RESEARCH ARTICLE

Editorial Process: Submission:03/30/2019 Acceptance:08/11/2019

The Utility of Urine-Based Sampling for Cervical Cancer Screening in Low-Resource Settings

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Abstract

Background: WHO has recommended Visual Inspection with Acetic acid (VIA) or Human Papillomavirus (HPV) DNA testing if feasible, for cervical cancer screening in low income countries. However, the number of women undergoing screening is very low as a result of limited information, inadequate infrastructure and invasive nature of sampling. **Methods:** A cross sectional study was carried out comparing HPV DNA detection by Polymerase Chain Reaction (PCR) in paired cervical and urine samples procured from histologically confirmed cervical cancer cases. **Results:** Amongst the samples collected from 114 cervical cancer cases, HPV DNA was tested positive in cervical samples of 89 (78.1%) and urine samples of 55 (48.2%) patients. The agreement between the two sampling methods was 66.7% and the kappa value was 0.35 indicating a fair agreement. The sensitivity of HPV detection using urine samples was 59.6% (95% confidence interval 49.16%-69.15%) and the specificity was 92% (95% confidence interval 75.0%-97.8%). **Conclusion:** Even though not acceptable as an HPV DNA screening tool due to low sensitivity, the urine sampling method is inexpensive and more socially acceptable for large epidemiological surveys in developing countries to estimate the burden.

Keywords: Cervical cancer- DNA- HPV- sampling- urine

Asian Pac J Cancer Prev, **20 (8)**, 2409-2413

Introduction

Premature death rates due to malignancies affecting the breast, cervix and ovary are higher among women from developing countries due to delayed diagnoses at advanced stages and inappropriate treatment. Cervical cancer with an extended pre-invasive stage is the second most common cancer among Indian women. This preventable cancer alone accounts for 17% of cancer related deaths among Indian women between 30 and 69 years (Bobdey et al., 2016). The main reasons behind higher cancer related mortality in developing countries are the inequities in screening, and treatment attributing to late detection at advanced stages. Even though there is evidence of decreased incidence of cervical cancer from developed countries after implementation of cytology (Adegoke et al., 2012; Sasieni et al., 2009; Nygård et al., 2002) the cytology-based cervical cancer screening coverage is only 2-6% in developing countries like India (Aswathy et al., 2012).

The recent update in cervical cancer screening was the 2015 US FDA approval of Roche Cobas HPV testing for women above 25 years without concurrent Pap testing (Flanagan, 2018). HPV testing is much more sensitive with a high negative predictive value compared to cytology as well as visual inspection with acetic acid. Another advantage is that noninvasive urine, as well as vaginal samples, can be tested by molecular assays which will further augment the acceptance rate in the community. Self-collected vaginal samples are not always culturally and socially feasible in conservative societies. In low-income countries, training of rural women regarding self-collection of vaginal samples using pamphlets or instructions may not be feasible as in developed countries. However, the incorporation of non-invasive sampling modalities into existing cervical cancer screening programme has improved the participation rate in developed countries (Tanzi et al., 2013). In France, a higher response rate was observed when women were instructed to provide self-collected urine samples (Payan

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et al., 2009). However, urine contains various polymerase chain inhibitors like urea, nitrites and other unknown agents and the presence of HPV DNA might indicate an infection of lower genital tract (Khan et al., 1991).

Urine based HPV assay has been introduced as an alternative mode for the screening of cervical cancer mainly for women from hard-to-reach areas (Munoz et al., 2013). The adoption of self-sampling can reduce the number of hospital visits and will be more acceptable to women with limited access to health care ensuring equity (Sabeena et al., 2016). From a household setting, urine samples can be transported to the laboratory in the cold chain. This approach is cost effective, acceptable and less embarrassing to the women from the low-income countries who demonstrate the lowest cervical cancer screening compliance despite being at a higher risk of HPV-associated malignancies. There is a dearth of comparative studies from India using urine and cervical samples from clinic-based settings. Our hypothesis was that urine self-sampling can be used as an alternative method for the detection and genotyping of high risk HPV DNA.

