The Role of Human Telomerase Catalytic Subunit mRNA Expression in Cervical Dysplasias

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Telomerase activity and human telomerase reverse transcriptase (hTERT) mRNA expression were investigated in cervical specimens and were correlated with cytologic findings and the presence of human papilloma virus (HPV) infection. Telomerase activity was evaluated by the telomeric repeat protocol assay and hTERT mRNA expression was evaluated by reverse transcriptase polymerase chain reaction (PCR). HPV DNA was detected by PCR, as well as restriction endonuclease digestion. HPV DNA was detected in all 82 specimens with abnormal cytologic findings and in 4 of 34 normal samples. Low-grade squamous intraepithelial lesions (LGSILs) were present in 74 of 82 specimens (90.2%) and high-grade squamous intraepithelial lesions (HGSILs) were present in 8 of 82 (9.75%) specimens. Seven of the eight HGSIL (87.5%) and 26 of 74 LGSIL (35.1%) specimens were hTERT positive, whereas all normal specimens were hTERT mRNA negative. Telomerase activity was detected in 21 of 74 (28.4%) LGSIL/atypical squamous epithelial cells of undetermined significance (ASCUS) and in five of eight (62.5%) HGSIL samples. A correlation was observed among telomerase activity, hTERT mRNA expression, and high-risk HPV infection in HGSIL samples ($P < 0.001$). High-risk HPV infection assessment showed 75% sensitivity and 72.2% specificity for HGSILs. Telomerase activity assessment in cervical smears showed sensitivity and negative predictive value (NPV) for HGSILs 62.5% and 96.7%, whereas specificity and positive predictive value (PPV) were 80.5% and 19.2%, respectively. hTERT mRNA expression assessment showed 87.5% sensitivity and 98.7% NPV for HGSILs, whereas specificity and PPV were 76% and 21.2%, respectively. Based on the above-described telomerase assessment values, it is suggested that the telomerase system might not be an appropriate diagnostic marker for cytology, given that the final evaluation must rely on a combination of all available test assessment data, clinical diagnosis, as well as the follow-up of all LGSIL samples that were positive for telomerase activation. Exp Biol Med 230:263–270, 2005

Key words: telomerase activity; hTERT; HPV; cervical cytology; cervical samples; cervical dysplasia

Introduction

Cervical cancer is a major cause of death and the second most common cancer in women worldwide. Epidemiologic and molecular studies performed in women from various races and ethnic minority groups have demonstrated convincingly that certain types of human papillomavirus (HPV) are etiologically related to the development of most cases of cervical cancer (1, 2). More than 100 different HPV types have been characterized by nucleotide sequence analysis and more than 40 types have been found to infect the anogenital region. HPV types have been divided into low and high risk according to their oncogenic potential. Women infected with high-risk HPV types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61, 66, and 68 are considered to be at a higher risk for the development of cervical cancer than those who are not infected with HPV or are infected with low-risk HPV types such as HPV 6, 11, 42, 43, and 44 (3, 4). The majority of cervical cancers are squamous cell carcinomas, which are preceded by intraepithelial precursor lesions composed of atypical or dysplastic cells (5). Cervical cytologic abnormalities are categorized in atypical squamous epithelial cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LGSILs) including condylomata acuminata and mild dysplasia, and high-grade squamous intraepithelial lesions (HGSILs) including moderate to severe dysplasia and carcinoma *in situ* (6). Although high-risk HPV strains have been detected in cervical smears with HGSILs, a small proportion of women with cytologic abnormalities or who are infected with high-risk HPV strains will eventually progress to invasive carcinoma. Therefore, additional criteria are needed to predict more

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accurately the clinical outcome of cervical lesions in individual women (6).

