# Telomerase activity in cervical scrapes of women with high-grade cervical disease: A nested case-control study

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**Abstract.** Epidemiological information on telomerase activity (TA) and development of cervical lesions is scarce. A nested case-control study was carried out within a cohort of Colombian women tested for Human Papillomavirus (HPV). Measurement of TA was done in cervical scrapes of 25 women who developed High Grade Squamous Intraepithelial Lesion (HGSIL) during the first 6 years of follow-up and was compared with that of 104 control women who maintained normal cytology during the entire follow-up. TA was measured by a telomerase repeat amplification protocol-ELISA. TA and HPV infections were significantly more frequent in cases than in controls. Likewise, 68% of the cases were positive for both TA and HPV compared with only 7.7% of the controls (P<0.0001). Factors independently associated with increased odds of HGSIL included TA, high risk HPV (hrHPV) infections and multiple parities. When restricted to hrHPV positive women, TA was strongly associated with increased odds of HGSIL (adjusted odds ratio=37.94, 95% confidence interval, 1.64-678.1). In addition to an infection with hrHPV, TA appears to be a significant cofactor for HGSIL.

## Introduction

Worldwide, cervical cancer is the third most common cancer found in women. There are 529,000 new cases diagnosed each year (1). In Colombia it has an incidence of 26.1/100,000 and is the most common cause of death from cancer in women (2). High-risk human papillomavirus (hrHPV) are detected in virtually all cervical carcinomas (3,4). However, other host cell factors are required for progression of hrHPV-induced precancerous lesions to invasive cancer.

Telomeres are specialized structures at the end of linear chromosomes. They consist of non-coding tandem repeated TTAGGG sequences which are involved in chromosomal stability. In somatic cells, during each cell division, telomeres are shortened progressively until reaching cellular senescence (5). Telomerase is a ribonucleoprotein enzyme complex containing several components, between them a catalytic subunit composed of hTERT (human Telomerase Reverse Transcriptase) and a RNA component (hTR). The hTR component acts as a template for elongation of telomeric DNA (6). Normal tissues and human somatic cells show low or undetectable levels of telomerase activity (TA), whereas immortalized cells and tumour cells from a variety of cancers show highly detectable TA (7,8). Telomerase activation, which is very important for cell immortalization, has been proposed as a critical step for the development of different types of cancer including cervical cancer (9).

Identifying molecular markers that will permit diagnosis and prognosis of women who have cervical lesions as well as predicting which precursor lesions are likely to become cancerous will lead to improvements in cervical cancer prevention. Several studies on women with normal cytology and/or different grades of cervical lesions have shown increased TA in higher grade lesions (10-13). Some studies have also shown that infections with HPV 16 and 18 are associated with increased TA (14,15). In addition a few studies have shown that expression of HPV16 E6, along with E6AP can induce hTERT transcription and TA (16-20). Gain of chromosome 3q, containing the sequence for the telomerase RNA component (TERC), and gain of chromosome 5p, containing the TERT gene, have been also associated with CIN2/CIN3+ in cervical tissue (21-23).

To the best of our knowledge, no previous epidemiological analysis has been performed on the association between TA and HPV in cervical scrapings, analyzing the role of TA as a possible risk factor for HGSIL adjusted for different known risk factors. Here we report the results of HPV and TA detection in a nested case control study within a cohort of Colombian women.

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*Key words:* telomerase, papillomavirus infections, risk factors, scraping, cervical intraepithelial neoplasia

## Materials and methods

*Study population*. Between November 1993 and November 1995 the Colombian National Cervical Cancer Institute conducted a population census in four health districts in Bogotá. Two thousand women aged 18-85 years were randomly identified and invited to participate in the cohort study. In order to acquire information on sexually active adolescents, 200 sexually-active women aged 13-17 years, all of whom had come to an adolescent clinic for contraceptive counseling without a doctor's referral, were also invited to participate. The ethics committee of INC approved the protocol. Methods of recruitment and data collection have been described elsewhere (24). Briefly, eligible women were those residing in Bogota, without history of cervical neoplasia, conisation or hysterectomy willing to participate and who gave written informed consent. At study entry and at each follow-up visit, participants responded to a questionnaire on risk factors for cervical cancer and underwent a gynaecological examination with collection of cervical cells for cytology and HPV DNA detection. Colposcopic examination of the cervix was performed in all women with repeated cytological diagnosis of Low Grade Squamous Intraepithelial Lesions (LGSIL) or with cytological evidence of High Grade Squamous Intraepithelial Lesions (HGSIL). Colposcopically guided cervical biopsies were performed in women with cytological or colposcopic evidence of HGSIL. Follow-up visits were scheduled every 6 months for up to 10 years until March 2004.

