Another 500 ul of buffer RPE was added to the RNeasy column and centrifuged for 2 minutes at 12,000 rpm to dry the RNeasy silica-gel membrane. For elution, the RNeasy column was transferred to a new 1.5 ml tube and 30 ul of RNase-free water was directly put onto the RNeasy silica-gel membrane and centrifuged for 1min at 12,000 rpm.

B. Synthesis of cDNA

2 ug of total RNA from each sample were reverse transcribed into cDNA, using the SuperScript™ III first strand synthesis system (Invitrogen, Carlsbad, CA, USA). 2 ug of total RNA was mixed with 1 ul Oligo(dT)$_{20}$ primer (0.5 ug/ul), 1 ul dNTPs and deionized water. The total volume of 10 ul was incubated at 65℃ for 5 min. After mixing with 10 x RT buffer 2 ul, 25 mM $MgCl_2$ 4 ul, 0.1M DTT 2 ul, RNase OUT TM (400 U/ul) 1ul and SuperScript™ III RT (200 U/ul) 1ul using thermal cycler at 50℃ for 50 minutes, the cycle was stopped at 85℃ for five minutes.

C. Real-time RT-PCR

The expression of hTERT mRNA was measured by real-time RT-PCR, based on TaqMan methodology, using LightCycler® instrument (Roche Diagnostics, Mannheim, Germany). The specific primers and probe nucleotide sequence for hTERT were forward primer 5`-TGACACCTCACCTCACCCAC-3`, reverse primer 5`-CACTGTCTTCCGCAAGTTCAC-3` and TaqMan probe 5`-(FAM)ACCCTGGTCCGAGGTGTGTCCCTGA(TAMRA)-3. The primers and probe for GAPDH were forward primer 5`-GAAGGTGAAGGTCGGA GTC-3`, reverse primer 5`-GAAGATGGTGATGGGATTTC-3` and Taq-Man probe 5`-(FAM)CAAGCTTCCCGTTCTCAGCC(TAMRA)-3`.

For each PCR run, a master mix was prepared on ice with Taq polymerase buffer, 4 mmol/L $MgCl_2$, 200 μmol/L deoxynucleotides, 300 nmol/L each primer, 150 nmol/L probe, 1U Taq polymerase and 20 ng cDNA. PCR was performed in a total volume of 20 μL. The thermal cycling conditions included preincubation for 10 minutes at 95℃,

followed by 40 cycles of 10 seconds at 95℃, 30 seconds at 63℃ and 1 second at 72℃.

To normalize the amount of total RNA present in each reaction, we amplified the housekeeping gene GAPDH. Our final result was expressed as a normalized hTERT (N hTERT) mRNA. The amount of target, which was normalized to the endogenous reference (GAPDH) and relative value to the calibrator was defined by ΔΔCt method. The formula is ; target amount = $2^{-\Delta\Delta Ct}$, where ΔΔCt ={[Ct(hTERT sample)-Ct(GAPDH sample)] - [Ct(hTERT calibrator)-Ct(GAPDH calibrator)]}. It was calculated by LightCycler software version 4.0.

3. Telomerase activity

Telomerase activity was determined according to the TRAP (telomeric repeat amplification protocol) assay that was first described by Kim et al.[18] TRAP assay has revolutionized the detection of telomerase and is composed of two steps. First, active telomerase adds telomeric repeats (TTAGGG) onto the 3` end of a substrate oligonucleotide, therefore the telomerase reaction is induced and telomeric repeat elongation is done. After that, the elongated products are amplified by PCR.

A. Preparation of tissue samples

Endometrial tissue samples were washed with phosphate buffered saline (PBS) and then suspended in lysis buffer. Tissues were then

homogenized and incubated on ice for 30 minutes. Cell homogenates were then centrifuged at 12000 rpm for 30 minutes at 4℃. A supernatant 160 μl was recovered and the protein concentration was measured using a BCA assay kit (Sigma-Aldrich, St. Louis, MO, USA).