Recent observations support the concept that a change of the enzyme telomerase may be a critical, if not obligate step in the development of cancer (7). Telomerase is a ribonucleoprotein DNA polymerase consisting of an RNA component, designated hTR, and a catalytic protein subunit, human telomerase reverse transcriptase (hTERT), involved in the synthesis of telomeric DNA repeats (8). Telomerase activity has been detected in high-grade cervical dysplasias as well as cervical carcinomas, whereas some investigators report telomerase activity in premalignant cervical lesions as well as normal cervical epithelial tissue (9–12). Given that HPV infection has been associated with the majority of cases of invasive cervical carcinoma, telomerase activation may be a central mechanism by which HPV infection results in malignant transformation of the cervical mucosa (11, 13). In recent reports telomerase activation has been detected in cervical biopsies infected with HPV types 16 and 18 (6, 11).

In the present study, we investigated telomerase activity and hTERT mRNA expression in cervical samples of different cytology. For the first time to our knowledge, hTERT mRNA expression was observed in cervical samples with low- and high-grade dysplasias infected with HPV types 45, 58, and 61 in addition to types 16 and 18, which have been reported previously. Furthermore, both telomerase activity and hTERT mRNA expression were observed in LGSILs and HGSILs and were absent in normal epithelial tissue. In the present study, we tested the specificity and sensitivity of telomerase activation and hTERT mRNA expression and correlated their detection with the presence of HPV infection in cervical samples with HGSILs and LGSILs.

Materials and Methods

Patients. A total of 116 cases were studied, including 82 cases with abnormal cytologic findings and 34 normal cases. The mean age of women with abnormal findings was 35 years (range, 19–54 years; SD, 8.35 years) and of control group was 38 years (range, 25–50 years; SD, 8.24 years). Cervical cytology samples were obtained at the colposcopy unit of University Hospital of Larissa (Larissa, Greece). All samples were collected at the time of colposcopic evaluation for management of previous abnormal cytology test result.

Two separate cervical specimens were obtained from each woman in appropriate collection vials using an endocervical cytobrush before the performance of colposcopic examination and cervical biopsy, and immediately after collection of the routine cervical sample. The study was double blind. The samples were kept on ice and were immediately processed in the laboratory (14). The first specimen was divided into equal parts: one for DNA extraction and subsequent genotyping of HPV-positive specimens, and another for telomeric repeat protocol assay (TRAP) analysis, which was placed in phosphate-buffered saline and was centrifuged at 3000 *g* for 5 min. Cells were counted using a hematocytometer (Neubauer chamber) and 2×10^5 cells were immediately stored at –80°C. The second cervical specimen was used for RNA extraction and hTERT mRNA expression. Cervical biopsies for histologic examinations were performed at the end of the colposcopy and were evaluated at the Pathology Laboratory of University Hospital of Larissa. The ethics committee of the University Hospital of Larissa approved the protocol.

HPV Analysis. Human genomic DNA was extracted from the scraped cervical cells using a proteinase K/phenol-chloroform protocol. HPV detection was performed by polymerase chain reaction (PCR) using the consensus primers MY09 and MY11 for the amplification of the L1 region of the HPV genome, with an expected product size of about 450 base pairs (bp). A total of 40 cycles were performed with a thermal profile 1 min at 94°C, 1 min annealing at 55°C, and 1 min at 72°C. A second round of amplification with general primers GP5 and GP6 positioned inside the MY09 and MY11 primers was performed and amplified a 150-bp fragment (35 cycles: 50 secs at 94°C, 50 secs annealing at 42°C, 50 secs at 72°C). Amplified MY09/11 PCR products were typed by restriction endonuclease digestion, using *Bam*HI, DdeI, *Hae*III, HinfI, PstI, RsaI, and Sau3aI enzymes for HPV types 6, 11, 13, 16, 18, 26, 31, 32, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69, MM4(W13B), MM7(PAP291), MM8(PAP155), MM9(PAP238A), LVX100, IS39, CP141, CP6108, CP8304, CP4173, and CP8061. Digestion products were electrophoretically separated on 3% agarose gels and visualized under UV light (15). Samples negative for MY09/11 that were positive with the GP5/6 PCR reaction were further processed with specific primers for HPV types 16, 18, 6/11, 31, and 33. Cloned plasmids with full HPV genomes (HPV types 6, 11, 16, 18, 31, and 33) served as positive PCR controls. Negative PCR controls were included in each experiment. PCR amplification of human β-globin gene fragment was used to determine the integrity of the specimens. Finally, samples that could not be typed with any of the above methods were processed with an HPV kit (Keymed S.r.l., Formello, Italy), which designates high- (16, 18, 31, 33, 35, 45, 42, and 58) and low-risk (6, 11, and 52) HPV types. Randomly selected PCR products, including the hTERT-positive sample infected with a low-risk HPV type (HPV-6), were sequenced to ensure the accuracy of the genotyping method being used.

