ORIGINAL RESEARCH

Efficacy of HPV E6/E7 mRNA assay, HPV DNA test and cytology in detection of high grade cervical lesions and invasive cancer at a tertiary care center in India

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Abstract

Background

Cervical cancer is caused by human papillomavirus (HPV) and remains a major public health issue. There are several methods for its diagnosis. Knowing their comparative usefulness may help in devising an appropriate strategy for early detection of this gynecological cancer in resource-constrained setting.

Aim

This study aims to evaluate the efficacy of human papillomavirus (HPV) E6/E7 mRNA assay, HPV DNA test and cytology in detection of high grade cervical lesions and invasive cervical carcinoma at a tertiary care center in India.

Methods

A total of 106 women were recruited in this hospital based study and underwent molecular tests (HPV DNA test and mRNA assay), cytological test and colposcopic-guided biopsy. Histopathological diagnosis was considered as the gold standard.

Results

We observed that 56 out of 106 participants had abnormal results in the terms of positive HPV DNA or E6/E7 mRNA with or without abnormal cytology or had histopathologically confirmed pre-malignant/malignant lesion. 47.2% (50/106) and 32% (34/106) women were positive for HPV DNA and E6/E7 mRNA respectively. 33% (35/106) women had abnormal cytology and 29.2% (31/106) had histologically confirmed CIN II and higher lesions. Sensitivity and specificity of cytology and HPV DNA for CIN III+ lesions were calculated as 92%, 90.4% versus 88% & 68% respectively. mRNA assay was found to be more sensitive (96%) and specific (93.3%) than other tests in detecting CIN II + lesions.

Conclusion

E6/E7 mRNA assay seems superior to HPV DNA test and cytology in detection of high grade cervical lesions and invasive carcinoma. It may be used as an alternative or adjunct to HPV DNA test or cytology for the purpose of cervical cancer screening and also be helpful in reducing the load for colposcopy.

Keywords: HPV DNA, mRNA assay, Cytology, Colposcopy, Cervical Cancer, Diagnosis, Bihar, India

Introduction

Cervical cancer remains a major public health issue and ranks the very second common gynecological malignancy in females despite significant efforts for screening of the disease as well as availability of vaccination against human papillomavirus (HPV), the causative agent[1]. In 2020, cervical cancer accounts for 604 127 new cases and 341 831 death among women living without HIV globally whereas India also witnessed a surge in the incidence rate for cervical cancer which estimates to 123,907 new cases and 77,348 death[1]. Certain HPV genotypes such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 are considered high risk (HR) and oncogenic[2]. Their linkage to high grade cervical lesion or invasive cervical cancer is well documented[3].

Although Pap smear test has been proven useful in screening of cervical cancer, it is crippled with relatively low sensitivity requiring skill for correct interpretation and liable for inter-observer variation[4]. Several countries in Europe such as Netherlands, Turkey, Italy, Sweden, Finland, Spain, United Kingdom etc. have implemented HPV DNA testing as a primary test for cervical cancer screening, but its full implementation is completed only in the Netherlands[5, 6]. Moreover, HPV DNA testing can reveal mere infection with HPV, but cannot distinguish transient or persistent HPV infection or the severity of pre-malignant lesion at times, which seems to be a major limitation.