B. Telomeric repeat amplification protocol (TRAP) assay

The SYBR Green real-time quantitative TRAP assay was conducted with a Quantitative Telomerase Detection kit (EBI, Frederick, MD, USA). The total volume of the reaction mixture was 25 μl which contained QTD (Quantitative Telomerase Detection) premix 12.5 μl, PCR qualified water 11.5 μl, and 1.0μl of tissue extract. 0.01 μg of protein extract was used for the TRAP assay. The reaction mixture was first incubated at 25℃ for 20 minutes to allow the telomerase in the protein extracts to elongate the TS primer (5‘-AATCCGTCGAGCAGAGTT-3') by adding a TTAGGG repeat sequence. After that, PCR was then performed at 95℃ for ten minutes followed by 45 cycles of amplification at 95℃ for 30 seconds, 60℃ for 30 seconds, and 72℃ for 30 seconds on a MiniOpticon real-time PCR machine (Bio-Rad, Hercules, CA, USA). The amplified fluorescence signal in each specimen was measured at the late extension step of each cycle and analyzed with Detector software. In the negative control, no fluorescent signal was observed. A standard curve was generated from serial dilutions of telomerase-positive TSR template (0.5, 0.1, 0.02, 0.004, 0.008, 0.00016, 0.000032, and 0.0000064 μg/μl). The telomerase activity of each specimen was calculated based on the standard curve. One unit of relative telomerase activity (RTA) was

defined as the activity equivalent to that in 100 molecules of TSR.

4. Statistical analysis

Comparison of values between endometriosis and control groups was done using the Student`s t-test. The levels of significance in correlation were calculated by Pearson`s correlation coefficient. Analysis according to the clinical disease stages in the endometriosis group was done using the Kruskall-Wallis test because a normal distribution was not obtained. All tests were performed with SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA) and a p-value <0.05 was considered statistically significant.

III. RESULTS

1. Clinical Features

A total of 30 samples from each group were examined. The mean age of the endometriosis group was 32.7 ± 6.8 years (mean ± SD) and that of normal control group was 34.6 ± 6.7 years (p=0.254). The BMI and hemoglobin level of each group were not significantly different. The level of serum CA 125 in the endometriosis group (56.5 ± 39.9) was significantly higher than in the control group (20.2 ± 10.5)(Table 1).

Table 1. Clinical characteristics of the study groups

	Control group (N=30)	**Endometriosis group (N=30)**	**P-value**
Age (yr)	34.6 ± 6.7	32.7 ± 6.8	0.254
BMI (kg/m^2)	22.0 ± 2.3	21.3 ± 2.2	0.240
Hb (g/dl)	12.3 ± 2.3	21.3 ± 2.2	0.785
Serum CA-125 (IU/ml)	20.2 ± 10.5	56.5 ± 39.9	<0.001*

BMI : body mass index, Hb : hemoglobin

Data is expressed as mean ± SD. t- test * P<0.05

2. Expression of hTERT mRNA

hTERT mRNA expression was detected in 27/30 (90%) specimens from the endometriosis group, and in 24/30 (80%) samples from the normal control group. The level of normalized hTERT mRNA was significantly higher in the endometriosis than the control group (52.4 ± 53.7 vs 22.4 ± 24.2, p=0.004) (Table 2).

Table 2. hTERT mRNA expression in the study groups

	Control group	Endometriosis group	p-value
Positive hTERT mRNA expression (%)	24/30 (80%)	27/30 (90%)	
Normalized hTERT mRNA (NhTERT)	22.4 ± 24.2	52.4 ± 53.7	0.004*

Data is expressed as mean ± SD. t- test * P<0.05

When comparing hTERT mRNA expression according to menstrual phase, the hTERT mRNA levels were highest in the proliferative phase of the endometriosis group and lowest in the secretory phase of the control group (Figure 1). In both groups, the level of hTERT mRNA was higher in the proliferative phase than in the secretory phase, but no significant difference was found. However, when the hTERT mRNA level during the secretory phase of the control group was compared to the endometriosis group, it was significantly higher in the latter (17.7 ± 25.1 vs 47.2 ± 40.1, p=0.036).