TRAP Assay. TRAP assay is considered a sensitive and specific PCR-based functional enzyme assay. It was performed using TeloTAGGG telomerase PCR ELISA^{PLUS} kit (Roche, Indianapolis, IN) in accordance with the manufacturer's instructions. Briefly, each frozen pellet was homogenized in 200 μl lysis reagent. After 30 mins incubation on ice, the lysate was centrifuged at 16,000 *g* for 30 mins at 4°C and the supernatant was aliquoted. Control templates were included in the kit, which contain positive-telomerase template DNA with the same sequence as a telomerase product with eight telomeric repeats. Lysates from Hela cells were used as positive controls. The PCR-

Table 1. Correlation of Cytology with HPV Infection[a]

	HPV infection			Biopsy findings			
Cytology findings	HPV negative	HPV positive (low risk[b] and unclassified HPV types)	High risk[c] HPV positive	Negative for dysplasia	CIN1	CIN2	CIN3
Normal	30/34 (88.2%)	3/34 (8.9%)	1/34 (2.9%)	34/34 (100%)	—	—	—
ASCUS	—	3/5 (60%)	2/5 (40%)	4/5 (80%)	1/5 (20%)	—	—
LGSIL	—	41/69 (55.4%)	33/69 (44.6%)	—	69/69 (100%)	—	—
HGSIL	—	2/8 (25%)	6/8 (75%)	—	—	5/8 (62.5%)	3/8 (37.5%)
Total	30/116 (25.9%)	46/116 (39.6%)	40/116 (34.5%)	38/116 (32.7%)	70/116 (60.3%)	5/116 (4.3%)	3/116 (2.6%)

[a] HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; ASCUS, atypical squamous epithelial cells of undetermined significance; LGSIL, low-grade squamous intraepithelial lesions; HGSIL, high-grade squamous intraepithelial lesion.
[b] 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61, 66, 68, 69.
[c] 6, 11, 42, 43, 44, 53, 83.

based analysis was carried out in a 30-µl reaction mixture containing 2 µl of cell extract. An internal standard was amplified by telomeric substrate primers, to avoid false-negative results. Telomerase-positive results were confirmed by repeat TRAP assay, with heat pretreatment (85°C for 15 mins) of cellular lysates to monitor for false-positive results. Lysis buffer reagent controls were included in each reaction to monitor for the possibility of reagent contamination. Positivity was detected when the substrate turned blue and then yellow on addition of the stop reagent; the color conversion maximized the sensitivity of the readings. Sample absorbance was measured within 30 mins using a spectrophotometer at 450 nm against a blank (reference wave length at 650 nm). Samples were also electrophoretically separated on a 12% polyacrylamide gel and samples were considered as positive when the difference between the absorbance of the sample and the absorbance of the negative control was higher than the 2-fold background activity and when they produced the characteristic 6-bp telomerase ladder. The sensitivity of the TRAP assay is extended to 10 Hela cell equivalents.