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Integration of HPV gene into host cell causes up-regulation of viral E6/E7 oncogenes and triggering the degradation of retinoblastoma protein (pRb) and p53 by binding it with E7 and E6 oncoprotein respectively[3]. This subsequently stimulates cell cycle and causes continuous morphological change by interfering with the mechanism of cell cycle regulation and immunologic escape, leading to malignant transformation and cervical carcinoma[3]. Recent studies showed that testing for E6/E7 mRNA of HPV types 16, 18, 31, 33 and 45 was more specific for identifying patients who developed high-grade cervical lesions rather than the detection of HPV DNA by PCR with GP5+/6+ consensus primers[7]. The HPV E6/E7 mRNA test has been shown to have a higher clinical specificity and positive predictive value (PPV) than HPV DNA test and thus, qualifies better than HPV DNA test for screening purpose[8]. Though, HPV E6/E7 mRNA test has better clinical performance than HPV DNA test for screening purpose[8], HPV DNA genotyping helps in discriminating the risk and predicting the outcome of infection during cervical cancer screening[9,10]. Triaging with specific E6/E7 mRNA test seems to reduce the referral burden for colposcopy for women who test positive for HR- HPV DNA or have abnormal cytology. HPV E6/E7 mRNA based testing kits such as PreTect Proofer, Aptima® HPV assay, Quantivirus® HPV assay, Fluorescence in situ hybridization (FISH), Optimygene HR-HPV RT-qDx assay, HPV OncoTect and RT-PCR assay have been used in the studies to detect CIN2+[11]. The Aptima® HPV assay is FDA approved and detects E6/E7mRNA of 14 oncogenic HPV genotypes but it does not discriminate between different high risk genotypes[12,13].

In the current scenario, lack of national organized screening, inadequate, limited health infrastructure and enormous cost of the screening tests to patients limit the access and effectiveness of screening at mass level. However, visual inspection with acetic acid or Lugol's iodine (VIA/VILI) has been proven as valuable and cost effective method of cervical cancer screening in resource constrained settings of India and other low- and middle- income countries (LMICs)[13], these tests lead to overtreatment and are liable to suffer from quality assurance. Hence, primary HPV DNA-based screening for cervical cancer is recommended by the World Health Organization (WHO)[14].

The present hospital based pilot study aimed to assess the clinical performance of HPV E6/E7 mRNA assay, HPV DNA test and cytology for the detection of high grade cervical pre-cancerous lesions and invasive cervical carcinoma among women attending a tertiary level healthcare hospital in Patna, Bihar, India. The clinical value of molecular tests and cytology was assessed in term of sensitivity and specificity.

Methods

Study population

This was a hospital based cross-sectional study carried out during the period of March 2018 to April 2020 at Gynecology Oncology Out Patient Department (OPD) at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna (Bihar, India), a tertiary care and referral center. This study was approved by the institutional ethics committee of IGIMS, Patna (reference number-288/IEC/2018/IGIMS) and conducted in accordance with the Declaration of Helsinki (1964). A total of 106 eligible sexually active and non-pregnant women aged 25-75 years visiting the OPD for gynecological problem or for screening were recruited in the study after obtaining their informed consent. The women younger than 25 years, hysterectomized or had previous surgical procedures on cervix and with history of receiving chemotherapy or radiotherapy were excluded from the study. The clinico-demographic details including name, age, marital duration, parity, education and socio-economic condition of all participants and results of screening tests along with clinical examination were recorded. The sample taken from each participant was labeled with unique ID provided by hospital patient registration system.

Cytology by conventional Pap smear

All participants underwent per speculum examination and Pap smear was taken by Ayers spatula, the slides were seen under microscope for any epithelial abnormalities and reported according to Bethesda classification varying from ASCUS, LSIL, ASC-H, to HSIL or invasive squamous cell carcinoma. Slides reported as unsatisfactory for interpretation were also included in the study.

HPV DNA detection and genotyping

Exfoliated cells from cervix of 106 women were collected in a collection tube containing a liquid transport medium and immediately transported to molecular biology laboratory for HPV DNA detection and genotyping using 15 High-risk Human Papillomavirus DNA Genotyping Diagnostic Kit (Sansure Biotech, China) following manufacturer's instructions. The Sansure HPV kit is based on novel one-tube fast release technology using real-time fluorescent quantitative PCR to recognize 15 HR-HPV genotypes including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 in patient's sample with at least 400 copies per mL of sample. The kit has been approved by the European Union Certificate (CE) and used in primary cervical cancer screening[15,16] with 100% and 72.0% sensitivity and specificity respectively[17]. Human β-globin gene was included as an internal control in all reactions.