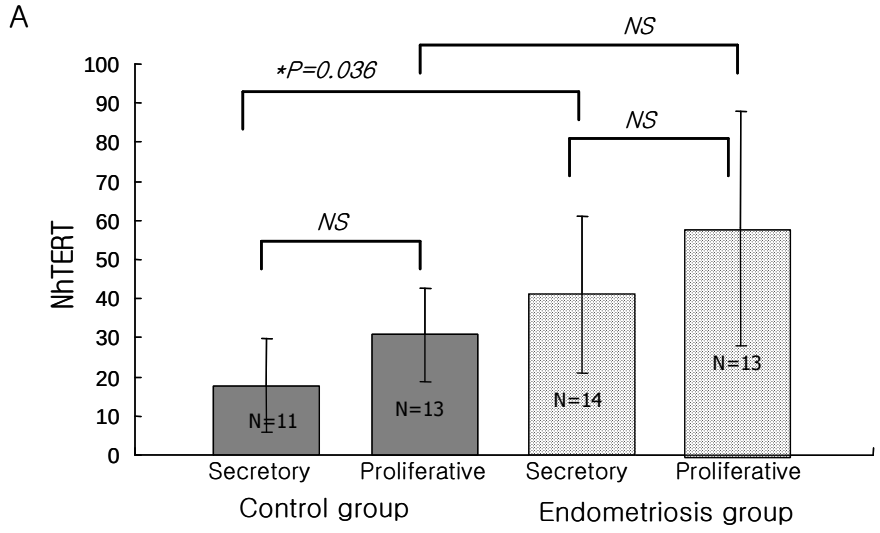

Figure 1. hTERT mRNA expression in the study group according to menstrual phase

(*NS* : not significant)

According to the clinical disease stages of the endometriosis group, hTERT mRNA expression increased by a statistically significant level as the disease became more severe (p=0.038). The mean normalized hTERT level in the endometriosis group was 10.40 (range 8.85-11.96, median 10.40) in stage I, 16.78 (range 8.75-26.78, median 14.83) in stage II, 47.64 (range 6.07-140.35, median 25.35) in stage III, and 74.62 (range 20.17-260.70, median 62.85) in stage IV (Figure 2).

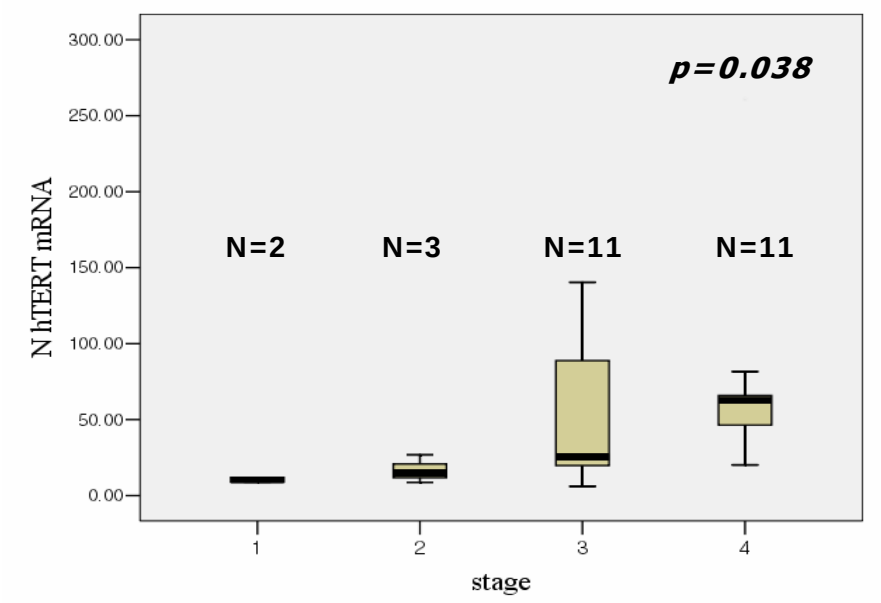

Figure 2. Comparison of hTERT mRNA expression between the clinical disease stages in the endometriosis group.

Although we could see a slight positive correlation between hTERT mRNA expression and the endometriotic cyst size in the endometriosis group, there was no significant correlation between the two variables (r=0.349, p=0.074).

Finally, when comparing the hTERT mRNA expression and the serum CA 125 level, no significant correlation was found (r=0.193, p=0.335).