The analysis described here is a nested case control study of the study cohort. Cases were defined as any women with cytological HGSIL or biopsy-confirmed CIN2/CIN3+ during the first six years of follow up. Controls were defined as any women that maintained normal cytology results during the entire follow up period.

All cases observed during the first six years of follow up were selected, while controls were randomly chosen from the participants of the study, but assuring a group age match (±2 years) with cases (at diagnosis). To maximize the statistical power of the study sample and considering the small number of cases diagnosed during follow up within the study, a case: control ratio of 1:4 was used.

From the 38 cases observed during follow up, only 25 cases (3 Invasive cancers, 10 CIN3, 5 CIN2 and 7 HGSIL) had available sufficient cervical scrape sample to analyze TA and 104 controls were selected with the defined criteria. HPV infections had previously been tested using GP5+/GP6+ PCR-EIA and genotyped using a Reverse Line Blot assay (RLB), as described previously (25). Samples were analyzed for the presence of 14 high risk HPV types (hrHPV) and 23 low risk HPV types (lrHPV).

*Sample processing for TA*. Samples were processed as described previously (10). Briefly, samples were centrifuged at 1,200 rpm. Pelleted cells were treated with trypsin at 37˚C for 5 min to separate cell clusters. Cells were counted with a haemocytometer, washed twice with PBS, and stored as pellets at -80°C until their use in a TRAP (Telomeric Repeat Amplification Protocol) assay.

*TRAP assay*. TA was measured by TRAP assay using a commercially available kit, Telo TAGGG Telomerase PCR enzyme-linked immunosorbent assay (ELISA) from Roche Applied Science, Mannheim, Germany. Assays were performed according to the manufacturer's specifications. In addition to the kit's positive control, cell extracts from HeLa cells were used as another positive control for each assay. Heat-treated cell extracts at 85°C for 10 min from positive controls and samples were used as negative controls. Final optical Densities (ODs) of samples were obtained by subtracting ODs of negative controls from those of the samples. Samples were regarded as telomerase positive if the difference in absorbance is higher than 0.2 $A_{450nm}$-$A_{690nm}$ units.

*Statistical analyses*. HPV infection, TA and cofactors related to cervical cancer aetiology (age, parity, smoking status and oral contraceptive use) were included in the analysis. Fisher's exact test and chi-squared test were used to compare HPV detection, TA and cofactors between cases and controls. Association of TA with HGSIL was evaluated using logistic regression analysis. As hrHPV infection is a necessary but not sufficient cause of cervical neoplasia, we also assessed the association of telomerase and HGSIL among women positive for hrHPV. Odds ratios (OR) and 95% confidence intervals (CIs) are reported for crude and adjusted analysis. All statistical analysis was done using STATA software (version 9, StataCorp, College Station, TX, USA).

## Results

*Characteristics of the population*. Characteristics of the cases and controls are summarized in Table I. Women included in this study were aged 14-68 years. While age and education were similar in both groups and no differences were evident in oral contraceptive use and smoking status, parity tended to be higher among cases, but the difference was not statistically significant.

*HPV detection and TA*. Among cases, 84% of the samples were HPV positive; all of these were positive for hrHPV genotypes (100%), predominantly of the alpha 7 and 9 species (Fig. 1). Among controls, 22.1% of the samples were HPV positive and 60.8% of these specimens were hrHPV positive. In this group of women, a mixture of hrHPV and lrHPV types were detected, predominantly of the alpha 3, 7 and 9 species (Fig. 2). HPV was detected in significantly more cases than controls (Table I).

TA was positive in 76% of cases, significantly higher than in controls, of which only 20.2% were positive (Table I).

Of the cases, 17 (68%) were positive for TA and HPV infection concurrently, 2 (8%) were positive only for TA, 4 (16%) had only HPV infection and 2 (8%) were negative for both. Of the controls, 8 (7.7%) were positive for TA and HPV infection simultaneously, 13 (12.5%) were positive only for TA, 15 (14.42%) had only HPV infection and 68 (65.38%) were negative for both (Fig. 3). Detection of concurrent TA and HPV was significantly higher in cases than in controls ($P<0.0001$) (Table I).