HPV E6/E7 mRNA assay

The HPV E6/E7 mRNA detection was carried out using Aptima® HPV assay (Gen-Probe Inc., San Diego, CA) as per manufacturer's instruction. The Aptima assay is a target amplification assay utilizing transcription-mediated amplification (TMA) approach for the in vitro qualitative detection of E6/E7 mRNA from 14 high-risk oncogenic types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens. Briefly, the cervical specimens were collected in ThinPrep Pap Test vials containing PreservCyt Solution. Subsequently, 1 mL aliquot of PreservCyt sample was transferred into a 2.9 mL APTIMA Specimen Transfer Tube containing specimen transport medium (STM) and mixed by gently inverting the tube for 2-3 min. Then, 400 µL aliquot of mixture was tested using semiautomated direct tube sampling (DTS) system. The assay includes an internal control for monitoring the nucleic acid capture, amplification and detection as well as operator or instrument error. The assay results were interpreted on the basis of analyte signal-to-cutoff (S/CO) ratios. The cervical samples with S/CO ratios ≥ 0.5 were considered positive.

Colposcopy and histopathology

All participants underwent colposcopy using Reid's score and guided cervical biopsy. Colposcopy guided cervical biopsy is included in the protocol of cervical cancer screening and

is a routine procedure being performed in Gynecology Oncology OPD at IGIMS, Patna. The procedure was performed by consultant of gynecology department. In case of abnormal or suspicious cervical lesions, a punch biopsy was taken. In case of normal looking cervix, four-quadrant cervical biopsy was taken and, from a subset of 13 patients, endocervical curettage was also taken. The tissue obtained on biopsy was transferred to pathology department in a vial containing formaline for histological reporting.

Cytopathologist and Histopathologist, who were working as consultants in the pathology department at IGIMS, examined the samples. They were kept blind for the result of tests the patients underwent.The four tests including cytology, molecular methods (HPV DNA and E6/E7 mRNA) and colposcopy guided biopsy were performed in the participants either on the same day or at follow- up visit if coming within 3 month of initial examination. Patients with abnormal screening tests or histopathological result were followed-up as per the American Society of Colposcopy and Cervical Pathology (ASCCP) guidelines.

Statistical analysis

All statistical analyses were performed using SPSS statistics software version 13.0 (Chicago, IL, USA). Comparison of demographic variables between the two groups was done by using Chi-square test. p-value <0.05 was considered statistically significant. True positive (TP), true negative (TN), false positive (FP) and false negative (FN) cases were seen for the cytology and molecular tests separately. Further, sensitivity (TP/TP+FP), specificity (TN/TN+FP), positive predictive value (PPV=TP/TP+FP) and negative predictive value (NPV=TN/FN+TN) of cytology, HPV DNA testing and E6/E7 mRNA assay were calculated for detecting CIN II+ lesions, considering histological diagnosis as gold standard. The overall agreement and percentage positive agreement test were also calculated to compare the performance of molecular tests and the cytological analysis.

Results

A total of 119 women of 25-75 years of age were enrolled in the study who got cytology and molecular tests done at their first visit. 11 out of them did not turn up for colposcopy and guided biopsy in the given period of 3 months following screening test. 2 patients had insufficient tissue in biopsied sample for histopathological reporting, however, they refused to undergo repeat cervical biopsy. Hence, data of only 106 women, who underwent all the four tests i.e. cytology, HPV DNA, E6/E7 mRNA assay and colposcopy guided biopsy, were considered for the analysis. Of these 106 women, 56 women were reported to have either abnormal screening reports or abnormal histopathological findings.

Molecular and conventional tests

Cervical samples from 106 women were subjected to HPV DNA genotyping, HPV E6/E7 mRNA assay, cytology and histopathology. Of 106 participants, 50 (47.2%) were positive for HPV DNA whereas HPV E6/E7 mRNA was found to be present in 34 women (32%) only (Table 1).

