Evaluation of HPV E6/E7 mRNA Detection in Clinically Suspected Cases of Cervical Cancer with Abnormal Cytology: Time to Upgrade the Screening Protocols

Bhawna Sharma[1]   Vikas Lakhanpal[2]   Kanwardeep Singh[1]   Loveena Oberoi[1]   Preet Kamal Bedi[3]
Pushpa Devi[1]

[1] Department of Microbiology, Government Medical College, Amritsar, Punjab, India
[2] Department of Internal Medicine, Government Medical College, Amritsar, Punjab, India
[3] Department of Obstetrics and Gynaecology, Government Medical College, Amritsar, Punjab, India

Address for correspondence Kanwardeep Singh, MD, Department of Microbiology, Government Medical College, Amritsar, Punjab, 143001, India (e-mail: kdmicrogmcasr@gmail.com).

Abstract

Background   Human papillomavirus (HPV) E6/E7 mRNA tests determine the onco-genic activity of the virus and represent a good clinical biomarker for predicting the risk of cervical cancer. So, the present study was conducted to know the role of HPV E6/E7 mRNA as a predictive biomarker for cervical carcinoma.

Methodology   The present study was conducted on 55 clinical samples of cervical scrapings and biopsy from the clinically suspected cases (based on signs and symptoms) of cervical cancer having abnormal PAP smear. The samples were processed in three steps—(1) HPV DNA detection, (2) HPV E6/E7 mRNA detection, and (3) histopathological analysis.

Results   Out of a total of 55 patients, 16 (29.09%) were positive for both HPV E6/E7 mRNA and HPV DNA and six were positive for only HPV DNA. So, a total of 22 (40%) patients were positive for HPV DNA. Out of these 22 samples, 10 (45.5%) were of HPV-16, six (27.3%) were of HPV-18, four (18.2%) were of HPV-31, and two (9.1%) were of HPV-45. Out of total 16 patients positive for HPV E6/E7 mRNA, 10 (62.5%) were of genotype 16 and six (37.5%) were of genotype 18. The patients who were found positive for HPV 31 and 45 genotypes did not have E6/E7 mRNA expression. On colposcopic-guided biopsy, among these 16 samples, eight (50%) were diagnosed with invasive squamous cell carcinoma, six (37.5%) with cervical intraepithelial neoplasia grade 3 (CIN3), and two (12.5%) with CIN2. Out of those six patients in whom only HPV DNA was positive, five had normal biopsy findings and one had CIN1.

Conclusion   The present study suggests that HPV E6/E7 mRNA detection could be more reliable than DNA testing for predicting the risk of progression of HPV-induced cervical lesions to cervical carcinoma and it can be used as a non-invasive tool for triage and patient follow-up.