In the seventeen cases that were positive for HPV infection and TA concurrently, the presence of hrHPV types in single and multiples infections was always detected and they were mainly types belonging to the Alpha 9 species. In the eleven cases with

Table I. Main characteristics of cases and controls. (A) The P-value was calculated from Fisher's exact test. (B) The P-value was calculated from Pearson's chi-square tests. hrHPV, high-risk human papillomavirus.

| | Cases | | Controls | | |
|---|---|---|---|---|---|
| Main characteristics | n=25 | (%) | n=104 | (%) | P-value |
| Education | | | | | |
| None | 1 | (4) | 1 | (1) | |
| Primary | 8 | (32) | 19 | (18) | |
| Secondary | 14 | (56) | 61 | (59) | |
| College/university | 1 | (4) | 10 | (10) | 0.21[a] |
| Missing | 1 | (4) | 13 | (12) | |
| Age (years): | | | | | |
| <20 | 1 | (11) | 11 | (11) | |
| 20-29 | 8 | (24) | 19 | (18) | |
| 30-44 | 14 | (58) | 69 | (66) | |
| 45-64 | 1 | (4) | 3 | (3) | |
| 65+ | 1 | (3) | 2 | (2) | 0.6[a] |
| Parity: | | | | | |
| 0-1 | 3 | (12) | 35 | (34) | |
| >2 | 21 | (84) | 69 | (66) | 0.07[a] |
| Missing | 1 | (4) | | | |
| Oral contraceptives: | | | | | |
| Never | 11 | (44) | 56 | (54) | |
| Ever | 14 | (56) | 48 | (46) | 0.49[b] |
| Smoking status | | | | | |
| Never | 18 | (72) | 72 | (69) | |
| Ever | 7 | (28) | 32 | (31) | 0.62[b] |
| HPV | | | | | |
| Positive | 21 | (84) | 23 | (22.1) | |
| Negative | 4 | (16) | 81 | (77.9) | <0.0001[b] |
| hrHPV | | | | | |
| Positive | 21 | (84) | 14 | (13.5) | |
| Negative | 4 | (16) | 90 | (86.5) | <0.0001[b] |
| Telomerase activity (TA) | | | | | |
| Positive | 19 | (76) | 21 | (20.2) | |
| Negative | 6 | (24) | 83 | (79.8) | <0.0001[b] |
| HPV and TA | | | | | |
| Concurrent | 17 | (68) | 8 | (7.7) | |
| No concurrent | 8 | (32) | 96 | (92.3) | <0.0001[b] |

[a]P-value was calculated from Fisher's exact test. [b]P-value was calculated from Pearson's chi-square tests.

single HPV infections, type 58 was found in one sample and HPV 16, 31, 33, 51 and 52 were found in two samples each. In the 6 cases with multiple HPV infections, different combinations were found (HPV 16 and 26, 31 and 33, 58 and 81 in one sample each; and the combination of HPV 31, 33 and 58 was detected in three different samples). In the eight controls that were positive for HPV and TA simultaneously, high and low risk HPV types were detected in single and multiple infections. In the five controls with single HPV infections, HPV 16, 58, 59, 81 and 84 were found in one sample each. In the three controls with multiple HPV infections, different combinations were found (HPV 39 and 42; HPV 43 and 68; and HPV 35, 52, 59, 40 and 84, in one sample each). There was no statistically significant interaction between TA and hrHPV infection (P=0.76).

*Risk factors for HGSIL*. Significantly increased odds for HGSIL were observed in women positive for hrHPV (OR=26.10, 95% CI 9.10-74.86) or TA (OR=12.51, 95% CI 4.44-35.24), or with multiple parities (OR=3.40, 95% CI 0.92-23.40). These associations decreased, but remained

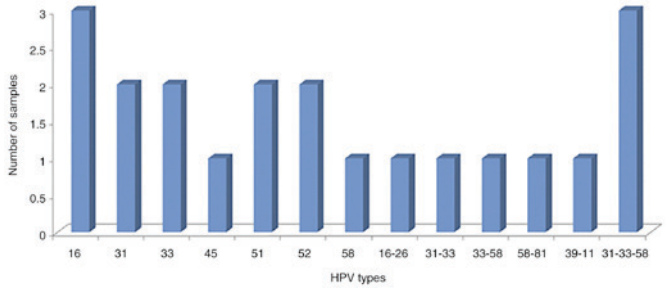


Figure 1. HPV genotypes detected in cases (n=25). Alpha 9 species contains the HPV types 16, 31, 33, 35, 52, 58 and 67. Alpha 7 species contains the HPV types 18, 39, 45, 59, 68 and 70. HPV, human papillomavirus.