hTERT mRNA Expression. Total cellular RNA was isolated by the guanidinium isothiocyanate method and transcribed to cDNA using the Moloney murine leukemia virus reverse transcriptase (RT) and random hexamers as primers. Retinoic acid receptor alpha cDNA sequences (RARα) were amplified in separate reactions as positive cDNA controls. Both in the RT reaction and in the ensuing amplification reactions, recommended measures to prevent cross-contamination of samples were followed. In addition, for each experiment, a control with no template was used to check for the presence of contaminants. hTERT cDNA sequences were amplified by PCR with the intron flanking primer pair A/B (A: 5′-CGGAAGAGTGTCTGGAGCAA-3′ and B: 5′-GGATGAAGCGGAGTCTGGA-3′) spanning between exons 3 and 4 of the corresponding gene to exclude cross-reactivity with genomic DNA. In contrast to other segments of this gene, no splice variants of hTERT mRNA have been detected in the genomic region selected for hTERT cDNA amplification (16, 17). K562 erythroleukemic cells, which express hTERT mRNA, served as positive PCR control. Direct DNA sequencing was used to identify specific PCR products.

Statistical Analysis. Diagnostic test characteristics (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]) were calculated by using the proportion of women with normal cytology, LGSIL, and HGSIL. All statistical analyses were performed by Fischer's exact test to evaluate the significance of the differences using the statistical analysis program SPSS version 10 (SPSS Inc., Chicago, IL). Only P values <0.05 were considered significant.

Results

HPV DNA was detected in all 82 specimens with abnormal cytologic findings (100%) and in 4 of 34 normal specimens (11.7%). A single genotype was detected in most HPV-positive specimens 77 of 82 (93.9%), whereas 5 of 82 positive specimens (6%) had multiple HPV infections. High-risk HPV types 16, 18, 31, 33, 45, 61, or 58 were present in 39 of 82 specimens with abnormal cytologic findings (47.5%), and in 1 of 34 normal specimens (2.9%). Low-risk HPV types 6, 11, 53, 54, CP141, or CP8304 were detected in 24 of 82 cases with abnormal cytologic findings (29.3%) and in 3 of 34 normal samples (8.8%). Nineteen of the 82 cytologically abnormal specimens (23.2%) were HPV positive (nested PCR) with unclassified HPV types. ASCUS and LGSILs were present in 74 of 82 (90.2%) and HGSILs were present in 8 of 82 (9.75%) of the cytologically abnormal samples. When ASCUS samples were grouped together with LGSILs, there were no discrepancies between cytologic findings and histologic outcome. When ASCUS samples (n = 5) were evaluated separately, one of five samples (20%) was classified as CIN1. All remaining 69 LGSIL samples were classified as CIN1, five of eight HGSILs (62.5%) were classified as CIN2, and three of eight HGSILs (37.5%) were classified as CIN3 (Table 1).

Telomerase activity was observed in 26 of 82 (31.7%) specimens analyzed, which were infected with HPV types 16, 18, 61, and 31. hTERT mRNA expression was observed

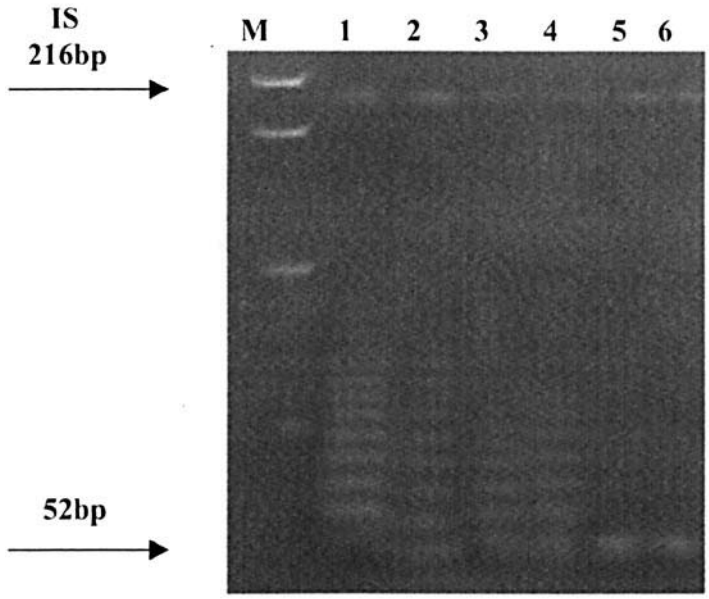

Figure 1. Telomeric repeat protocol assay of representative human papilloma virus (HPV)-infected and normal samples. M, marker. Lane 1, positive control high (provided by the kit); lane 2, positive control low (provided by the kit); lane 3, positive HPV-16 sample; lane 4, positive HPV-18 sample; lane 5, negative HPV-54 sample; lane 6, negative normal sample.