Table 1: Correlation of cytology and histology with HPV DNA and HPV E6/E7 mRNA status

Variables	Total (n=106)	HPV DNA+ (n=50)	HPV DNA- (n=56)	E6/E7 mRNA + (n=34)	E6/E7 mRNA - (n=72)
Cytology					
NILM	68	18	50	5	63
ASCUS	4	3	1	3	1
LSIL	4	3	1	1	3
ASC-H	2	2	0	2	0
HSIL	15	11	4	12	3
SCC	10	10	0	10	0
Unsatisfactory	3	3	0	1	2
Histology					
Normal/Cervicitis	64	13	51	2	62
CIN I	11	11	0	3	8
CIN II	6	4	2	5	1
CIN III	14	12	2	13	1
SCC	11	10	1	11	0

NILM: negative for intraepithelial lesion or malignancy, **ASCUS:** Atypical squamous cell of undetermined significance, **ASC-H:** atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion **(HSIL**). **LSIL**: low-grade squamous intraepithelial lesion, **HSIL:** high-grade squamous intraepithelial lesion, **CIN:** cervical intraepithelial neoplasia, **SCC:** squamous cell cancer

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Table 2: HPV DNA genotype, HPV E6/E7mRNA, cytology and associated histopathology outcome in 56 women

Patient No.	HPV Genotype	HPV E6/E7 mRNA	Cytology	Biopsy
1.	16	+	SCC	SCC
2.	-	+	HSIL	CIN-II
3.	16	-	NILM	BENIGN
4.	16	-	LSIL	BENIGN
5.	16	-	NILM	BENIGN
6.	16	+	ASC-H	CIN-III
7.	16	+	SCC	SCC
8.	16	+	HSIL	CIN-III
9.	16	+	SCC	SCC
10.	16	-	HSIL	CIN-I
11.	18	-	NILM	CIN-I
12.	16	-	NILM	BENIGN
13.	39	-	NILM	BENIGN
14.	16	+	SCC	SCC
15.	-	-	HSIL	CIN-III
16.	16	-	LSIL	CIN-I
17.	33	+	HSIL	CIN-III
18.	16	+	HSIL	CIN-III
19.	16	-	Unsatisfactory	CIN-I
20.	18	+	SCC	SCC
21.	16	+	HSIL	CIN-II
22.	39	-	Unsatisfactory	BENIGN
23.	16	+	NILM	SCC
24.	16 & 52	+	NILM	CIN-I
25.	16	+	ASCUS	CIN-II
26.	31	-	NILM	BENIGN
27.	16	+	NILM	CIN-III
28.	16	+	SCC	SCC
29.	16 &52	+	HSIL	BENIGN
30.	18	+	Unsatisfactory	CIN-II
31.	31	-	NILM	BENIGN
32.	-	+	ASCUS	SCC
33.	-	-	HSIL	CIN-II
34.	16	+	SCC	SCC
35.	56	+	SCC	SCC
36.	16	+	LSIL	CIN-II
37.	-	-	LSIL	BENIGN
38.	16	-	ASCUS	CIN-I
39.	51 & 56	+	SCC	SCC
40.	16	+	ASC-H	CIN-III
41.	16	+	ASCUS	CIN-I

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Table 2 Cont....

42.	16	-	NILM	BENIGN
43.	16	+	HSIL	CIN-III
44.	18	-	NILM	BENIGN
45.	39 & 51	+	HSIL	CIN-III
46.	16	-	NILM	CIN-I
47.	18	-	NILM	CIN-I
48.	16	-	NILM	CIN-I
49.	16	-	NILM	BENIGN
50.	-	+	HSIL	CIN-III
51.	16	+	NILM	BENIGN
52.	16	+	HSIL	CIN-III
53.	16 & 52	+	NILM	CIN-I
54.	18	+	HSIL	CIN-III
55.	16	+	HSIL	CIN-III
56.	31	+	SCC	CIN-III

Table 3: Concordance of HPV DNA with cytology and HPV E6/E7 mRNA

	HPV DNA+	HPV DNA-	Overall percentage agreement	Percent positive agreement
LSIL+	23	4	72.8%	45.1%
LSIL-	24	52		
E6/E7 mRNA +	31	3	79.2%	58.4%
E6/E7 mRNA -	19	53		

LSIL+ lesion included ASC-H, HSIL and SCC, whereas **LSIL-** lesions included NILM, ASCUS and LSIL on cytology reporting.

