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Can human papillomavirus DNA testing of self-collected vaginal samples compare with physician-collected cervical samples and cytology for cervical cancer screening in developing countries?

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ABSTRACT

Background: To determine human papillomavirus (HPV) types by polymerase chain reaction (PCR)-reverse line blot assay and examine the concordance between HPV by Hybrid Capture 2 (HC2) and PCR on self-collected vaginal and physician-collected cervical samples and cytology. *Methods*: This was a cross-sectional study of 546 sexually active women aged ≥30 years with persistent vaginal discharge, intermenstrual or postcoital bleeding or an unhealthy cervix. Participants self-collected vaginal samples (HPV-S) and physicians collected cervical samples for conventional Pap smear and HPV DNA (HPV-P) testing and performed colposcopy, with directed biopsy, if indicated. HPV testing and genotyping was done by HC2 and PCR reverse line blot assay. Concordance between HC2 and PCR results of self- and physician-collected samples was determined using a Kappa statistic (κ) and Chi-square test. *Results*: Complete data were available for 512 sets with 98% of women providing a satisfactory self-sample. PCR detected oncogenic HPV in 12.3% of self- and 13.0% of physician-collected samples. Overall, there was 93.8% agreement between physician-collected and self-samples (κ = 76.31%, 95% confidence interval [CI]: 64.97–82.29%, $p = 0.04$)—complete concordance in 473 cases (57 positive, 416 negative), partial concordance in seven pairs and discordance in 32 pairs. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of self-sampling for detection of cervical intraepithelial neoplasia (CIN)2+ disease were 82.5%, 93.6%, 52.4% and 98.4%, respectively; for physician-sampling they were 87.5%, 93.2%, 52.2% and 98.9%, respectively; and for cytology they were 77.5%, 87.3%, 34.1% and 97.9%, respectively. Concordance between HC2 and PCR was 90.9% for self-samples (κ = 63.7%, 95% CI: 55.2–72.2%) and 95.3% for physician-collected samples (κ = 80.4%, 95% CI: 71.8–89.0%). *Conclusions*: Self-HPV sampling compares favourably with physician-sampling and cytology. A rapid, affordable, HPV self-test kit can be used as the primary method of cervical cancer screening in low-resource situations.

1. Introduction

Cervical cancer is the second most common cancer among women worldwide and the most common cause of cancer among women in India [1]. There are facilities for opportunistic screening but no regular screening programmes are in place. It is known that persistent infection with high-risk types of human papillomavirus (HR-HPV) is a major cause of cervical cancer [2] and that HPV DNA testing of cervical samples has higher sensitivity for detection of high-grade cervical intraepithelial neoplasia (CIN) and invasive cancer than the Pap smear test [3,4]. HPV testing has been recommended for primary cervical screening and with the introduction of a rapid, affordable test may be possible, even in low-resource situations [5,6]. Physician-obtained HPV samples also require gynaecological examination, which self-collected vaginal sampling can obviate in remote areas. The majority of studies have reported equivalent or less than equivalent sensitivity of self-sampling as compared to physician-sampling in the detection of high-grade lesions [7–11]. The present study aimed to compare the HPV types, test characteristics and concordance

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between self- and physician-collected samples as well as conventional cytology, to understand how a rapid test may perform in this setting.

2. Materials and methods

This cross-sectional study was carried out in the Gynaecology Outpatient Department (OPD) from January 2003 through to June 2005. Women presenting with complaints of persistent vaginal discharge, irregular menstrual bleeding, postcoital bleeding, or those found on examination to have an unhealthy cervix were invited to participate in a cancer-screening programme. Exclusion criteria were: age <30 years; unmarried; hysterectomised; prior surgical procedures on cervix; gross tumour on the cervix; and pregnancy. Informed written consent was taken from the women. Ethical clearance was obtained from the Institutional Review Board. A total of 625 potential participants were recruited, of which 74 were found ineligible and 5 refused to participate; thus 546 eligible women were enrolled and an enrolment questionnaire completed.

2.1. Clinical examination and investigation

Patients underwent the following tests in sequence: (1) self-collection of vaginal sample for HPV testing, (2) conventional Pap smear, (3) physician-collected cervical sample for HPV testing, and (4) colposcopy.

2.1.1. Procedure of self-sampling

The procedure of self-sampling was first explained to the patient with the help of a chart. A pre-labelled Digene HPV collection tube containing Specimen Transport Medium (STM, Qiagen Gaithersburg, Inc., USA) and a cervical sampler were then provided to the patient. She was instructed to introduce the cervical sampling brush into the vagina till she met with resistance, rotate the brush 3–5 times, remove it and place it in the tube containing the collection medium. The extra length of the brush was snapped off, the bottle re-capped and deposited with the doctor. The collection procedure was supervised.