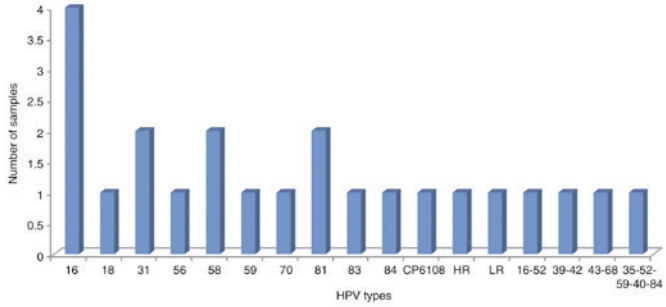


Figure 2. HPV genotypes detected in controls (n=104). Alpha 9 species contains the HPV types 16, 31, 33, 35, 52, 58 and 67. Alpha 7 species contains the HPV types 18, 39, 45, 59, 68 and 70. Alpha 3 species contains the HPV types 61, 72, 81, 83, 84. HR, unidentified high risk HPV genotype(s); LR, unidentified low risk HPV genotype(s); HPV, human papillomavirus.

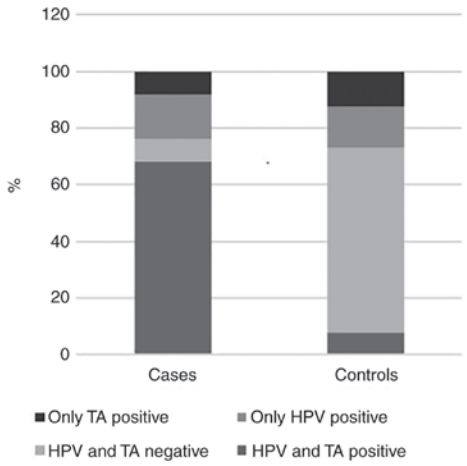


Figure 3. Detection of HPV and Telomerase activity in cases and controls. TA, telomerase activity; HPV, human papillomavirus.

significant, when adjusting for relevant cofactors; (OR=18.26, 95% CI 4.43-76.92), (OR=5.52, 95% CI 1.43-21.30), and (OR=3.50, 95% CI 1.03-10.30), respectively (Table II). When considering only women with hrHPV infection, the association between TA and HGSIL was also significant (OR=7.7, 95% CI 1.63-35.79). When adjusting for age and other cofactors, this association remained significant (OR=37.94, 95% CI 1.64-678.1). No other risk factors were clearly associated with the risk of HGSIL (Table III).

## Discussion

To the best of our knowledge, no epidemiological studies have been performed so far analysing association of TA with HPV in cervical scrapes and its role as a possible risk factor for HGSIL adjusted for different known risk factors. Our results show that TA is a risk factor for high grade cervical lesions. Furthermore, TA was associated with increased odds of high grade cervical lesions in hrHPV infected women. We propose that TA can be added to the list of HPV cofactors previously described (26). Moreover, in addition to the mentioned cofactors, TA can be measured objectively, as can HPV infection.

TA was detected in 76% of the cases studied, and in 21.1% of the controls. These results are in agreement with previous studies which have detected TA in cancer and precancerous lesions while showing undetectable or low TA levels in normal tissue (12,13). Similar results were observed for the detection of HPV DNA. As expected, HPV DNA was detected in 84% of the cases and 22.1% of the controls. Multiple studies have shown a high prevalence of HPV (particularly with high risk genotypes) in women with HGSIL and a low prevalence in women without lesions (27-29).

When both TA and HPV status were considered, we found a higher percentage of TA and HPV in cases (68%) than in controls (7.7%). These results are in agreement with previous studies on cervical biopsies, in which TA and HPV was detected in a higher percentage of cases with CINII or III than in normal cervical specimens (7,15).