Table 4: Performance of screening tests (cytology, HPV DNA and HPV E6/E7 mRNA

Screening tests	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI	+ LR	- LR	Accuracy
Molecular											
HPV E6/E7 mRNA for CIN II	83.3	35.8-99.5	93.3	85.1-97.8	50	28.5-71.9	98.5	92.1-99.7	12.5	0.2	92.5
HPV E6/E7 mRNA for CIN III+SCC	96	79.6-99.9	93.3	85.1-97.8	82.7	67.2-91.8	98.5	91.1-99.7	14.4	0	94
HPV DNA for CIN II	66.6	22.2-95.6	68	56.2-78.3	14.2	7.9-24.2	96.2	89.0-98.7	2.1	0.5	67.9
HPV DNA for CIN III+SCC	88	68.7-97.4	68	56.2-78.3	47.8	39.0-56.7	94.4	85.3-98.0	2.8	0.2	73
Cytology											
ASCUS + Cytology for CIN II	100	47.8-100	90.4	81.2-96.0	41.6	26.1-59.1	100	-	10.4	0	91.0
ASCUS+ Cytology for CIN III+ SCC	92	73.9-99.0	90.4	81.2-96.0	76.6	61.6-87.0	97	89.7-99.2	9.5	0.1	90.8
LSIL + Cytology for CIN II	80	28.3-99.4	93.1	84.7-97.7	44.4	23.5-67.4	98.5	92.1-99.7	11.6	0.2	92.3
LSIL + Cytology for CIN III+ SCC	88	68.7-97.4	93.1	84.7-97.7	81.4	65.1-91.2	95.7	88.67-98.5	12.8	0.1	91.8
HSIL + Cytology for CIN II	60	14.6-94.7	97.2	90.4-99.6	60	24.2-87.5	**97.2**	92.3-99.1	21.9	0.4	94.8
HSIL + Cytology for CIN III+ SCC	80	59.3-93.1	97.2	90.4-99.6	90.9	71.5-97.5	**93.4**	86.6-96.8	29.2	**0.2**	92.8

PPV: Positive predictive value, **NPV**: Negative predictive value, +**LR**: Positive likelihood ratio, -**LR:** Negative likelihood ratio

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The most commonly detected genotype in the present study was HPV 16 in 64% (32/50) followed by HPV 18 in 12% (6/50) HPV positive women participants. Other HR-HPV genotypes detected were HPV 31 and HPV 39 in 6% (3/50) and 4% (2/50) respectively whereas each of HPV 33 and HPV 56 was found in 2% (1/50) participants. Infection with multiple strains of HPV as (16 and 52), (39 and 56) and (51 and 56) was also detected in 10% of the HPV positive women (Table 2).

On cytological evaluation, 68 women (64.1%) had NILM report and 4 (3.7%) had each of ASCUS and LSIL. ASC-H and HSIL were found in 2 (1.9%) and 15 women (14.1%) respectively whereas invasive cervical cancer was detected in 10 women (9.4%) (Table 1). Histopathological examination revealed that out of 106 participants, 64 (60.3%) had normal histology or cervicitis, 31 (29.2%) had cervical intraepithelial neoplasia (CIN I in 11, CIN II in 6 and CIN III in 14) and 11 cases (10.3%) with squamous cell carcinoma (Table 1).

Cytology and histopathology report in relation with HPV DNA and E6/E7 mRNA

26.4% (18 /68) women reported with NILM were found to be HPV positive whereas only 0.7% (5/68) of them showed presence of HPV E6/E7 mRNA. Worse lesion than LSIL which included ASC-H and HSIL was found in 17/56 women in the study group out of which 13 (76.4%; 13/17) were HPV DNA positive whereas HPV E6/E7 mRNA positivity was seen in 14 women (82.3%; 14/17). All cases of invasive cervical cancer (10/56) detected by cytology were also found to be positive for HR-HPV DNA as well HPV E6/E7 mRNA. 3 out of 106 women in the study had unsatisfactory cytology but were positive for HR-HPV DNA and one of them was detected positive for E6/E7 mRNA also (Table 1).