Materials and Methods

Methodology

A cross sectional study was carried out to compare the detection rate of HPV DNA urine samples and cervical samples collected from histologically confirmed cervical cancer cases. One hundred and fourteen cervical cancer patients prior to surgical management or chemoradiation attending the Gynecologic Oncology and Radiotherapy Departments of Government Medical College, Kozhikode were enrolled in the study. Women were provided with a subject information sheet in the local language and a written informed consent was taken in the local language. The study was reviewed and approved by the Institutional Ethical Committee of Manipal Academy of Higher Education (MUEC/011/2017) and Institutional Ethical Committee of Government Medical College, Kozhikode (GMCKKD/RP 2017/IEC/160).

Before the pelvic examination, study participants were instructed to collect about 20 ml first-void (first-stream) urine in wide-mouthed containers. On speculum examination, cervical samples were collected using sterile polypropylene swab under aseptic precautions and transported in 2 ml sterile normal saline at 4-8^{o}C to Manipal Institute of Virology (MIV). At MIV, the urine samples were subjected to modified aliquoting prior to DNA extraction (Tanzi et al., 2013). The pellets obtained after centrifugation was suspended in phosphate buffered saline (PBS) and viral DNA was extracted using Qiagen viral DNA extraction kit (Qi Amp DNA Mini kit) as per manufacturer's instructions. Multiplex real-time PCR was performed in an ABI 7500 cycler (Applied Biosystems) for the amplification of the LCR/E6/E7 regions of four high risk HPV types-16, 18, 31 and -45 (Schmitz et al., 2009). The remaining untyped samples were subjected to conventional nested PCR with PGMY09/11 primer sets for the first round PCR and GP5+/GP6+ primer sets for the second round (Gravitt et al., 2000; de RodaHusman et al.,1995). The purified PCR products were sequenced using GP5+/GP6+ primer set and BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in a 3500 XL Genetic Analyzer (Applied Biosystems).

Statistical analysis

The statistical analysis was carried out using SPSS 15.0 for Windows (SPSSTMInc, Chicago, IL, USA). The demographic data and baseline characteristics were summarised by frequency and percentages. Meanwhile, continuous variables were represented by mean with standard deviation for normally distributed parameters and median with interquartile range for parameters not normally distributed. The sensitivity and specificity of HPV DNA detection in urine samples was calculated with cervical sampling as the gold standard. The values were reported as percentages with 95% confidence interval (95% CI), positive predictive value (PPV) and negative predictive value (NPV). Kappa index was used to determine the level of agreement between the paired samples. A Kappa value between 0.00 and 0.20, 0.21-0.40, and 0.81-0.99 was considered poor agreement, fair agreement and almost perfect agreement respectively (Landis and Koch, 1977).

Results

The mean age of the study participants was 56 years (SD=10.7). In the present cross sectional study, eighty-two women had attained menopause (71.9%) and the mean age of menopause was 46.9 years (SD=5.7). The mean age at marriage was 19.8 years (SD=4). As shown in Table 1, three women (2.6%) were nulliparous and thirty-five (66.7%) women had four or more children. Only one lady reported the presence of genital warts whose cervical and urine sample were tested negative for HPV DNA. The rest of the study participants denied the presence of any skin or genital warts among themselves as well as sexual partners. More than one lifetime sexual partner was reported by three (2.6%) women and five (4.4%) women reported extramarital relations of their spouse. The most predominant symptom was post-menopausal bleeding observed in 69 patients (60.5%) followed by vaginal discharge in 60 (52.6%) and post coital bleeding in 17 (14.9%) cases. Clinically, based on FIGO staging (Bhatla et al., 2018) fifty-six (49.1%) cases were classified under stage IIb and thirty-two (28.1%) patients were staged as III b.