Keywords
► human papillomavirus
► E6/E7 mRNA
► cervical cancer

Introduction

Invasive cervical cancer is the second most common cancer in women worldwide and the most common cause of cancer-related mortality among women in India.[1] The etiology and pathogenesis of cervical cancer have been clearly linked to persistent infection with human papillomavirus (HPV).[2] Approximately, 30 distinct HPV types preferentially infect the anogenital mucosa, resulting in lesions that range from condyloma to cervical intraepithelial neoplasia (CIN)/squamous intraepithelial lesion (SIL) and invasive carcinoma.[3] The most common oncogenic HPV type in preinvasive and invasive cervical cancer is HPV 16 and is detectable in more than 50% of women with CIN3/cervical cancer followed by HPV 18 which accounts for 10 to 15% of cervical cancers.[4] HPV DNA detection is one of the most important screening triage available for the detection of cervical cancer nowadays along with PAP smear. There are many clinical applications of HPV DNA testing like triage of females with atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL) findings on PAP smear; as a primary screening test; as a prognostic tool for a therapeutic outcome after the treatment of an intraepithelial lesion; and for determining the prevalence of certain genotypes of HPV at regional, national, or global levels.[5] However, the major limitation of this modality is inability to differentiate between progressive and regressive lesions as the former can lead to carcinogenesis in the future. HPV E6/E7 mRNA messenger ribonucleic acid tests determine the oncogenic activity of the virus and represent a good clinical biomarker for predicting the risk of developing cervical cancer.[6] On the molecular level, the conversion of an acute HPV infection into a transforming HPV infection is characterized by the substantially increased expression of viral oncogenes E6/E7 in the basal cells that retain the capacity to replicate.[7] The deregulated expression of these oncogenes induces chromosomal instability that is the predominant hallmark of cervical carcinogenesis.[8] So, E6/E7 mRNA expression is a powerful indicator for the progression of cervical carcinoma. The major advantages of HPV E6/E7 mRNA test over HPV DNA test are is a more specific and non-invasive test, has a greater positive predictive value (greater prognostic significance),[9] correlates better with cytology and histology,[10] and directly detects the expression level of oncogenic HPV E6 and E7 (mRNA).[11] However, currently available HPV DNA test generally targets the conserved L1 region and it cannot discriminate between regressive and progressive HPV infections.[12] However, since most women have self-resolving HPV infections that will not develop into cancer, a positive HPV test result provides little actionable information and causes a psychological burden on the patient and also may cause over referral to colposcopy. So, in the given study, we tried to compare both HPV DNA and HPV E6/E7 mRNA with histopathological findings in females with clinical suspicion (based on signs and symptoms) of cervical carcinoma.

Materials and Methods

The given study was conducted from 1st January 2016 to 30th June 2017. The study comprised of 55 samples of cervical scrapings from the suspected cases of carcinoma cervix, attending obstetrics and gynecology outpatient department /inpatient department of the institute. The detailed history in relation to demographic features, symptoms such as abnormal vaginal discharge, post-coital bleeding, post-menopausal bleeding, menstrual and obstetric history, personal history, contraception used, and any significant past medical/surgical history was enquired as a routine protocol and all these details were entered in a pretested proforma. Written informed consent was obtained from all subjects. Ethical clearance was obtained from the institute's ethical committee with reference number IEC/GMC/Th 00134. Female patients with age more than 18 years who were sexually active with clinical signs and symptoms of carcinoma cervix along with abnormal PAP smear were included in the study. Pregnant female patients and females having a prior history of cervical carcinoma were excluded.

Collection and Transportation of Samples

The specimen was collected by introducing a bivalve speculum into the vagina for a complete visualization of the external os and ectocervix. The samples were collected in the specimen collection tube containing phosphate-buffered saline. The specimen was sent to the microbiology laboratory for further processing. The samples were processed on the same day, and in case of delay, samples were stored at −80°C. A colposcopic-guided biopsy sample was taken for histopathological analysis.

Processing of Samples

The samples were proceeded in two—(1) HPV DNA detection and (2) HPV E6/E7 mRNA detection.

- HPV DNA detection:
  - *DNA extraction*
  Each sample was processed for DNA extraction according to the instruction manual given along the QIAamp DNA by Qiagen (Hilden, Germany).
  - *DNA amplification*
  The sequence of primers is given in ►**Table 1**. Details are provided in **Supplementary Material**.
  Polymerase chain reaction (PCR) products, thus, obtained were subjected to gel electrophoresis for HPV detection.

- HPV E6/E7 mRNA detection
  - *RNA extraction by a spin column technique*
  Each sample was processed according to the instructions manual given along the RNeasy mini kit by Qiagen.
  - *Reverse transcription and amplification*
  Both reverse transcription and amplification of cDNA occurred in a single step by Qiagen one-step reverse

Table 1 Sequence of primer GP5+ and GP6+

HPV genotype	Amplicon (bp)	Sequence (5′-3′)
GP5+	140	FP- TTT GTT ACT GTG GTA GAT AC
GP6+	140	RP- GAA AAA TAA ACT GTA AAT CA

Abbreviation: HPV, human papillomavirus.