While considering histopathological analysis among women with abnormal tests in the study, 25% (14/56) cervical biopsies were benign but 92.8% (13/14) of them were HR-HPV DNA positive whereas only 14.2% (2/14) showed presence of HPV E6/E7 mRNA. 11 patients had CIN I, all HR-HPV +ve but only 3 of them (3/11) were detected with HPV E6/E7 mRNA. CIN II and III were diagnosed in 20 out of 56 patients (35.7%) and positivity for HPV DNA and E6/E7 mRNA among them was seen in 80% (16/20) and 90% (18/20) respectively. Invasive cervical cancer was confirmed histopathologically in 11/56 women (19.6%). All of them were found positive for HPV E6/E7 mRNA whereas HPV DNA could be detected in 10/11 women (90.4%) (Table 1).

Comparison of clinical performance of cytology, HPV DNA and E6/E7 mRNA tests

Concordance of HPV DNA test with cytology and E6/E7 mRNA test was calculated in terms of overall percentage agreement and it was found to be 72.8% and 79.2% respectively with percent positive agreement 45.1% and 58.4% respectively (Table 3). Comparing the performance of screening tests to detect CIN II and CIN III+ lesion, sensitivity of cytology was found to be 100% and 92% versus 66.6% and 88% of HPV DNA test respectively and the highest was seen for E6/E7 mRNA as 83.3% and 96%. The specificity of cytology, HPV DNA and E6/E7 mRNA tests for detection of CIN III+ lesion were 90.4%, 68% and 93.3% respectively. The PPV and NPV of E6/E7 mRNA assay was observed higher for CIN III+ lesion i.e. 82.7% and 98.5% in comparison to HPV DNA test (47.8% and 94.4%) and cytology (76.6% and 94.4%) (Table 4).

Discussion

Following establishment of HR-HPV infection as primary causative agent of cervical cancer, several molecular and epidemiological studies have been conducted to obtain information regarding HPV prevalence and its genotyping and consequently the potential burden of cervical cancer in concerned population[18,19]. Also, studies have been carried out to see the performance of E6/E7 mRNA assay as primary screening test or for triaging[20,21]. The present study was conducted to test the potential of three major assays namely cytology, HPV DNA and HPV E6/E7 mRNA in detection of pre-cancerous lesions and invasive cervical carcinoma among women attended a tertiary healthcare center in Bihar, India.

In this study, HR-HPV DNA positivity rate among the patients with benign biopsy (inflammatory and CIN I) was found to be 32 % (24/75) and rising to 66.6% in CIN II cases (4/6), 85.7% in women with CIN III (12/14) to 91% in women with cervical carcinoma (10/11) which were in agreement with data published previously by Deodhar and co-workers[22]. Similar to the findings of Sowjanya and colleagues[23], HPV-16 and -18 were the most frequent infecting genotypes among HPV positive women participated in the present study.

Although in the general population, HPV DNA test is the recommended primary screening test[14], few studies have demonstrated HR- HPV DNA testing as an effective triage method for women with equivocal cytologic abnormalities[24,25]. High sensitivity, combined with a reasonable specificity for triage, makes HPV DNA testing the recommended option for the management of ASCUS cytology[25]. In the present study, strong association was observed between HPV infection and abnormal cytology and the overall percentage agreement and percent positive agreement between these two was found to be 72.8% and 45.1% respectively.

The performance of cytology in terms of sensitivity, specificity, PPV and NPV for detection of CIN III and higher lesions was found as 92%, 90.4%, 76.6% and 97% (Table 4). Katki and group[26] observed sensitivity, specificity, PPV and NPV of cytology for predicting CIN II+ lesion as 53.1%, 96.5%, 9.7% and 99.7% whereas Szarewski et al.[27] reported greater sensitivity 93.8% and lesser specificity 34.7% of cytology with PPV and NPV 36.6% and 93.3% respectively.