Amongst the 114 cervical cancer cases enrolled in the present cross sectional study, the prevalence of HPV DNA was 78.1% (95% confidence interval I 69.2%- 85%) in cervical samples and 48.3% in urine samples (95% confidence interval 39.4%-57.3%) as shown in Table 2. The overall agreement between the two sampling methods was 66.67% and the kappa value was 0.35 (p-value <0.01) indicating a fair agreement. The sensitivity of HPV detection using urine samples was 59.55% (95% confidence interval 49.16%-69.15%) and the specificity was 92% (95% confidence interval 75.03%-97.78%) with cervical sampling as the gold standard. The positive

Table 1. Table Depicting the Sociodemographic and Maternal Factors of Study Participants (N=114)

	N (%)	HPV DNA positive In cervical sample (n=89, 78.1%)	HPV DNA positive Urine sample (n=55, 48.2%)
Age group			
<30	1 (0.9%)	0	0
30-35	1 (0.9%)	1	0
36-45	11 (9.6%)	6	3
46-55	40 (35.1%)	36	19
56-65	35 (30.7%)	28	16
66-75	21 (18.4%)	15	14
76-85	5 (4.4%)	3	3
Marital status			
Married	76 (66.7%	60	36
Separated	5 (4.4%)	5	2
Widow	32 (28.1%)	23	16
Divorced	1 (0.9)	1	1
Unmarried	0 (0)	0	0
Parity (n=114)			
0	3 (2.6)	3	2
1-3	76 (66.7)	60	30
≥4	35 (30.7)	26	23
Living children (n=114)			
0	3 (2.6)	3	2
1-3	76 (66.7)	60	30
≥4	35 (30.7)	26	23
Menopause attained			
Yes	82	67	44
No	32	22	11

predictive value of HPV detection in urine samples was 96.4% and negative predictive value 39%. The sensitivity for detection of the most common high risk genotype, HPV-16 was 42.98%in urine samples. Meanwhile, a very low sensitivity of 11.1% was observed for the next common high risk genotype, HPV-18 (Table 3). The most common histopathology reported among the study participants was squamous cell carcinoma large cell keratinising (n=54, 47.3%) out of which forty-two were HPV DNA positive in cervical samples. HPV-16 was observed to be the most common high risk type detected in seventy-seven study participants (67.5%) followed by HPV-18 in cervical samples (n=9, 7.9%). Among the fifty-four cases with squamous cell carcinoma large cell keratinising, HPV-16 was detected in thirty-four cervical samples and twenty-three urine samples. The cervical sample of one patient with cervical adenocarcinoma was tested positive for both HPV-16 and HPV-18 whose urine sample was positive for only HPV-16. In the present study, high risk genotypes other than HPV-16 and -18 were not detected. There was concordance between high risk genotypes detected in the cervical sample and urine samples of fifty-five patients.

Discussion

In the present cross sectional study, modified aliquoting of urine samples was carried out to increase the sensitivity. We observed low sensitivity and high speciicty of urine samples for HPV DNA detection in comparison to cervical sample. Previous studies carried out amongst high risk women also had observed a low sensitivity and high specificity for urine based HPV detection (Mendez et al., 2014; Hagihara et al., 2016). However, we observed vast heterogeneity in the methodology of studies resulting in contradictory outcomes. The overall concordance percentage reported in the present study was in accordance with the study carried out in Thailand (Nilyanimit et al., 2017). There are reports of good agreement of HPV DNA detection in paired urine and cervical samples (Tanzi et al., 2013; Sahasrabuddhe et al., 2014; Stanczuk et al., 2003; Nicolau et al., 2014; Nilyanimit et al., 2013; Bernal et al., 2014). Another study carried out among thirty cervical cancer cases, urine samples of 28 cases were HPV DNA positive, while all the thirty cervical samples were tested

Table 2. The Accuracy of Urine HPV DNA Detection in Comparison to Cervical HPV DNA Detection among Cervical Cancer Cases (n=114)

		Cervical Sample		Sensitivity %	Specificity %	PPV %	NPV %	Kappa
		HPV (+)	HPV(-)					
Urine	HPV (+)	53	2	59.55	92	96.36	39	0.35*
Sample	HPV (-)	36	23					

Agreement %, 66.67; * Fair agreement

Table 3. Comparison of Urine High Risk HPV DNA Positivity as Per Real Time Multiplex PCR Assay with Cervical HPV DNA Detection (n=114)