Table 2 Primers designed for HPV 16, 18, 31, and 45 E6/E7 mRNA detection

HPV	Region	Size	Sequence
16	E6	±659 pb	5′ TAAAACTAAGGGCGTAACCG 3′ 5′ TCTATTTCATCCTCCTCCTCTG 3′
16	E7	±491 pb	5′ ACTGTGTCCTGAAGAAAAGCAA 3′ 5′ AACCATCCATTACATCCCGT 3′
18	E6	±608 pb	5′ TAGGTTGGGCAGCACATACT 3′ 5′ ATACTTGTGTTTCTCTGCGTCG 3′
18	E7	±358 pb	5′ CGACGCAGAGAAACACAAGTAT 3′ 5′ ATTGTTGCTTACTGCTGGGAT 3′
31	E6	±606 pb	5′ AAGTAGGGAGTGACCGAAAGT 3′ 5′ ACAGTGGAGGTCAGTTGCC 3′
31	E7	±437 pb	5′ AGGCACGGCAAGAAAGACT 3′ 5′ AAAGAACCAGCCATTACACC 3′
45	E6	±659 pb	5′ ATACTACATAAAAAAGGGTG 3′ 5′ TCGTAACACAACAGGTCAACA 3′
45	E7	±439 pb	5′ AGGCACGGCAAGAAAGACT 3′ 5′ AAAGAACCAGCCATTACACC 3′

Abbreviation: HPV, human papillomavirus.

transcription (RT)-PCR Kit 210212. Details are given in **Supplementary Material**.

- Type-specific PCR for HPV 16, 18, 31, and 45 oncogenes E6 and E7 mRNA

Next PCR reactions were performed to ascertain the presence of E6/E7 mRNA of the following HPV types: 16, 18, 31, and/or 45. The reaction was performed separately for each HPV genotype using specifically designed primers (►**Table 2**). For details of cycling condition, refer **Supplementary Material**.

- Analysis of amplified products

Amplified products were subjected to gel electrophoresis and analyzed on a gel documentation system.

Cytopathological Analysis

All samples for cytology were taken using the Thin Prep PAP smear cytology and were analyzed in the pathology laboratory of the institute. Cytological results were classified according to the revised Bethesda classification,[13,14] such as ASC-US; ASC, cannot exclude a high-grade squamous intraepithelial lesion (HSIL); LSIL (CIN grade 1[CIN1]); HSIL (CIN2; CIN3; carcinoma in situ) and invasive squamous cell carcinoma.

Histopathological Analysis

Colposcopic-guided biopsy samples for histopathological analysis were taken and were sent to the histopathological department of the institute, and diagnoses were made according to the CIN reporting system.[15]

Statistical Analysis

Different parameters like age and parity were presented as mean ± standard deviation (SD). A *p*-value <0.05 was considered statistically significant. Fisher's exact test was used when the number of observations in a cell was less than 5, and to compare between the continuous variables like age at presentation and marital age, independent *t*-test was used. In others, the chi-square test was used to study the associations between various risk factors and HPV E6/E7 mRNA expression. The minimum sample size was calculated as 45 using a formula: $n = Z^2P(1 - P)/d^2$, where *n* is the sample size, *Z* is the statistic corresponding to the level of confidence (taken as 95%), *P* is expected prevalence, and *d* is precision after taking the prevalence of cervical cancer as 3% in Punjab region.[16] Data were analyzed using Microsoft Excel version 16 (Microsoft Corp., Richmond, CA, United States), and statistical analysis was performed using GraphPad Prism V.6.0 (GraphPad Software, La Jolla, CA, United States). Diagnostic performance in terms of sensitivity, specificity, positive, and negative predictive values (confidence interval was taken as 95%) were calculated using MedCalc version 12.1.4 (MedCalc Software bvba, Mariakerke, Belgium).