3. Telomerase activity detection

Telomerase activity was found in 27/30 (90%) endometriosis patients and in 27/30 (90%) normal patients. The level of relative telomerase activity was higher in the endometriosis group than in the control group, but no significant

difference was noted. However, when only the moderate-to-severe endometriosis group was compared with the control group, the difference was statistically significant (21.8 ± 33.0 vs 7.6 ± 13.2, p=0.048)(Table 3).

Table 3. Telomerase activity in the study groups

	Control group	Endometriosis group	p value
Telomerase activity expression(%)	27/30 (90%)	27/30 (90%)	
Relative telomerase activity(RTA)	7.6 ± 13.2	17.8 ± 30.8	0.122
	7.6 ± 13.2	**III-IV EMS (n=22)**	0.048*
		21.8 ± 33.0	

III-IV EMS : moderate-to-severe endometriosis.

Data is expressed as mean ± SD. t- test * P<0.05

As shown in figure 3, telomerase activity was revealed to have a pattern similar to the level of hTERT mRNA expression when assessed by menstrual phase. In each group, telomerase activity was higher in the proliferative phase than in the secretory phase, but no significant difference was found. Also, when each menstrual phase was compared, the difference was not significant.

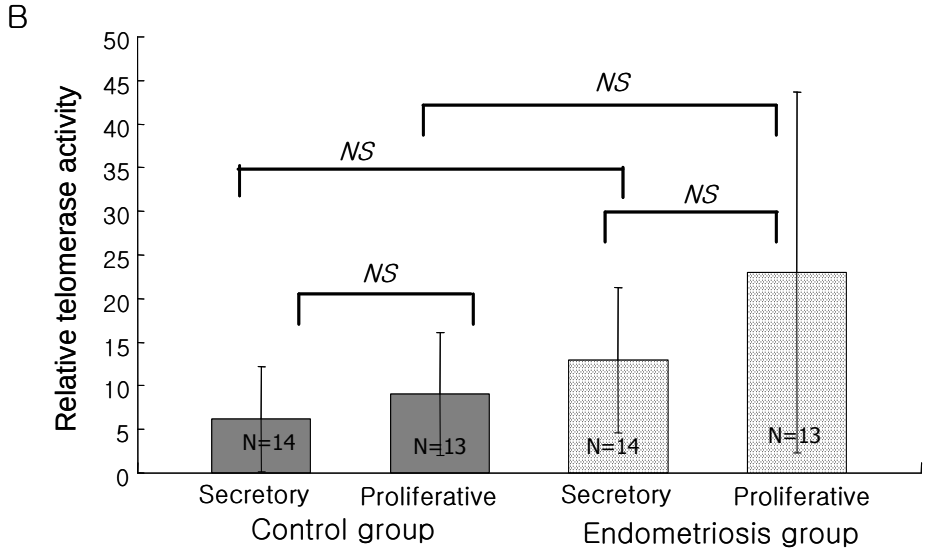

Figure 3. Telomerase activity in the study group according to menstrual phase

(*NS* : not significant)

We analyzed the level of relative telomerase activity according to clinical disease stage in the endometriosis group and found a trend of increasing telomerase activity with increasing stage of endometriosis (Figure 4). This result was similar to the results of hTERT mRNA and was statistically significant (p=0.016).