Our results indicate increased odds of HGSIL in women positive for hrHPV, in women positive for TA and in women with more than 2 parities. The epidemiological association of HPV infection and parity with HGSIL and cervical cancer have been widely reported (30-33). However epidemiological information on TA with HGSIL is scanty. A systematic review analyzing the accuracy of telomerase assay in cervical lesions showed a positive association between a positive TA test and high grade cervical lesions with a diagnostic odds ratio (DOR) of 5.8, which is similar to our results (34). However in that review the authors did not take into account other important risk factors, such as HPV infection, of pivotal importance for the development of the disease. When only women with hrHPV infection were included in our analysis, TA showed a positive association with HGSIL in both crude and adjusted models. It was observed that the odds ratio increases when TA detection is adjusted to additional cofactors, although it is important to note that the CI is very wide.

Research into the biological relationship between HPV infection and TA are ongoing. Some studies have shown a relationship between the activation of the enzyme and HPV infection, especially HPV 16 and 18 (14,15). Telomerase activation by HPV has been principally attributed to hrHPV E6 oncoprotein because this protein can act as a transcription factor at the hTERT promoter and upregulate its expression (16,19,35,36). The E7 protein and the pRB pathway have been also found to be involved in the maintenance and induction of TA (37). The fact that amongst cases that were positive for TA, different HPV types of the alpha 9 species were detected, i.e. HPV-16, 31, 33, 52, and 58, could suggests that other hrHPV types can activate telomerase either directly or indirectly. Recently one *in vitro* study published

Table II. Crude and adjusted OR and 95% CI of telomerase activity and relevant risk factors.

| | | | Crude | | Adjusted | |
|---|---|---|---|---|---|---|
| Risk Factors | Cases n=25 | Controls n=104 | OR | (95% CI) | OR[a] | (95% CI) |
| High Risk HPV infection | | | | | | |
| No | 4 | 90 | 1 | | | |
| Yes | 21 | 14 | 26.1 | (9.10-74.8) | 18.2 | (4.33–76.9) |
| Telomerase Activity | | | | | | |
| No | 6 | 83 | 1 | | | |
| Yes | 19 | 21 | 12.5 | (4.44-35.2) | 5.5 | (1.43–21.3) |
| Age (years): | | | | | | |
| <30 | 9 | 30 | 1 | | | |
| ≥30 | 16 | 74 | 1.8 | (0.70-4.7) | 1.2 | (0.46–3.5) |
| Parity: | | | | | | |
| 0-1 | 3 | 35 | 1 | | | |
| >2 | 21 | 69 | 3.4 | (0.92-23.4) | 3.5 | (1.03–10.3) |
| Oral Contraceptives: | | | | | | |
| Never | 11 | 56 | 1 | | | |
| Ever | 14 | 48 | 1.5 | (0.69-3.5) | 1.4 | (0.61–3.5) |
| Smoking status | | | | | | |
| Never | 18 | 72 | 1 | | | |
| Ever | 7 | 32 | 0.6 | (0.27-1.6) | 0.8 | (0.33–2.3) |

OR, Odds ratio crude; OR[a], Odd ratio adjusted for age and all cofactors; CI, confidence interval.

Table III. Association of telomerase and main cofactors with HGSIL, in presence HR HPV infection.

| | | | Crude | | Adjusted | |
|---|---|---|---|---|---|---|
| HPV HR Cofactors | Controls n=14 | Cases n=21 | OR | 95% CI | OR[a] | 95% CI |
| Viral load (tertiles) | | | | | | |
| I | 4 | 1 | 1 | | 1 | |
| II | 2 | 2 | 4.0 | (0.56-28.3) | 0.4 | (0.01-20.3) |
| III | 8 | 18 | 4.8 | (0.84-27.2) | 7.0 | (0.52-95.7) |
| Telomerase activity | | | | | | |
| No | 9 | 4 | 1 | | 1 | |
| Yes | 5 | 17 | 7.7 | (1.63-35.7) | 37.9 | (1.64-678.1) |
| Parity: | | | | | | |
| 0-1 | 8 | 3 | 1 | | 1 | |
| >2 | 8 | 19 | 5.0 | (1.17-14.6) | 2.3 | (0.12-50.3) |
| >3 | 2 | 2 | 3.5 | (0.37-32.9) | 2.4 | (0.11-53.0) |
| Oral contraceptives: | | | | | | |
| Never | 8 | 8 | 1 | | 1 | |
| Ever | 6 | 13 | 2.2 | (0.54-8.5) | 10.1 | (0.74-137.5) |
| Smoking status | | | | | | |
| Never | 9 | 16 | 1 | | 1 | |
| Ever | 5 | 5 | 0.8 | (0.16-3.6) | 0.2 | (0.02-2.6) |

HR HPV, High risk human Papillomavirus; HGSIL, high-grade squamous intraepithelial lesion; OR, crude odds ratio; OR[a], Odds ratio adjusted for age and all cofactors; CI, confidence interval.

by Schutze *et al*, 2014 showed that cell lines generated using various hrHPV types were able to activate telomerase after a period in culture (38). However more functional assays are needed to establish the mechanism used by these genotypes.