in 33 of 82 (40.2%) specimens analyzed, which were infected with HPV types 16, 18, 61, and 58, and in one case with HPV type 6, which was also positive for telomerase activity. All normal samples were negative for both telomerase activity and hTERT mRNA expression. Among the 33 samples positive for hTERT mRNA expression, 24 were also positive for telomerase activity (72.7% concordance). Two samples positive for telomerase activity were found to be hTERT negative. Figure 1 shows telomerase activity in cervical samples revealing the progressive 6-bp ladder. Figure 2 shows hTERT mRNA expression relative to RARα mRNA control in a representative number of HPV-infected and normal samples.

Correlation Between HPV Infection Status and Cytology. HPV test results were compared with the cytology of the samples. Four of the 34 normal specimens (11.7%) and all of 82 LGSIL and ASCUS and HGSIL specimens (100%) were HPV positive. The proportion of HPV-infected specimens differed significantly between normal samples and samples with either LGSIL and ASCUS or HGSIL ($P < 0.001$; Table 1).

High-risk HPV types 16, 18, 31, 33, 45, 61, or 58 were identified in one of 34 (2.9%) normal samples, in 33 of 74 (44.6%) LGSIL and ASCUS, and in six of eight (75%) HGSIL samples. Low-risk HPV types 6, 11, 53, 54, CP141, or CP8304 were identified in 3 of 34 (8.9%) normal samples, 41 of 74 (55.4%) LGSIL and ASCUS, and two of eight (25%) HGSIL samples. The most common HPV types detected were types 16 and 18, which were detected in 15 of 86 (18.6%) of positive cases. Among HPV-infected specimens, a significant difference was observed between viral risk type and cytologic findings. More specifically, a significant difference was observed between high-risk HPV types and HGSIL and low-risk HPV types and LGSIL ($P < 0.05$; Table 1).

Correlation Between Telomerase Activity, hTERT mRNA Expression, and Cytology. Telomerase activity was detected in five of eight HGSIL (62.5%) and 21 of 74 LGSIL and ASCUS (28.4%) samples and was not detected in samples with normal cytology. Seven of eight HGSIL (87.5%) and 26 of 74 LGSIL and ASCUS (35.1%) specimens were positive for hTERT mRNA expression. Telomerase activity and hTERT mRNA expression differed

Table 2. Correlation of Telomerase Activity and hTERT mRNA Expression with Cytology[a]

Cytology	Telomerase positive	Telomerase negative	hTERT positive	hTERT negative
Normal	0/34 (0%)	34/34 (100%)	0/34 (0%)	34/34 (100%)
LGSIL	21/74 (28.4%)	53/74 (71.6%)	26/74 (35.1%)	48/74 (64.9%)
HGSIL	5/8 (62.5%)	3/8 (37.5%)	7/8 (87.5%)	1/8 (12.5%)
Total	26/116 (22.4%)	90/116 (77.6%)	33/116 (28.4%)	83/116 (71.6%)

[a] hTERT, human telomerase reverse transcriptase; LGSIL, low-grade squamous intraepithelial lesions; HGSIL, high-grade squamous intraepithelial lesion.