Results

- Demographic details and risk factors
  The mean age of the females in the given study was 34 ± 10 years. Mean parity in the patients was 2.1 ± 0.7. The mean age of the patients at the time of marriage was 25 ± 8 years. The majority (96.36%) of the patients had a single sexual partner, and only 3.64% gave the history of multiple sexual partners. Out of total 55 patients, partners of majority patients, i.e., 26 (54.17%) were using male condoms. Oral contraceptive pills (OCPs) were used by 16 (33.33%) patients, two (4.17%) patients were using a copper intrauterine device, one (2.08%) practiced coitus interruptus, and three (6.25%) patients gave a history of tubectomy done in the past. Seven patients had no history of use of contraception since they were in the postmenopausal state.

- Clinical signs and symptoms
  Out of the 55 patients, majority (27) presented with foul-smelling discharge per vaginum. The next common complaint was pain in the lower abdomen (24) followed by irregular bleeding per vaginum (18) and post-coital bleeding (12). Only eight patients presented with post-menopausal bleeding. The majority of the patients had multiple complaints.
  On examination, the majority of them (34) had cervical erosions followed by hypertrophied cervix with discharge in 20 patients. Only 10 patients had polypoidal growth. Multiple findings were observed in some of the patients

- Cytopathological (PAP smear) findings
  All the patients had abnormal PAP smear findings. Out of 55 patients, 36 patients had ASC-US on their cytology, one had LSIL, eight had HSIL, eight had invasive squamous cell carcinoma, and only two patients had atypical glandular cells of undetermined significance.

- HPV DNA detection and its genotype
  Out of the total, 22(40%) were positive and 33(60%) were negative for HPV DNA. Out of these 22 patients, 10 (45.5%)

Table 3 Comparison between HPV E6/E7 mRNA and HPV DNA vis-à-vis the histopathological and cytological finding

	HPV DNA (*n*)		HPV E6/E7 mRNA (*n*)	
	Positive (22)	Negative (33)	Positive (16)	Negative (39)
Cytology (n)				
ASC-US (36)	4	32	0	36
AGUS (2)	1	1	0	2
LSIL (1)	1	0	0	1
HSIL (8)	8	0	8	0
Invasive squamous cell carcinoma (8)	8	0	8	0
Histopathology (n)				
Normal (38)	5	33	0	38
CIN 1 (1)	1	0	0	1
CIN 2 (2)	2	0	2	0
CIN 3 (6)	6	0	6	0
Invasive squamous cell carcinoma (8)	8	0	8	0

Abbreviations: AGUS; atypical glandular cells of undetermined significance; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesions; *n*, number of the patients.

were of HPV-16, six (27.3%) were of HPV-18, four (18.2%) were of HPV-31, and two (9.1%) were of HPV-45.

- HPV E6/E7 mRNA expression and its specific genotype
  Out of total 55 patients, 16 (29.09%) were positive and 39 (70.91%) were negative for HPV E6/E7 mRNA. Out of total 16 patients positive for HPV E6/E7 mRNA, 10 (62.5%) were of genotype 16 and 6 (37.5%) were of genotype 18. The patients who were found positive for HPV 31 and 45 genotypes did not have E6/E7 mRNA expression. So, in total, 16 patients were positive for both HPV-DNA and HPV E6/E7 mRNA, whereas six were positive for only HPV DNA (►**Table 3**).

- Histopathological findings
  On histopathological analysis, 38 had normal findings, eight had invasive squamous cell carcinoma, six had CIN3, two had CIN2, and one had CIN1. Out of all HPV DNA-positive cases (22), only 16 were positive for HPV E6/E7mRNA. Among those 16, eight (50%) were diagnosed with invasive squamous cell carcinoma, six (37.5%) with CIN3, two (12.5%) with CIN2. Whereas out of those six patients in whom only HPV DNA was positive, five had normal biopsy findings and one had CIN1. Sensitivity, specificity, positive predictive value, and negative predictive value of HPV DNA and HPV E6/E7 mRNA against gold-standard histopathology method were calculated (►**Table 4**).