In the present study, we observed samples with HPV infection without TA and samples with TA without HPV infection, and analysis between TA and hrHPV show no statistically significant interaction. This suggests that there are other factors aside from HPV infection that can activate the enzyme. Factors including HPV integration state can influence TA (39). When the viral genome is maintained as an episome, the E2 protein is expressed and can inhibit expression of hTERT. In contrast, when HPV is integrated into the host genome, E6 and E7 expression increases producing the expression of hTERT. Epigenetic regulation of the hTERT gene could also be important to evaluate, as some researchers have shown that methylation play a key role in the regulation of hTERT, although contradictory results have been reported (40-43). Recently we have published an association between type specific HPV infections and hTERT methylation in patients with cervical cancer but additional studies in patients with HGSIL have to be done (44). In addition, lifestyle aspects including diet, exposure to stress, and level of physical activity can also produce changes in TA (45-47). Unfortunately, we did not assess these factors in this study to have a more complete picture of the different mechanisms involved in TA in cases and controls. However our results show clearly that TA is a risk factor for HGSIL, with the strongest association in women who are hrHPV positive.

Our study suggests that detection of both HPV infection and TA at the same time could have a higher predictive value for HGSIL than either HPV detection or TA detection independently. The next step of this study is to evaluate TA in a larger cohort as a diagnostic test in the triage of HPV positive women.

Limitations of this study include low sample size resulting in very wide confidence intervals when adjusting for relevant cofactors.

In summary, this is the first epidemiological study that shows an independent association of TA with HPV as a risk factor for HGSIL when adjusted for different risk factors. TA could be used as an adjunct tool, in addition to HPV DNA detection, for identifying patients with higher risks of progression to cervical neoplasia.