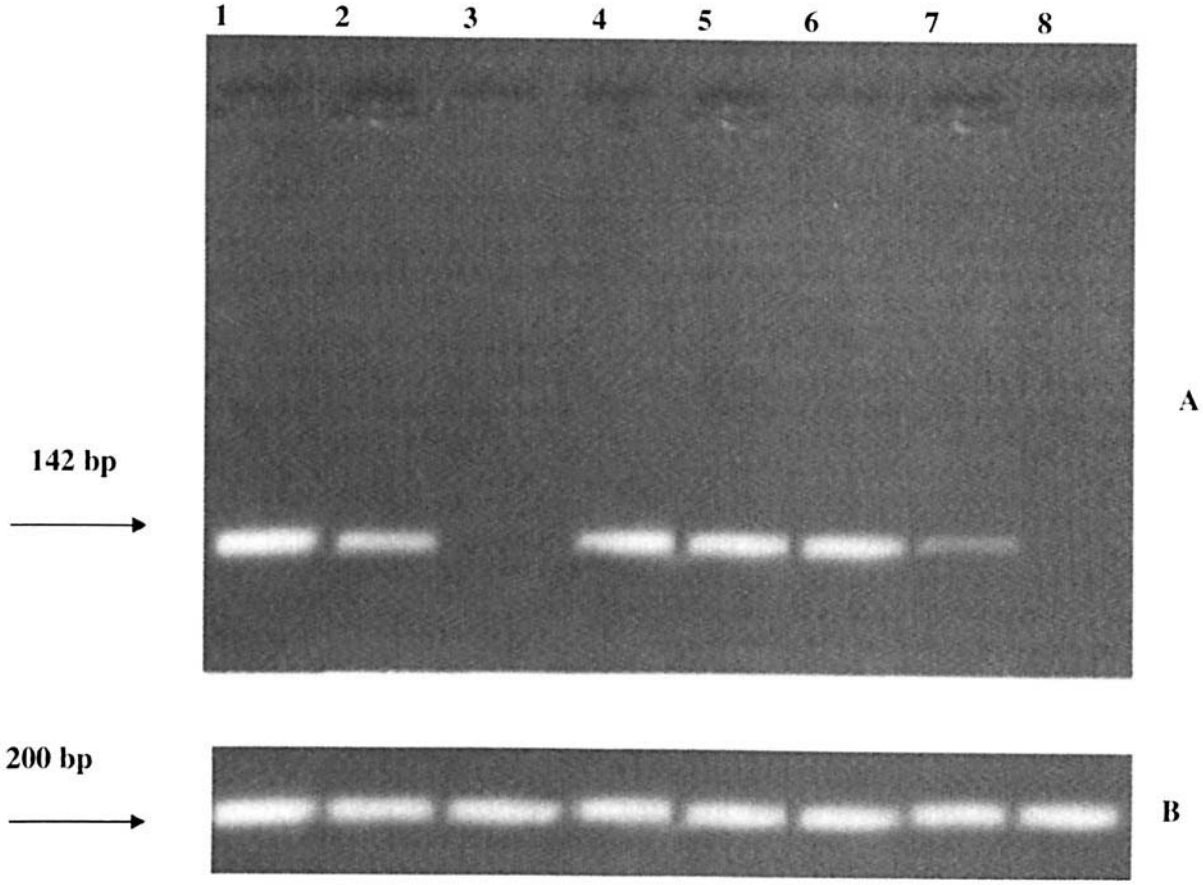

Figure 2. Reverse transcriptase polymerase chain reaction (PCR) analysis of representative human papilloma virus (HPV)-infected and normal cervical specimens for the presence of human telomerase reverse transcriptase (hTERT) mRNA (panel A) relative to retinoic acid receptor alpha (RARα) mRNA control (panel B). Lanes 1 and 2, HPV-16 sample; lane 3, normal sample; lane 4, HPV-45; lane 5, HPV-58; lane 6, HPV-61; lane 7, HPV-6b; lane 8, HPV-6. The PCR products of K562 positive control are shown below.

significantly between LGSIL and ASCUS and HGSIL ($P = 0.004$ and $P < 0.05$, respectively; Table 2).