- Association between various risk factors and HPV E6/E7mRNA expression
  The positivity rate of HPV E6/E7 mRNA between different age groups was compared along with various other risk factors (►**Table 5**).

Discussion

The given study highlights the role of detection of E6/E7 mRNA of HPV for predicting the risk of progression of HPV-induced cervical lesions to cervical carcinoma as all the patients in the given study showing expression of HPV E6/E7 mRNA had features of cervical cancer on histpthology. It shows that the detection of HPV E6/E7 mRNA is more specific than HPV DNA detection.

In our study, HPV infection was detected in 40% of patients which is quite less than the previous studies.[17] The difference may be attributed to the smaller sample size taken in our study. According to the International Agency for Research on Cancer analysis, the five most common HPV genotypes with decreasing frequency are, HPV-16, -18, -45, -31, and -33, found in 82.9% of the cases.[18] Similarly, according to one study,[17] the most prevalent genotype was HPV 16 (87.28%) followed by HPV 18 (24.56%) and HPV 51(3.46%). Similarly, our study depicted that among HPV DNA-positive patients, HPV 16(45.45%) was the most prevalent type followed by

Table 4 Sensitivity, specificity, positive predictive value, and negative predictive value of HPV DNA and HPV E6/E7 mRNA against histopathology

	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
HPV DNA	100 (80.5–100)	86.84(71.9–95.6)	19.03((9.4–34.7)	100
HPV E6/E7 mRNA	94.12 (71.3–99.8)	100 (90.7–100)	100	99.82 (98.8–99.8)

Abbreviations: CI, confidence interval; HPV, human papillomavirus.

Table 5 Association of various risk factors with HPV E6/E7 mRNA positivity

Demographic/Risk factors (total number)	HPV E6/E7 mRNA positive (16)	HPV E6/E7 mRNA negative (39)	p-Value
Age at the time of presentation, in years (n)			
18–30 (23)	9	14	0.0023
31–40 (22)	1	21	
>40 (10)	6	4	
Age at the time of marriage, in years (n)			
18–25 (30)	13	17	< 0.05
26–30 (20)	3	17	
31–35 (5)	0	5	
Parity (n)			
Para 1 (10)	1	9	0.0046
Para 2 (28)	5	10	
Para 3 (17)	10	7	
Number of sexual partners (n)			
Single (53)	14	39	0.0245
Multiple (2)	2	0	
Contraceptives used (n)			
Male condom (26)	1	25	0.0001
OCPs (16)	12	4	
Tubectomy (3)	1	2	
Cu-T (2)	1	1	
Coitus interruptus (1)	1	0	

Abbreviations: Cu-T, copper intrauterine device; HPV, human papillomavirus; *n*, number of the patients; OCPs, oral contraceptive pills.
Note: *p* = 0.0001, statistically significant.

HPV 18(27.27%), HPV 31(18.18%), and HPV 45(9.10%). The present study showed that the positivity of HPV E6/E7 mRNA in women with abnormal PAP smear was 29.09%. Origoni et al,[19] Ho et al,[20] and Molden et al[21] demonstrated the positivity of HPV E6/E7 mRNA among women with abnormal cytology as 26, 21, and 22.8%, respectively. However, in a recent study by Dabeski et al[22] high-risk HPV E6/E7 mRNA infection was detected in 60.94% of patients with squamous cell abnormalities of the cervix. These differences could be due to different sample sizes in different studies along with different methodologies used. In a study by Cuschieri and Wentzensen[23] HPV E6/E7 mRNA was found to be significantly associated with the severity of cervical lesions. The similar fact was proven by a study of Liu et al,[24] which found that 25.7% of LSIL cases and 71.9% of HSIL+ cases had positive E6/E7 mRNA tests, and as the severity of cervical lesions increased, the positive rate of HPV E6/E7 mRNA also increased. The findings were similar in our study also as in the patients who have normal biopsy findings, HPV E6/E7 mRNA is negative and as the severity of cervical lesions increases on biopsy, positivity of HPV E6/E7 mRNA also increases and all the patients who had HSIL+ lesions had HPV E6/E7 mRNA expression also. A study by Salimović-Bešić et al[25] on 105 women with abnormal cervical cytological findings showed low HPV E6/E7 mRNA positivity in women with cytological findings of ASC-US on the PAP test. In our study, 36 patients