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## References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893-2917, 2010.
2. Pardo C and Cendales R: Incidencia estimada y mortalidad por cancer en Colombia, 2002-2006. Instituto Nacional de Cancerología, Legis S.A, 2010.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ and Muñoz N: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189: 12-19, 1999.
4. Muñoz N: Human papillomavirus and cancer: The epidemiological evidence. J Clin Virol 19: 1-5, 2000.
5. Blackburn EH: Structure and function of telomeres. Nature 350: 569-573, 1991.
6. Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ and Reddel RR: Protein composition of catalytically active human telomerase from immortal cells. Science 315: 1850-1853, 2007.
7. Snijders PJ, van Duin M, Walboomers JM, Steenbergen RD, Risse EK, Helmerhorst TJ, Verheijen RH and Meijer CJ: Telomerase activity exclusively in cervical carcinomas and a subset of cervical intraepithelial neoplasia grade III lesions: Strong association with elevated messenger RNA levels of its catalytic subunit and high-risk human papillomavirus DNA. Cancer Res 58: 3812-3818, 1998.
8. Shay JW and Wright WE: Senescence and immortalization: Role of telomeres and telomerase. Carcinogenesis 26: 867-874, 2005.
9. Holt SE and Shay JW: Role of telomerase in cellular proliferation and cancer. J Cell Physiol 180: 10-18, 1999.
10. Ault KA, Allen HK, Phillips SL, Zimmerman MB and Klingelhutz AJ: Telomerase activity as a potential diagnostic marker for triage of abnormal Pap smears. J Low Genit Tract Dis 9: 93-99, 2005.
11. Sharma A, Rajappa M, Saxena A and Sharma M: Telomerase activity as a tumor marker in Indian women with cervical intraepithelial neoplasia and cervical cancer. Mol Diagn Ther 11: 193-201, 2007.
12. Liu H, Liu S, Wang H, Xie X, Chen X, Zhang X and Zhang Y: Genomic amplification of the human telomerase gene (hTERC) associated with human papillomavirus is related to the progression of uterine cervical dysplasia to invasive cancer. Diagn Pathol 7: 147, 2012.
13. Castro-Duque AF, Loango-Chamorro N, Ruiz-Hoyos BM and Landázuri P: Telomerase activity associated with progression of cervical lesions in a group of Colombian patients. Rev Bras Ginecol Obstet 37: 559-564, 2015.
14. Pinto-Tang J, Castro T and Premoli G: Detection of telomerase activity in cervical lesions by non-radioactive telomeric repeat amplification protocol (TRAP). Invest Clin 46: 255-263, 2005 (In Spanish).
15. Kailash U, Soundararajan CC, Lakshmy R, Arora R, Vivekanandhan S and Das BC: Telomerase activity as an adjunct to high-risk human papillomavirus types 16 and 18 and cytology screening in cervical cancer. Br J Cancer 95: 1250-1257, 2006.
16. Veldman T, Liu X, Yuan H and Schlegel R: Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. Proc Natl Acad Sci USA 100: 8211-8216, 2003.
17. Jeong Seo E, Jung Kim H, Jae Lee C, Tae Kang H and Seong Hwang E: The role of HPV oncoproteins and cellular factors in maintenance of hTERT expression in cervical carcinoma cells. Gynecol Oncol 94: 40-47, 2004.
18. Liu X, Yuan H, Fu B, Disbrow GL, Apolinario T, Tomaic V, Kelley ML, Baker CC, Huibregtse J and Schlegel R: The E6AP ubiquitin ligase is required for transactivation of the hTERT promoter by the human papillomavirus E6 oncoprotein. J Biol Chem 280: 10807-10816, 2005.
19. Katzenellenbogen RA, Vliet-Gregg P, Xu M and Galloway DA: NFX1-123 increases hTERT expression and telomerase activity posttranscriptionally in human papillomavirus type 16 E6 keratinocytes. J Virol 83: 6446-6456, 2009.
20. Zhou Y, Xu F, Tao F, Feng D, Ling B, Qian L, Yang X, Wang Q, Wang H, Zhao W, *et al*: GRIM-19 restores cervical cancer cell senescence by repressing hTERT transcription. J Interferon Cytokine Res 36: 506-515, 2016.
21. Koskimaa HM, Kurvinen K, Costa S, Syrjänen K and Syrjänen S: Molecular markers implicating early malignant events in cervical carcinogenesis. Cancer Epidemiol Biomarkers Prev 19: 2003-2012, 2010.