Correlation Between Telomerase Activity, hTERT mRNA Expression, and HPV Infection. The results of telomerase detection and hTERT mRNA expression were compared with the detection of HPV (Table 3). Telomerase activity was detected in 20 of 39 (51.3%) cases infected with high-risk HPV types and in 6 of 43 (14%) cases infected with low-risk types or with unclassified HPV types. hTERT mRNA expression was observed in 25 of 39 (64.1%) cases positive for high-risk HPV type and in 8 of 43 cases (18.6%) positive for low-risk HPV types. A significant difference was observed between telomerase activity, hTERT mRNA expression, and HPV infection status ($P < 0.001$).

Assessment of Telomerase Activity, hTERT mRNA Expression, and HPV Infection Status for the Detection of HGSIL. Sensitivity and NPV for telomerase activity assessment for HGSILs were 62.5% and 96.7%, respectively, whereas specificity and PPV were

Table 3. Correlation of Telomerase Activity and hTERT mRNA Expression with HPV Infection[a]

Infection type		Telomerase positive	Telomerase negative	hTERT positive	hTERT negative	Total
Normal	High-risk HPV	—	1/1 (100%)	—	1/1 (100%)	1
	Other types[b]	—	3/3 (100%)	—	3/3 (100%)	3
	Negative	—	30/30 (100%)	—	30/30 (100%)	30
LGSIL	High-risk HPV	16/33 (48.5%)	17/33 (51.5%)	19/33 (57.6%)	14/33 (42.4%)	33
	Other types[b]	5/41 (12.2%)	36/41 (87.8%)	7/41 (17%)	34/41 (83%)	41
HGSIL	High-risk HPV	4/6 (66.7%)	2/6 (33.4%)	6/6 (100%)	—	6
	Other types[b]	1/2 (50%)	1/2 (50%)	1/2 (50%)	1/2 (50%)	2
Total		26	90	33	83	116

[a] hTERT, human telomerase reverse transcriptase; HPV, human papilloma virus; LGSIL, low-grade squamous intraepithelial lesions; HGSIL, high-grade squamous intraepithelial lesion.
[b] Low risk and unclassified HPV types.

Table 4. Test Characteristics of Telomerase Components and HPV When Used for the Detection of HGSIL[a]

Telomerase components and HPV	Sensitivity	Specificity	Positive predictive value	Negative predictive value
TRAP	62.5%	80.5%	19.2%	96.7%
hTERT	87.5%	76%	21.2%	98.7%
High-risk HPV	75%	68.5%	15%	97.4%

[a] HPV, human papilloma virus; HGSIL, high-grade squamous intraepithelial lesion; TRAP, telomeric repeat protocol assay; hTERT, human telomerase reverse transcriptase.

80.5% and 19.2%, respectively. hTERT mRNA expression assessment showed a high sensitivity (87.5%), 98.7% NPV, 76% specificity, and 21.2% PPV for HGSIL (Table 4)

Discussion

We investigated the relation between telomerase activity, hTERT mRNA expression, HPV infection status, and cytologic findings in cervical epithelial cells and evaluated telomerase activity and hTERT mRNA expression assessment in cervical smears with low- and high-grade lesions.

We studied the overall telomerase activity as well as hTERT mRNA expression, which correlate closely to telomerase activity, and not the RNA component (hTR), or telomerase-associated proteins (TP1/TLP1), given that the latter are expressed in both normal cervical tissue and neoplastic samples, and therefore are nonspecific for clinically significant cervical disease (10).

HPV infections are the single most potent insult leading to the development of cervical cancer and are present in more than 90% of such cases (2, 5, 9). The mechanism of malignant transformation by HPV appears to involve the integration of HPV DNA into the host cellular genome, resulting in constitutive expression of oncoproteins E6 and E7, which in turn inactivate tumor suppressor proteins and lead to the development of immortalizing or transforming phenotypes (13, 18–21). Testing for oncogenic types of HPV in cervical specimens is another possible method to triage women with low abnormalities on cytologic screening and has been found to have sensitivity and specificity ranging from 65% to 98% and from 52% to 74%, respectively, for detecting high-grade lesions (22). In our study, the sensitivity and specificity for high risk HPV types were 75% and 68.5%, respectively, for the detection of HGSIL, which is in agreement with previous studies (22–24).