had ASC-US cytological findings on PAP smear, but none of them showed HPV E6/E7 mRNA expression and also these patients had normal biopsy findings. A recent study done by Zhang et al[26] showed that HPV DNA, mRNA, increased with the severity of histopathology diagnosis, from 25.5%, 19.1%, in normal to 100.0% in squamous cell carcinoma, respectively. In our study HPV E6/7 mRNA has higher specificity (100%) and positive predictive value (100%) as compared with HPV DNA. Previous literature also showed that HPV E6/E7 mRNA testing has higher specificity and positive predictive than HPV DNA testing.[22,27] In our study, out of the total patients who had normal biopsy findings (38), five had HPV DNA positive and none of them had the expression of HPV E6/E7 mRNA showing that E6/E7 mRNA expression is more specific for cervical carcinogenesis as compared with the presence of only HPV DNA. Yao et al also concluded in their study that the HPV E6/E7 mRNA test were highly concordant with cytology test results, and both the diagnostic modalities resulted in similar sensitivity, specificity, PPV, and NPV while identifying HSIL+ lesions.[28] Various studies showed that HPV E6/E7 mRNA expression can reflect the stage of HPV infections, and its positive pattern can predict the development direction of CINs, so guiding the accurate treatment protocols for these patients.[29,30] Nowadays various studies are being done on the detection of HPV E6/E7 mRNA expression by different methods including both quantitative and qualitative. A study

done by Wang et al[31] evaluated the performance of the Optimygene HR-HPV RT-qDx assay, which is an HPV E6/E7 mRNA-based assay, to detect 16 HR-HPV subtypes, concluded that the higher specificity and positive predictive value of this assay are valuable for predicting insignificant HPV DNA infections among patients with a borderline cytological diagnosis. Recently, a study done by Liu et al[32] on quantitative HPV E6/E7 mRNA assay using QuantiVirus HPV E6/E7 mRNA assay (Kodia, Henan, China) to compare the expression levels of HPV E6/E7 mRNA in HSIL and LSIL showed that the median E6/E7 mRNA copy number in HSIL (median, 14,684 copies/mL) was far higher than that in LSIL (median, 936.00 copies/mL) ($p < 0.001$). In our study, when we compared the positivity of HPV E6/E7 mRNA with various risk factors, it has been seen that out of 16 patients positive for HPV E6/E7 mRNA, 56.3% patients were of <30 years of age, 81.2% had marriage in the age of 18 to 25 years, 62.5% were of para 3, and 75% were using OCPs making them all as risk factors for cervical cancer, as shown by previous studies also.[33–36] Recently, the American Cancer Society recommends cervical cancer screening for females from age of 25 to 65 years with an HPV-DNA test alone every 5 years. If HPV testing alone is not available, females can get screened with an HPV/Pap co-test every 5 years or a Pap test every 3 years.[37] Our data suggest that HPV E6/E7 mRNA detection may be a useful non-invasive tool for the screening of HPV-induced cervical neoplasia. The smaller sample size was the major limitation of our study. Hence, further studies on HPV-E6/E7 mRNA with a large sample size are needed for establishing this diagnostic modality as a routine screening test for ruling out cervical cancer in patients with borderline cytology. The smaller sample size was the major limitation of our study, and also the patients in which only HPV DNA was present without the expression of HPV E6/E7 mRNA were not followed-up further.