22. Li T, Tang L, Bian D, Jia Y, Huang X and Zhang X: Detection of hTERC and c-MYC genes in cervical epithelial exfoliated cells for cervical cancer screening. Int J Mol Med 33: 1289-1297, 2014.
23. Zhao XY, Cui Y, Jiang SF, Liu KJ, Han HQ, Liu XS and Li Y: Human telomerase gene and high-risk human papillomavirus infection are related to cervical intraepithelial neoplasia. Asian Pac J Cancer Prev 16: 693-697, 2015.
24. Molano M, Posso H, Weiderpass E, van den Brule AJ, Ronderos M, Franceschi S, Meijer CJ, Arslan A and Munoz N; HPV Study Group HPV Study: Prevalence and determinants of HPV infection among Colombian women with normal cytology. Br J Cancer 87: 324-333, 2002.
25. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ and Snijders PJ: GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 40: 779-787, 2002.
26. Castellsagué X and Muñoz N: Chapter 3: Cofactors in human papillomavirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr: 20-28, 2003.
27. Hamlin-Douglas LK, Coutlée F, Roger M, Franco EL and Brassard P: Prevalence and age distribution of human papillomavirus infection in a population of Inuit women in Nunavik, Quebec. Cancer Epidemiol Biomarkers Prev 17: 3141-3149, 2008.
28. Berois N, Heard I, Fort Z, Alonso R, Sica A, Moerzinger P, Rodriguez G, Sancho-Garnier H, Osinaga E and Favre M: Prevalence of type-specific HPV infection in Uruguay. J Med Virol 86: 647-652, 2014.
29. Ogembo RK, Gona PN, Seymour AJ, Park HS, Bain PA, Maranda L and Ogembo JG: Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: A systematic review and meta-analysis. PLoS One 10: e0122488, 2015.
30. Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, Shah KV, Meijer CJ and Bosch FX; International Agency for Research on Cancer. Multicentric Cervical Cancer Study Group: Role of parity and human papillomavirus in cervical cancer: The IARC multicentric case-control study. Lancet 359: 1093-1101, 2002.
31. Almonte M, Albero G, Molano M, Carcamo C, García PJ and Pérez G: Risk factors for human papillomavirus exposure and co-factors for cervical cancer in Latin America and the Caribbean. Vaccine 26 (Suppl 11): L16-L36, 2008.
32. Muñoz N, Hernandez-Suarez G, Méndez F, Molano M, Posso H, Moreno V, Murillo R, Ronderos M, Meijer C and Muñoz A; Instituto Nacional de Cancerología HPV Study Group: Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer 100: 1184-1190, 2009.
33. Roura E, Travier N, Waterboer T, de Sanjosé S, Bosch FX, Pawlita M, Pala V, Weiderpass E, Margall N, Dillner J, *et al*: Correction: The influence of hormonal factors on the risk of developing cervical cancer and pre-cancer: Results from the EPIC cohort. PLoS One 11: e0151427, 2016.
34. Rosa MI, Medeiros LR, Bozzetti MC, Fachel J, Wendland E, Zanini RR, Moraes AB and Rosa DD: Accuracy of telomerase in cervical lesions: A systematic review. Int J Gynecol Cancer 17: 1205-1214, 2007.
35. Klingelhutz AJ, Foster SA and McDougall JK: Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature 380: 79-82, 1996.
36. Veldman T, Horikawa I, Barrett JC and Schlegel R: Transcriptional activation of the telomerase hTERT gene by human papillomavirus type 16 E6 oncoprotein. J Virol 75: 4467-4472, 2001.
37. Liu X, Roberts J, Dakic A, Zhang Y and Schlegel R: HPV E7 contributes to the telomerase activity of immortalized and tumorigenic cells and augments E6-induced hTERT promoter function. Virology 375: 611-623, 2008.
38. Schütze DM, Kooter JM, Wilting SM, Meijer CJ, Quint W, Snijders PJ and Steenbergen RD: Longitudinal assessment of DNA methylation changes during HPVE6E7-induced immortalization of primary keratinocytes. Epigenetics 10: 73-81, 2015.
39. Lee D, Kim HZ, Jeong KW, Shim YS, Horikawa I, Barrett JC and Choe J: Human papillomavirus E2 down-regulates the human telomerase reverse transcriptase promoter. J Biol Chem 277: 27748-27756, 2002.
40. Kyo S, Takakura M, Fujiwara T and Inoue M: Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. Cancer Sci 99: 1528-1538, 2008.
41. de Wilde J, Kooter JM, Overmeer RM, Claassen-Kramer D, Meijer CJ, Snijders PJ and Steenbergen RD: hTERT promoter activity and CpG methylation in HPV-induced carcinogenesis. BMC Cancer 10: 271, 2010.
42. Jiang J, Zhao LJ, Zhao C, Zhang G, Zhao Y, Li JR, Li XP and Wei LH: Hypomethylated CpG around the transcription start site enables TERT expression and HPV16 E6 regulates TERT methylation in cervical cancer cells. Gynecol Oncol 124: 534-541, 2012.
43. Daniel M, Peek GW and Tollefsbol TO: Regulation of the human catalytic subunit of telomerase (hTERT). Gene 498: 135-146, 2012.
44. Molano M, Moreno-Acosta P, Morales N, Burgos M, Buitrago L, Gamboa O, Alvarez R, Garland SM, Tabrizi SN, Steenbergen RD and Mejía JC: Association between type-specific HPV infections and hTERT DNA methylation in patients with invasive cervical cancer. Cancer Genomics Proteomics 13: 483-491, 2016.
45. Ludlow AT, Zimmerman JB, Witkowski S, Hearn JW, Hatfield BD and Roth SM: Relationship between physical activity level, telomere length, and telomerase activity. Med Sci Sports Exerc 40: 1764-1771, 2008.
46. Zhu H, Guo D, Li K, Pedersen-White J, Stallmann-Jorgensen IS, Huang Y, Parikh S, Liu K and Dong Y: Increased telomerase activity and vitamin D supplementation in overweight African Americans. Int J Obes (Lond) 36: 805-809, 2012.
47. Deng W, Cheung ST, Tsao SW, Wang XM and Tiwari AF: Telomerase activity and its association with psychological stress, mental disorders, lifestyle factors and interventions: A systematic review. Psychoneuroendocrinology 64: 150-163, 2016.