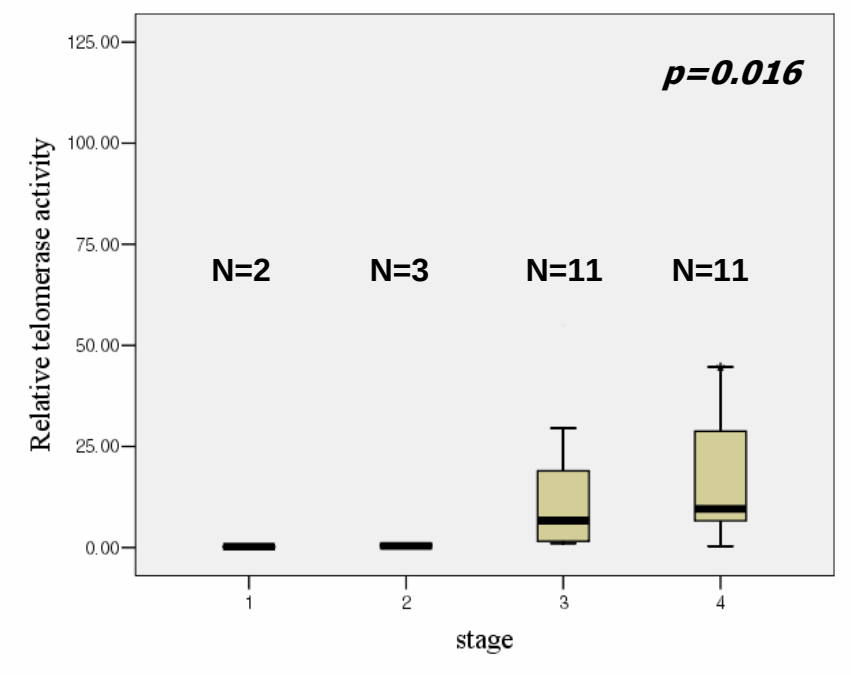

Figure 4. Comparison of telomerase activity between the clinical disease stages in the endometriosis group.

There was no significant correlation between telomerase activity and the size of the endometriotic cyst in the endometriosis group (r=0.178, p=0.197). Moreover, when the level of telomerase activity and serum CA 125 were compared, no significant correlation was detected (r=0.199, p=0.219).

4. Correlation of hTERT mRNA and telomerase activity

We examined the relationship between hTERT mRNA expression by quantitative real-time RT-PCR and telomerase activity by TRAP assay in

all samples. A significant, positive correlation was found, and the levels of hTERT mRNA and telomerase activity showed a linear association ($r=0.778$, $p<0.001$)(Figure 5).

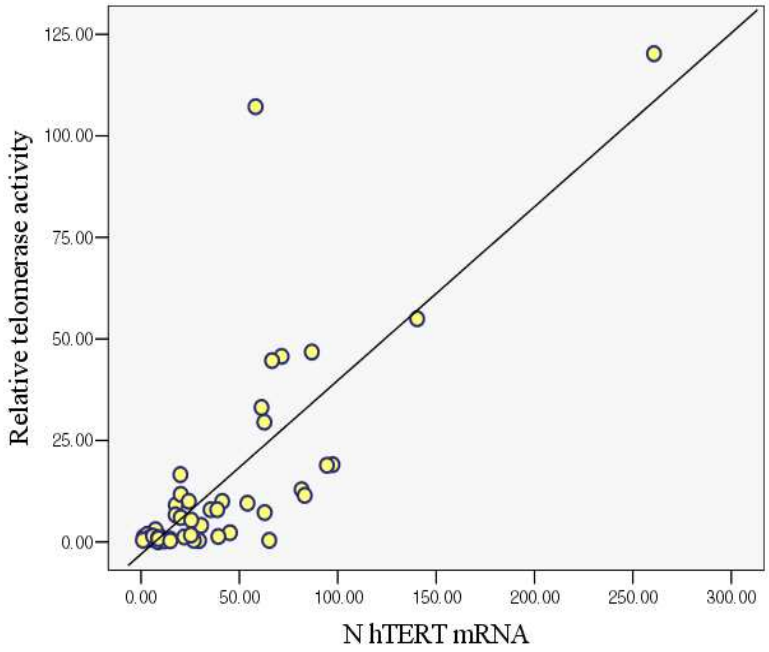

Figure 5. The correlation between hTERT mRNA expression and telomerase activity

IV. DISCUSSION

Endometriosis is characterized by the growth of endometrial tissue outside the uterus, but the etiology and pathogenesis of this disease remain unclear. Endometriosis tends to recur and progress, and the cumulative recurrence rate is about 40% after five years, regardless of medical or surgical treatment.[19] Therefore, it is very important to determine the pathogenesis of this disease.

Several studies have focused on the overexpressed genes in the endometrium of endometriosis patients. Persistent expression of matrix metalloproteinases (MMPs), abundant expression of several angiogenic factors, including interleukins 1, 6, and 8, and fibroblast growth factors could contribute to establishment of endometriotic lesions in the peritoneal cavity.[20,21] Up-regulation of anti-apoptotic genes and down-regulation of pro-apoptotic genes has been observed in the endometrium of endometriosis patients.[22] Likewise, Giudice et al[7] reported that there was an intrinsic abnormality in eutopic endometrium in women with endometriosis that permits these tissues to attach, survive, invade and establish a blood supply in the peritoneun.