2.1.2. Physician-collected sampling

Patients were asked to lie in the dorsal position and a Cusco bivalve vaginal speculum was introduced. A Pap smear was taken with an Ayre spatula and endocervical brush. The cervical brush sampler was then introduced inside the endocervix with the lowermost bristles touching the ectocervix, rotated 3–5 times in a counter-clockwise direction and then placed in the Digene specimen collection tube as described for self-sampling.

2.1.3. Colposcopy

All women underwent a colposcopic examination by an experienced gynaecologist. Biopsies were taken from all lesions with a Reid score ≥ 0. Women were considered to be free of disease if CIN or invasive cancer were ruled out after biopsy or if colposcopy was normal, thereby obviating the need for taking a biopsy.

2.1.4. Sample storage and processing and HPV testing

Both the samples collected in Digene STM were divided into two aliquots and stored at −70 °C till further processing. One aliquot was tested for 13 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) by HC2 as per the manufacturer's recommendation (Qiagen Gaithersburg, Inc., USA). The second aliquot underwent testing by polymerase chain reaction (PCR) amplification with the use of the PGMY09/11 L1 consensus primer system and a reverse line blot detection strip that individually identifies 22 high-risk types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and its sub-type ISO39) and 15 low-risk HPV types (6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 71, 72, 83, 84 and 89) [12]. The sample was processed as previously described [13,14]. In brief, 150 μl of the sample were digested with 15 μl of 10× digestion buffer (containing 700 μl of 20 mM Tris–HCl—1 mM EDTA (TE) buffer, 100 μl 10% Tween-20 and 200 μl of 20 mg/ml proteinase K) at 65 °C for 1 h followed by heat inactivation at 95 °C for 10 min. The DNA was precipitated with ethanol and ammonium acetate at −20 °C overnight. After centrifugation at 21,000 × *g* for 30 min at 4 °C for pelleting the DNA, the pellet was dried, resuspended in 75 μl of TE and stored at −20 °C until amplification for HPV testing.

The specimen DNA was amplified using PGMY 09/11 HPV-specific primers that amplify the 450 bp fragment of L1 ORF of genital HPV. Human β-globin target was co-amplified with HPV consensus primers to determine adequacy of the specimen. The PCR products were denatured and hybridised to an immobilised HPV probe array on strips (*kind gift of Roche Molecular Systems, Alameda, CA, USA*). Positive hybridisation was detected by colour precipitation at the probe site and the type determined by reading from a reference overlay. Each amplification run included HPV DNA positive controls (SiHa cell line/HeLa cell line) as well as no HPV DNA negative controls.

For analysis purposes, samples were considered sufficient for HPV determination if the β-globin probe was detected. All β-globin negative samples were excluded from further analysis.

2.1.5. Statistical analysis

Overall agreement with a 95% confidence interval was computed. The discordance between the two methods and between self- and physician-collected specimens was tested by Mc-Nemar Chi-square test. Chance corrected agreement was assessed by Kappa statistic along with a 95% confidence interval. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the self- and physician-collected samples and cytology were calculated taking lesions ≥CIN2 on biopsy as the reference standard for disease positivity. All analyses were performed using Stata 9.1.

3. Results

The median age of women enrolled in the study was 36 years, with 62.3% in the age group of 30–40 years; 39.2% of the women had no formal education, 26.0% had received some primary education and 23.7% of women had received high school or higher education. The majority of women belonged to lower (47.1%) and middle (49.2%) socio-economic class; 187 (36.4%) reported having had four or more births. The mean age at first coitus was 19.0 ± 3.3 years.

Out of 546 women enrolled and questionnaires completed, six absconded after being handed the specimen collection tube for self-sampling. The remaining 540 women were asked to provide a self-collected vaginal sample and physician-collected cervical sampling was also performed. In five women, the self-collection tubes were found to contain no fluid so HPV DNA could not be tested. In six samples, the β-globin gene could not be amplified (two physician-collected samples, three self-collected samples, and, in one case, in both physician and self-collected samples) so it was not possible to comment on presence and type of HPV. Therefore, 96.9% (529/546) of women enrolled were able to provide a satisfactory sample for testing. However, four women refused colposcopy, PCR results were missing in nine women and HC2 results were missing in four women. Thus, complete results were available for 512 pairs of HPV DNA samples (self- and physician-collected). Colposcopy was performed in all these cases and a biopsy taken in 315 cases. Biopsy-positive CIN or invasive cancer was present in 66 women (CIN1—26; CIN2—13; CIN3—19; invasive cancer—8).