The overall HPV E6/E7 mRNA positivity rate in present study was observed as 32% (34/106) increasing to 93.5% (29/31) in women with histologically confirmed CIN II and higher lesions including invasive cancer. E6/E7 mRNA positivity was observed in 100% (11/11), 92.8% (13/14), 83.3% (5/6), and 6.6% (5/75) in histologically diagnosed cases of cervical cancer, CIN III, II, I and benign pathology respectively. Yao et al.[28] observed HPV E6/E7 mRNA positivity rate in high-grade lesions as 100%, 89.11%, 77.05%, and 44.05% for histologically confirmed cancer, HSIL, LSIL, and normal cases respectively. A study by Cattani et al.[29] reported the presence of HPV E6/ E7 mRNA in 25% of patients with normal cytology, however, the positivity rate increased progressively with increasing severity of the lesions to 50% in LSILs, 96% in HSILs or ASC-H patients and 100% of Squamous cell carcinoma (SCC) cases. The present study too showed similar findings of HPV E6/ E7 mRNA positivity i.e. in 7.3% among women with NILM cytology

(5/68), increasing to 25% in LSIL (1/4) and 80% (12/15) in HSIL cases whereas in 100% cases of ASC-H (2/2) and SCC (10/10) (Table 1).

In the present study, HPV E6/E7 mRNA assay was found more sensitive than HPV DNA test for detection of CIN II (sensitivity 83.3% versus 66.6%) and CIN III+ lesions (sensitivity 96% versus 88%). The specificity of mRNA assay for CIN II and higher lesions was found significantly greater at 93.3% as compared to HPV DNA test of 68%, which is similar to the findings of CLEAR study[30] reporting 96.2% specificity of Aptima® HPV test for CIN III and lower lesions in comparison to HPV DNA test. Lower specificity of mRNA assay (in the range of 42-79%) in comparison to HPV DNA test was also reported for CIN II+ lesions[31,32]. A plethora of evidences suggested that E6/ E7 mRNA detection may be a more reliable and acceptable modality for screening of cervical cancer than HPV DNA and other conventional tests like Pap smear since mRNA assay has strong potential for offering triage and follow-up to the patients by predicting the risk of progression towards high grade cervical lesion[33,34]. Some studies have reported that inadequacy of the sample, error in sample storage, absence of cancer cells in the sample, HPV-DNA integration or absence of HPV DNA in the sample may lead to false negative HPV test results[35-37] and these may be the possible reasons behind comparatively low detection of HPV DNA in our samples.

Due to lack of awareness in people of the state for cervical cancer screening and prevention as well as fear associated with screening procedure, the study faced challenges during the recruitment of the participants, resulting in enrollment of considerably small number of subjects. High cost of tests and irregular lab services also contributed to a protracted recruitment process. Despite these loco-regional challenges, the present study is an attempt to generate preliminary evidences for exploring the feasibility of molecular tests with conventional Pap smear in diagnosis/screening of cervical cancer particularly in this understudied state of India. Another drawback of the study was selection of the participants from hospital-based population who were not real representative of the general community and the possible risk of bias in interpretation of results could not be denied. Henceforth, we recommend a larger sample based study to validate the findings of the present study by screening women for cervical cancer at general community level.

Conclusion

In the present hospital based study, we found that E6/E7 mRNA assay has higher sensitivity and specificity than other molecular and conventional tests including HPV DNA testing and Pap smear for detecting the CIN II+ lesions in women. We suggest that E6/E7 mRNA assay can be useful as an alternative or adjunct to HPV DNA test or cytology for the purpose of cervical cancer screening at mass level. However, before reaching to any conclusion, the findings of present pilot study need to be validated in larger samples as well in various healthcare settings.

Conflict of interest

The authors declare no conflict of interests.

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Contribution of authors

Conceptualization: BJ; Supervision and Guidance: RKS, BJ, AX; Study conduct: SP; Data collection: SP, JR, KA, PK, SK; Data analysis: SP, JR, KA, SK, BJ; Data interpretation: SP, JR, BJ; Preparation of first draft of manuscript: SP, JR, KA; Review and editing of first draft of manuscript: RKS, BJ; Final editing: VC, NRB, BJ; Revision: SP, JR, KA, BJ; Support for conduct of study: SKS, AK. All authors read and approved the manuscript.

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