In the present study, we examined hTERT mRNA expression and telomerase activity in order to evaluate the limitless replication potential of the endometrium in endometriosis patients. Higher levels of both parameters were detected in endometriosis patients when compared with the normal control group.

The hTERT mRNA was not expressed in 10% (3/30) of the

endometriosis and 20% (6/30) of the control group. These results were similar to previous other studies, regarding hTERT mRNA expression in normal endometrium.[15,16] All samples without expression were from the secretory phase. Telomerase activity was not detected in 10% of samples from both groups. This expression rate was somewhat higher than that of other studies because we used a real-time TRAP assay instead of the conventional TRAP assay.[23,24] The conventional TRAP assay requires complex post-amplification procedures, such as polyacrylamide gel electrophoresis, autoradiography and densitometry, for measurement of telomerase products, whereas a real-time TRAP assay reduces the risk of carryover contamination and provides a more rapid and reliable quantification of telomerase activity.[25] The samples without telomerase activity were all from the secretory phase. Particularly, within the endometriosis group, all samples without telomerase activity were those of minimal-to-mild disease.

Our experiment demonstrated a significant difference in hTERT mRNA level between the two groups. However, when the level of telomerase activity was compared, we found a prominent differences only between moderate-to-severe endometriosis group and the control group. These results reflect the presence of multiple mechanisms for regulating telomerase activity. Posttranscriptional regulation of the hTERT gene, such as alternative splicing of hTERT transcripts and protein kinase C-mediated phosphorylation of hTERT can modulate the enzyme activity.[26,27]

In this study, the patterns of hTERT mRNA and telomerase activity ,thoughout the menstrual phase, were consistent with a previous study.[21]

This suggests that telomerase activation is closely associated with cellular proliferative activity and self-regeneration of the endometrium. In addition, Belair et al[28] reported that telomerase activity is a biomarker of cell proliferation, rather than that of malignant transformation. Estrogen may play a role in the regulation of telomerase activity as a potent inducer of endometrial proliferation. Telomerase expression, in fact, declines rapidly after ovulation when the estrogen level decreases. Furthermore, it is suppressed by progesterone.[29] The interesting feature of this study was that the hTERT mRNA level during the secretory phase in the endometriosis group was significantly higher than that of the control group although the difference was not significant for the proliferative phase. This may indicate that endometriotic tissue is different from normal endometrium, which undergoes estrogen–induced cell proliferation that is suppressed by progesterone.

This study revealed that there was a statistically significant association between hTERT mRNA level and telomerase activity in both groups. However, we found discordant results in some cases; a few patients had relatively high telomerase activity with a low hTERT mRNA level, while some cases demonstrated the inverse association. The former result may result from inefficient RNA extraction or RNA degradation in the clinical samples , while the latter result may reflect suboptimal extraction of telomerase or enzyme instability. In addition, other mechanisms, such as posttranscriptional regulation may play an important role in telomerase activity.

We found that hTERT mRNA expression and telomerase activity were significantly different according to different endometriosis stages. This finding

suggests that hTERT mRNA expression and telomerase activity may reflect the severity of the disease. However, we had a small number of samples in the minimal-to-mild disease state. Therefore, further study with a large number of cases are needed to confirm this result.

V. CONCLUSION

We measured hTERT mRNA expression level and telomerase activity to evaluate limitless replicative potential of endometrium in patients with endometriosis.

1. The hTERT mRNA expression in the endometrium of endometriosis patients was significantly higher than that of normal control group ($p=0.004$).
2. The telomerase activity in the endometriosis group was higher than normal control group and the difference was statistically significant only in moderate to severe endometriosis group ($p=0.048$).
3. According to the menstrual phase, both hTERT expression and telomerase activity were higher in proliferative phase than secretory phase. The hTERT mRNA level of secretory phase in endometriosis group was significantly higher than that of control group although the difference was not significant for proliferative phase ($p=0.036$).
4. The levels of hTERT mRNA expression and telomerase activity increased as the disease became more severe ($p=0.038$, $p=0.016$).
5. The telomerase activity and hTERT mRNA were significantly correlated ($p<0.001$).