Conclusion

The present study suggests that HPV E6/E7 mRNA detection could be more reliable than DNA testing for predicting the risk of progression of HPV-induced cervical lesions to cervical carcinoma as the former had 100% specificity as compared with latter (86.8%). Hence, it can be used as a non-invasive tool for triage and patient follow-up. However, large-scale studies with proper cost factor analysis are further needed to substantiate the findings of our study for better management of this growing menace of cervical carcinoma.

References

1 Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No.11 [Internet]. Lyon, France:: International Agency for Research on Cancer;; 2013

2 Stanley M. HPV—immune response to infection and vaccination. Infect Agent Cancer 2010;5:19

3 Muñoz N, Bosch FX, de Sanjosé S, et al; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(06):518–527

4 Herrero R, Hildesheim A, Bratti C, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst 2000;92(06):464–474

5 Abreu ALP, Souza RP, Gimenes F, Consolaro ME. A review of methods for detect human Papillomavirus infection. Virol J 2012;9:262

6 Hwang SJ, Shroyer KR. Biomarkers of cervical dysplasia and carcinoma. J Oncol 2012;2012:507286

7 zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 2000;92(09):690–698

8 Scheurer ME, Tortolero-Luna G, Guillaud M, et al. Correlation of human papillomavirus type 16 and human papillomavirus type 18 e7 messenger RNA levels with degree of cervical dysplasia. Cancer Epidemiol Biomarkers Prev 2005;14(08):1948–1952

9 Coquillard G, Palao B, Patterson BK. Quantification of intracellular HPV E6/E7 mRNA expression increases the specificity and positive predictive value of cervical cancer screening compared to HPV DNA. Gynecol Oncol 2011;120(01):89–93

10 Sørbye SW, Arbyn M, Fismen S, Gutteberg TJ, Mortensen ES. HPV E6/E7 mRNA testing is more specific than cytology in post-colposcopy follow-up of women with negative cervical biopsy. PLoS One 2011;6(10):e26022

11 Kraus I, Molden T, Holm R, et al. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. J Clin Microbiol 2006;44(04):1310–1317

12 Molden T, Kraus I, Skomedal H, Nordstrøm T, Karlsen F. PreTect HPV-Proofer: real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses. J Virol Methods 2007;142(1-2):204–212

13 Zerat L. La nouvelle terminologie de Bethesda: quellschangements? Rev Prat Gynecol Obstet. Numero Special 2002:3–10

14 Solomon D, Davey D, Kurman R, et al; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287(16):2114–2119

15 Buckley CH, Butler EB, Fox H. Cervical intraepithelial neoplasia. J Clin Pathol 1982;35(01):1–13

16 Kaur H, Kaur S, Goyal L, Kaur P. Prevalence of carcinoma cervix in rural Punjab and need to create awareness regarding cervical cancer. Indian J Public Health Res Develop 2013;4(03):111

17 Senapati R, Nayak B, Kar SK, Dwibedi B. HPV genotypes distribution in Indian women with and without cervical carcinoma: implication for HPV vaccination program in Odisha, Eastern India. BMC Infect Dis 2017;17(01):30

18 Bzhalava D, Eklund C, Dillner J. International standardization and classification of human papillomavirus types. Virology 2015;476:341–344

19 Origoni M, Cristoforoni P, Carminati G, et al. E6/E7 mRNA testing for human papilloma virus-induced high-grade cervical intraepithelial disease (CIN2/CIN3): a promising perspective. Ecancermedicalscience 2015;9:533

20 Ho CM, Lee BH, Chang SF, et al. Type-specific human papillomavirus oncogene messenger RNA levels correlate with the severity of cervical neoplasia. Int J Cancer 2010;127(03):622–632

