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Human papillomavirus viral load on *care*HPV testing of self-collected vaginal samples vs. clinician-collected cervical samples

Q1 L. Satyanarayana *, A. Smita

Q2 *Division of Epidemiology and Biostatistics, Institute of Cytology and Preventive Oncology, Indian Council of Medical Research, Department of Health Research, Nagar, Uttar Pradesh, India*

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ABSTRACT

Objective: To compare viral load on *care*HPV DNA testing in self-collected vaginal (VHPC) and clinician-collected cervical (CHPC) samples for the detection of high-grade cervical intra-epithelial neoplasia (CIN).

Study design: Cross-sectional study. Ever-married women aged 30–59 years were targeted for cervical screening. On attendance for screening, vaginal self-sampling was performed by the woman, and an auxiliary nurse midwife subsequently performed a per-speculum examination, collected a CHPV sample and a Pap smear, and made a visual inspection of the cervix with acetic acid. The ratio of viral load expressed in relative light units to positive controls set at a cut-off of 1 pg/ml was used for *care*HPV quantitative assessment. The median viral load was compared using non-parametric tests. Receiver operating characteristic (ROC) curves were constructed for the detection of CINII+ and CINIII+ in CHPV and VHPV samples.

Results: Overall, the median viral load in the 4658 women screened was higher in CHPV samples compared with VHPV samples (9.8-fold higher in cases of high-grade CIN). The median viral load was significantly higher among Pap-positive women compared with Pap-negative women in both CHPV and VHPV samples ($p < 0.01$). Assessment by ROC analysis for the detection of high-grade CIN did not differ significantly between CHPV and VHPV samples.

Conclusion: Viral load on *care*HPV testing was comparable between self- and clinician-collected samples for the detection of high-grade CIN. The self-sampling approach may be an option for screening in low-resource countries.

Introduction

Q3 More than 85% of cases of cervical cancer occur in low-resource countries [1]. In high-income countries, regular Papanicolaou (Pap) testing has reduced the number of cases of cervical cancer, but other concerns exist. The need for robust cervical screening and management to reduce the burden of cervical cancer in low-resource countries is well recognized. Cervical human papillomavirus (HPV) DNA testing [2,3] has been shown to be reliable for the detection of high-grade cervical intra-epithelial neoplasia (CIN), and the possibility of using self-collected vaginal samples has been suggested [4]. The concept of introducing HPV self-sampling as an alternative means for screening for cervical cancer and its related issues has been addressed recently [5–7]. Randomized controlled trials among women who have not attended for cervical screening [8,9] have targeted self-sampling, and suggested the need for the approach to be investigated in different geographic and demographic settings.

*Care*HPV testing is a simple, low cost and robust method for HPV testing, and a new variant of traditional hybrid capture II (HCII) HPV testing [10] demonstrated its usefulness for the detection of CIN. Several studies have reported the use of *care*HPV testing [10–15], some of which compared its performance with HCII testing [10,13,14]. Other studies [16–21] have reported quantitative assessment in terms of HPV viral load using HCII testing, and demonstrated an association with cervical lesions, but to the authors' knowledge, no studies have been performed using *care*HPV testing to date. Previously, the authors undertook a qualitative study in a rural Indian centre [11] to compare *care*HPV testing in self-collected cervical and vaginal samples, along with

* Corresponding author at: Division of Epidemiology and Biostatistics, Institute of Cytology and Preventive Oncology, Indian Council of Medical Research, I-7, Sector-39, Noida, Uttar Pradesh, India. Tel.: +91 120 2446917; fax: +91 120 2579473.
E-mail address: satyanarayanalabani@yahoo.com (L. Satyanarayana).

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other conventional methods of screening. Although useful, qualitative assessment does not utilize the full spectrum of data. Quantitative assessment, based on viral load, provides a better opportunity to improve understanding and correlate different grades of cervical lesions by comparison of *care*HPV testing between self-collected and clinician-collected samples. As such, this study compared *care*HPV viral loads for self-collected vaginal samples and clinician-collected cervical samples from women screened in a rural low-resource community setting.

Subjects and methods

A cross-sectional study was undertaken in a rural tehsil in Uttar Pradesh, India between September 2010 and April 2012. This was part of a multicentre study [12] conducted in rural and urban settings in four centres in India, Uganda and Nicaragua. A door-to-door baseline community survey of all ever-married women was conducted to identify women aged 30–59 years, and these women were invited to attend a health centre for screening. Women who attended for screening were counselled, screening procedures were explained, and written informed consent was obtained. An auxiliary nurse midwife (ANM) and multipurpose healthcare workers were trained in screening procedures, motivational aspects and follow-up counselling. Health education materials such as flip charts, information brochures and pamphlets were used.

First, a vaginal careHPV (VHPV) sample was self-collected by the woman. Next, the ANM performed a per-speculum examination, collected a cervical *care*HPV (CHPV) sample and a Pap smear, and made a visual inspection of the cervix with acetic acid (VIA). Both vaginal and cervical samples were collected using a *care*HPV cervical sampler and Digene Co-collection Media (QIAGEN, Gaithersburg, MD, USA) with the brush provided by the manufacturer. Laboratory technicians received 1 week of training in *care*HPV testing procedures. The ratio of viral load expressed in relative light units (RLU) and positive controls set at a cut-off (CO) of 1 pg/ml was used for *care*HPV quantitative assessment. Ratios (RLU/CO) were presented as group frequency, median and interquartile range. RLU/CO values <0.5, 0.5–1.0 and >1.0 were classified as lower viral load, intermediate viral load and high viral load, respectively. RLU/CO ≥1.0 was considered positive for the detection of histological CINII+ and CINIII+. The Bethesda system was used for the analysis of Pap smears [22]. A Pap smear result of atypical squamous cells of undetermined significance (ASCUS) or more was considered positive. VIA was performed by applying 5% acetic acid to the cervix with a cotton swab, and allowing sufficient time (1 min) for colour change in the transformation zone. VIA was considered positive if a white colour could be observed against the pinkish background of normal epithelium; other samples were considered negative [23]. Women with any positive screening tests were referred to colposcopy and directed biopsy.