Our results represent the first evidence that telomerase activity and hTERT mRNA expression are correlated with the proliferative potential of the endometrium in endometriosis patients. In addition, it may have an important role in the pathogenesis of endometriosis.

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ABSTRACT(IN KOREAN)

자궁내막증 환자의 자궁내막 조직에서 *telomerase* 활성도 및 h*uman telomerase reverse transcriptase* (h*TERT*) mRNA 발현 분석

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목적 : 자궁내막증은 가임기 여성의 7-15%에서 발생하는 비교적 흔한 양성질환으로 종양화 과정과 유사한 특징을 가지고 있다. 주요 병리기전으로 생리혈의 역류현상과 유전적 또는 환경적 요인등이 중요하게 인식되었으나, 아직까지 자궁내막증의 착상 및 증식등에 관련해 정확히 밝혀진 바가 없다. 최근 연구에서 일부 유전자 또는 유전자 산물들이 자궁내막증 환자의 자궁내막 조직에서 발현이 증가되어있음이 보고되고 있다. human telomerase reverse transcriptase (hTERT) mRNA는 telomerase의 핵심 구성요소로서, telomerase와 함께 다양한 악성 조직에서 발현이 증가되어 있으며, 증식능 (replicative potential)과도 관련있는 것으로 알려져있다. 따라서, 본 연구에서는 정량적 hTERT mRNA와 telomerase 활성도를 이용해 자궁내막증이 있는 환자에서 자궁내막 조직의 증식능을 살펴보고자 한다.

대상 및 방법 : 본 연구는 수술중 자궁내막 조직검사를 이용해 채취한 자궁내막증 환자들의 자궁내막 조직과 자궁내막증이 없으며, 다른 자궁내막내의 병변이 없는 환자들의 정상 자궁내막 조직 각각 30개씩을 이용하였다. 각각의 군은 다시 생리주기와 자궁내막증 병기에 따라 분류하였다. hTERT mRNA의 발현은 Taq-Man fluorescence methodology에 근거한 real-time RT-PCR을 이용해 측정하였다. Telomerase activity 측정은 telomerase repeat amplification protocol (TRAP) assay를 이용하였다.

결과 : hTERT mRNA의 발현양상을 살펴보면, 정상군에서는 80% (24/30)의 발현율을 보였으며, 자궁내막증군에서는 90% (27/30)가 발현되었다. 그 발현값은 정상군에 비해 자궁내막증군에서 통계적으로 유의하게 높았다 (p=0.004). Telomerase activity는 두 군 모두에서 90% (27/30)에서 관찰할 수 있었으며, 그 절대값은 정상군에 비해 자궁내막증군이 높았으나, 유의한 차이는 없었다. 그러나, 정상군과 자궁내막증 병기 III-IV 군만을 비교해 보았을때는 통계적으로 유의한 차이를 확인할 수 있었다 (p=0.048). 생리주기별로 증식기와 분비기로 각각 나누어 두 군의 hTERT mRNA 발현을 살펴보았을때, 자궁내막증 분비기의 값이 정상군의 분비기의 값에 비해 유의하게 증가되어 있었으며 (p=0.036), 증식기에서는 두 군간에 차이가 없었다. telomerase 발현도 역시 hTERT mRNA와 유사한 양상으로 나타났으나, 각 군간에 통계적으로 유의한 차이는 없었다. hTERT mRNA와 telomerase activity 모두 자궁내막증군에서 병기가 증가할 수록 그 값이 증가하는 양상을 관찰할 수 있었다 (p=0.038, p=0.016).

결론 : 본 연구는 자궁내막증 환자의 자궁내막 조직에서 hTERT mRNA 및 telomerase activity가 정상군에 비하여 발현도가 증가되어있음을 보여주었다. 이는 자궁내막증의 자궁내막 조직이 정상조직과는 다른

증가된 증식능이 있음을 의미하는 것이고, 이것이 자궁내막증 병리기전에 관여할 것이라 사료된다.

핵심되는 말 : 자궁내막증, 병리기전, *tel*ome*ra*se,
h*uma*n *tel*ome*ra*se *re*v*e*r*s*e *tra*n*scripta*se (h*T*ER*T*) mRNA