21 Molden T, Nygård JF, Kraus I, et al. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: a 2-year follow-up of women with ASCUS or LSIL Pap smear. Int J Cancer 2005;114(06):973–976

22 Dabeski D, Duvlis S, Basheska N, et al. Comparison between HPV DNA testing and HPV E6/E7 MRNA testing in women with squamous cell abnormalities of the uterine cervix. Prilozi (Makedon Akad Nauk Umet Odd Med Nauki) 2019;40(01):51–58(Makedon Akad Nauk Umet Odd Med Nauki)

23 Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2008;17(10): 2536–2545

24 Liu TY, Xie R, Luo L, et al. Diagnostic validity of human papillomavirus E6/E7 mRNA test in cervical cytological samples. J Virol Methods 2014;196:120–125

25 Salimović-Bešić I, Tomić-Čiča A, Smailji A, Hukić M Comparison of the detection of HPV-16, 18, 31, 33, and 45 by type-specific DNA- and E6/E7 mRNA-based assays of HPV DNA positive women with abnormal Pap smears. J Virol Methods 2013;194(1-2):222–228

26 Zhang S-K, Guo Z, Wang P, et al. The potential benefits of HPV E6/E7 mRNA test in cervical cancer screening in China. Front Oncol 2020;10:533253

27 Duvlis S, Popovska-Jankovic K, Arsova ZS, Memeti S, Popeska Z, Plaseska-Karanfilska D. HPV E6/E7 mRNA versus HPV DNA biomarker in cervical cancer screening of a group of Macedonian women. J Med Virol 2015;87(09):1578–1586

28 Yao YL, Tian QF, Cheng B, Cheng YF, Ye J, Lu WG. Human papillomavirus (HPV) E6/E7 mRNA detection in cervical exfoliated cells: a potential triage for HPV-positive women. J Zhejiang Univ Sci B 2017;18(03):256–262

29 Hui C, Bai H, Liu J, et al. Accuracy of HPV E6/E7 mRNA examination using in situ hybridization in diagnosing cervical intraepithelial lesions. Diagn Pathol 2021;16(01):13

30 Sun J, Yue Y, Li R, et al. Detection of HPV E6/E7 mRNA in the diagnosis of cervical cancer and precancerous lesions after kidney transplantation. Am J Transl Res 2021;13(06):7312–7317

31 Wang HY, Kim H, Park KH. Diagnostic performance of the E6/E7 mRNA-based Optimygene HR-HPV RT-qDx assay for cervical cancer screening. Int J Infect Dis 2019;78:22–30

32 Liu J, Yang T, Hu Y, Ye C. The value of HPV E6/E7 mRNA quantitative analysis in distinguishing high-grade cervical squamous intraepithelial lesions from low-grade cervical squamous intraepithelial lesions. J Virol Methods 2021;289:114014

33 Aggarwal R, Gupta S, Nijhawan R, et al. Prevalence of high-risk human papillomavirus infections in women with benign cervical cytology: a hospital based study from North India. Indian J Cancer 2006;43(03):110–116

34 Asiaf A, Ahmad ST, Mohammad SO, Zargar MA. Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. Eur J Cancer Prev 2014;23(03):206–224

35 Rama CH, Roteli-Martins CM, Derchain SF, et al. [Prevalence of genital HPV infection among women screened for cervical cancer]. Rev Saude Publica 2008;42(01):123–130

36 Remschmidt C, Kaufmann AM, Hagemann I, Vartazarova E, Wichmann O, Deleré Y Risk factors for cervical human papillomavirus infection and high-grade intraepithelial lesion in women aged 20 to 31 years in Germany. Int J Gynecol Cancer 2013;23(03):519–526

37 Available at: https://www.cancer.gov/news-events/cancer-currents-blog/2020/cervical-cancer-screening-hpv-test-guideline. [cited Oct 3, 2021]