The tight connection between HPV infection and cervical neoplasia has been supported by the presence of hTERT transcripts in human keratinocytes expressing the E6 protein of HPV type 16. In some reports a negative association between telomerase activity and HPV infection has been observed (6, 9, 12, 25, 26), whereas other studies report a positive association between telomerase activity and high-risk HPV types in cervical lesions (13, 27).

Until now, the evaluation of telomerase activity and hTERT mRNA expression as potential predictors of cervical cancer has been inconclusive. Some reports have detected telomerase activity in normal cervical tissues as well as in benign LGSIL and ASCUS (6, 11, 22, 23), whereas some others have detected telomerase activity in HGSIL only (25, 28–31). hTERT mRNA expression has been observed in premalignant as well as in malignant lesions of cervical epithelium (6, 7, 9, 26, 27, 32, 33). In our study we observed hTERT mRNA expression in the majority of HGSIL (87.5%) samples and in 35% of LGSIL and ASCUS samples, whereas telomerase activity was observed in 62.5% of HGSIL and in 28.4% of LGSIL and ASCUS samples, in agreement with previous reports (6, 9, 10, 21, 26). However, the number of HGSIL samples in our study was too small to allow definite conclusions. We also observed, for the first time, hTERT mRNA expression in cervical samples with low- and high-grade dysplasias, infected with HPV types 45, 58, and 61, in addition to previously reported types 16 and 18. In agreement with Cheah et al (25) and Kawai et al (27), who reported the presence of low telomerase activity in four cases infected with non–high-risk HPV types using the TRAP assay (8, 27), we observed hTERT mRNA expression and telomerase activity in one specimen infected with low-risk HPV-6. The sample was sequenced and was found to be an HPV-6b variant. Overall, in our study a correlation was observed between telomerase activity, hTERT mRNA expression, and HPV infection status in cervical samples with low- and high-grade abnormalities.

Routine cervical cytology is the most effective screening test for cancer, but the practice of cervical cytology is limited by problems of false-negative diagnosis of HGSILs because of errors of interpretation and of poor specificity for clinically significant lesions in ASCUS and LGSILs. Reports of false-negative rates in cervical cytology vary form 1.6% to 28%, with mean sensitivity of 58% and specificity of 69% (10, 34). Because of this low sensitivity and specificity, the use of a second test in parallel with the Papanicolaou smear might be very useful. The issue of the clinical utility of telomerase assay and hTERT mRNA expression in cervical samples with low- and high-grade abnormalities has been addressed in few recent reports and there are conflicting results, with sensitivity ranging from 3.7% to 100% (12, 31, 35–37). Telomerase activity assessment in our study showed a relatively high sensitivity and a high specificity for HGSILs. hTERT mRNA assessment showed even higher sensitivity for HGSILs. More

specifically, the sensitivity and NPV of telomerase activity were 62.5% and 96.7% for HGSILs, whereas specificity and PPV were 80.5% and 19.2%, respectively. hTERT mRNA sensitivity was 87.5% and NPV 98.7%, whereas specificity and PPV were 76% and 21.2%, respectively. Although the sensitivity and specificity values of telomerase activity and hTERT assessment for HGSILs were high, the observed high NPV suggests that the telomerase system might not be a useful marker for predicting biologic behavior of a cervical lesion.

In conclusion, our findings suggest that HPV infection either alone or in association with other insults activates the expression of the telomerase gene in the cervical mucosa. Telomerase activation appears to be an early event in the malignant transformation of cervical epithelium, and based on our telomerase assessment values, it is suggested that the final evaluation of telomerase test performance must rely on a combination of all available test assessment data, cytologic and histologic diagnosis, and the follow-up of all women with low-grade lesions that expressed telomerase activity.

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