Colposcopic diagnosis was made in accordance with the guidelines of the International Agency for Research on Cancer (IARC) [22]. Biopsy/endocervical curettage was performed wherever necessary. Precancerous lesions were treated in accordance with the IARC guidelines [22]. CINII+ cases were treated with cryotherapy, where eligible, by a doctor, or referred to a tertiary care hospital for surgical procedures and radiotherapy. Quality control of screening tests, colposcopy and histological evaluations was ensured by retraining ANMs, doctors and independent external review histopathologists, respectively. Receiver operating characteristic (ROC) curves were constructed for the detection of CINII+ and CINIII+ using CHPV and VHPV samples, as well as other parameters. Non-parametric methods (Wilcoxon's signed rank and rank sum tests) were performed using SPSS Version 21.0 (IBM Corp., Armonk, NY, USA) for analysis of data related to viral load comparisons between CHPV and VHPV samples.

Results

In total, 7761 women were invited for screening, and 5032 (64.8%) reported at a screening centre. After exclusion of women

Table 1
Frequency distribution of *care*HPV viral load in self-collected vaginal (VHPV) and clinician-collected cervical (CHPV) samples according to different parameters.

Parameter	VHPV RLU/CO				CHPV RLU/CO			
	<0.5 *n* (%)	0.5–1.0 *n* (%)	>1.0 *n* (%)	Median (IQR)	<0.5 *n* (%)	0.5–1.0 *n* (%)	>1.0 *n* (%)	Median (IQR)
Age (years)								
<40	2685 (93.4)	110 (3.8)	80 (2.8)	0.24 (0.21–0.30)	2633 (91.6)	145 (5.0)	97 (3.4)	0.28 (0.23–0.34)
40–50	1193 (94.6)	50 (4.0)	19 (1.5)	0.24 (0.21–0.30)	1172 (92.9)	62 (4.9)	27 (2.1)	0.27 (0.23–0.33)
50–60	495 (95.0)	14 (2.7)	12 (2.3)	0.26* (0.22–0.31)	492 (94.4)	16 (3.1)	13 (2.5)	0.27 (0.23–0.32)NS
Menstrual history								
Premenopausal	3598 (93.4)	154 (4.0)	100 (2.6)	0.24 (0.21–0.30)	3536 (91.8)	194 (5.0)	121 (3.1)	0.28 (0.23–0.34)
Postmenopausal	774 (96.1)	20 (2.5)	11 (1.4)	0.26** (0.22–0.31)	760 (94.4)	29 (3.6)	16 (2.0)	0.27 (0.23–0.32)NS
VIA								
Positive	231 (89.9)	14 (5.4)	12 (4.7)	0.26* (0–165)	226 (87.9)	14 (5.4)	17 (6.6)	0.29** (0–224)
Negative	4142 (94.1)	160 (3.6)	99 (2.2)	0.24 (0–384)	4071 (93.5)	209 (4.7)	120 (2.7)	0.28 (0–404)
Pap								
Positive	104 (78.8)	11 (8.3)	17 (12.9)	0.26* (0.22–0.43)	103 (78.0)	4 (3.0)	25 (18.9)	0.30* (0.24–0.44)
Negative	3936 (94.3)	155 (3.7)	82 (2.0)	0.25 (0.21–0.30)	3864 (92.6)	206 (4.9)	102 (2.4)	0.28 (0.23–0.36)
Histological diagnosis								
Cancer, *n* (%)	1 (25.0)	1 (25.0)	2 (50.0)	7.03 (0.35–15.89)	1 (25.0)	0 (0)	3 (75.0)	40.6 (1.90–171.93)NS
CINIII, *n* (%)	3 (33.3)	1 (11.1)	5 (55.5)	7.29 (0.35–37.32)	1 (1.1)	0 (0)	8 (88.9)	71.7 (9.56–234.26)
CINII, *n* (%)	9 (47.4)	4 (21.0)	6 (31.6)	0.53 (0.24–9.27)	12 (63.1)	1 (5.3)	6 (31.6)	0.39 (0.26–19.37)NS
CINI, *n* (%)	51 (64.5)	19 (24.0)	9 (11.4)	0.31 (0.23–0.66)	48 (60.7)	16 (20.2)	15 (19.0)	0.34 (0.26–0.65)
Negative, *n* (%)	4309 (94.8)	149 (3.3)	89 (1.9)	0.24 (0.21–0.30)	4235 (93.1)	206 (4.5)	105 (2.3)	0.28 (0.23–0.34)

RLU/CO, ratio of relative light units to positive controls set at a cut-off of 1 pg/ml; IQR, interquartile range; Pap, Papanicolaou; VIA, visual inspection of cervix with acetic acid; CIN, cervical intra-epithelial neoplasia; NS, comparison between VHPV and CHPV not significant.
* $p < 0.05$.
** $p < 0.01$.

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