		Cervical Sample				
		HPV16	HPV18	Untyped	Negative	HPV16 and HPV18
Urine Sample	HPV16	49	0	0	0	1
	HPV18	0	1	0	0	0
	Untyped	1	0	1	2	0
	Negative	27	8	1	23	0

Sensitivity % for HPV 16, 42.98; Sensitivity % for HPV 18, 11.11

positive (Gupta et al., 2006). An almost perfect agreement was observed between urine and cervical samples of cases with high grade cervical intraepithelial neoplasia in another study (Piyathilake et al., 2016). A recent study from China observed high concordance between the cervical samples and pellet fraction of initial stream urine samples collected from healthy women attending cancer screening clinics. The sensitivity and specificity for all HPV DNA in the pellet fractions of urine with cervical samples as reference were 68.4% and 99.9% (Hagihara et al., 2016). Another study from Colombia reported an overall HPV prevalence of 60.00% in cervical samples and 64.72% in urine samples with HPV-16 being the moc in both specimens (Cómbita et al., 2016) So far only one study employed urine samples for primary cervical cancer screening and observed lower HPV positivity rate of 11.6% in urine compared to 14.7% in cervical samples. (Stanczuk et al., 2003). Another study from Thailand observed a higher sensitivity and specificity using urine samples in high grade lesions (Khunamornpong et al., 2016).

HPV exhibits tissue tropism to the squamocolumnar junction of the cervix and anogenital areas with no predilection to the urinary tract. The HPV detection in urine represents exfoliation from the cervix, vagina or vulva which will be more frequent in high grade lesions and cancers (Sahasrabuddhe et al., 2014). Urine based screening is not ideal and a 35% loss of sensitivity in comparison to recommended screening practices is not satisfactory (Mendez et al., 2014). As the negative HPV test does not necessarily rule out HPV infection repeat testing has to be carried out. However, there is a better acceptance rate especially from women from remote areas and urine sampling is culturally acceptable in comparison to self-collected vaginal sampling. There are considerable variations of HPV detection in urine samples which is mainly attributed to lack of standardisation of urine collection, aliquoting, DNA extraction and amplification techniques (Senkomago et al., 2016; Vorsters et al., 2014). A mete-analysis reported a 22-fold reduction in accuracy when random or midstream urine samples were used for HPV detection (Pathak et al., 2014). Urine sampling is appropriate for women who do not prefer vaginal examination and also for monitoring sexually unexposed adolescents after HPV vaccination (Cuschieri et al., 2011). The optimisation and standardisation of the procedure are essential as the standards for the processing of cervical samples may not be applicable to urine. Acceptance rate may be higher among ethnic groups, post-menopausal elderly women.

In conclusion, even though not acceptable as an HPV DNA screening tool due to low sensitivity, the urine sampling method is inexpensive and more socially acceptable for large epidemiological surveys in developing countries to estimate the burden.

Strength of the study

Women with histologically confirmed cervical cancer cases were enrolled for the study. Most of the study participants were at advanced stages of malignancy and were instructed to collect an initial stream of urine (first part of a urine void) before pelvic examination. Throughout sample transport, cold chain was ensured and modified aliquoting of urine samples incorporating two-step centrifugation was employed. A validated PCR based assay was used for HPV DNA detection in both cervical and urine samples.

Limitations

We collected random urine samples with no preservative or DNA conservation medium.

Acknowledgements

We acknowledge our sincere gratitude to all the study participants. We would also like to place on records our gratitude to Mrs Laveena Kotian, senior laboratory technologist who played a vital role in sample reception and processing.

Funding

This work was supported by the Indian Council of Medical Research (ICMR) (Project No: 5/8/7/15/2010 ECD-1).

Ethical approval

This study was carried out in accordance with the ethical standards of the Helsinki Declaration (1964, amended in 2008) of the World Medical Association. The study was approved by the Institutional Ethical Committee of both the institutions (MUEC/011/2017, GMCKKD/RP 2017/IEC/160). Informed written consent was obtained from all the study participants.

Conflicts of interest

None.

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