Human Papilloma Virus Testing of Vaginal Smear Obtained with a Novel Self-sampling Device

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Sir,

Screening for cervical cancer aims to detect cytological changes, follow-up their development and, if needed, remove them surgically before they progress to invasive cancer. The Swedish National Board of Health and Welfare recommends that all women aged 23–50 years be offered cytological screening with Papanicolaou smear test (Pap smear) every 3 years and women aged 51–60 every 5 years.

Despite a well-organized screening programme, about 500 women each year in Sweden are diagnosed as having cervical cancer (1). The vast majority of cervical carcinomas are squamous cell carcinomas, arising from the transformation zone or ectocervix. Adenocarcinomas deriving from the columnar epithelium in the endocervical canal are more rare (2).

The major risk factors for cervical cancer are early age at first intercourse, multiple sex partners, and sexually transmitted infections. Human papilloma virus (HPV) is of significant importance for development of cervical carcinoma and is found in almost 100% of cervical carcinomas (2, 3, 4). Studies show that HPV tests have a high sensitivity for cervical intra-epithelial neoplasia lesions, while the Pap smear has a higher specificity than HPV tests (5, 6). Additional research is being done to find out whether HPV testing can replace the Pap smear as a primary screening test (7, 8).

Most women (approximately 50%) who are diagnosed as having cervical cancer have no prior cytology-based screening history, because for various reasons they have not attended cytological screening (9, 10). To reach these non-attending women a novel self-sampling device (SSD) for vaginal samples has been designed (Aprovix AB, Uppsala, Sweden).

This study was conducted to determine the agreement between HPV test results on self-collected samples obtained from fornix vaginae using SSD and clinician-obtained samples collected from cervix using a cytobrush. In order to determine how women accept self-sampling their attitudes were surveyed.

MATERIALS AND METHODS

The study enrolled 44 women aged 23–58 years (mean age 35.8 years), who visited the gynaecological clinic at the University Hospital of Uppsala or the maternity clinics in Årsta and Svartbäcken, Uppsala, Sweden, between September 2004 and May 2005. The women were admitted for further examination due to previous abnormal cytology observed at gynaecological screening. During the appointment the women were asked to collect a vaginal sample using the SSD. The self-sampling kit consists of the SSD, a dry test tube and instructions for collection of vaginal samples. When collecting samples the SSD is inserted into the bottom of the vagina and rotated one turn. After sampling, the upper part of the SSD was inserted into the test tube and cut off by bending it against the tube. The test tube was closed and sent to a laboratory for analyses (Fig. 1). After this procedure an additional sample was collected from the cervix by the clinician using a cytobrush (Medscand, Malmö, Sweden). The women also answered a questionnaire about their attitudes towards self-collection of vaginal samples.

The study has been approved by the ethical committee at Uppsala University.

Human papilloma virus analysis

HPV tests were performed on samples from the vagina and cervix with Digene's Hybrid Capture 2 (HC2) technology (Digene Corporation, Gaithersburg, Maryland, USA) The self-sampled material was sent dry to the HPV laboratory, where the self-samplers upper part was washed into Digene Specimen Transport Medium and the DNA was denatured by adding denaturation fluid and heating.

After denaturation the ssDNA is pipetted, together with 8 controls (3 negative, 3 positive and 2 quality controls) on a microtitre plate. A RNA probe cocktail, containing probes for 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) that bind to target DNA, is added.

Samples taken by clinicians from cervix using cytobrush were also HPV-tested using HC2 technology. The cervix material was

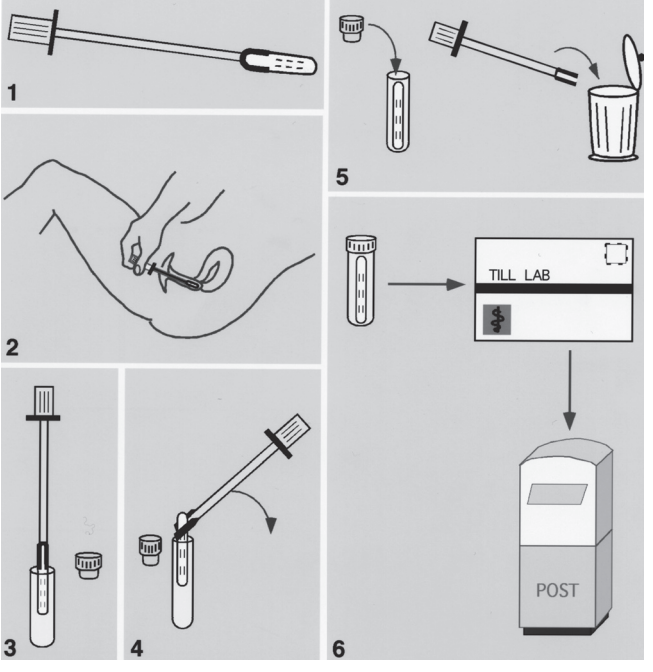

Fig 1. Procedure for self-collecting a vaginal smear at home using a SSD-kit (self-sampling device). Reproduced from Aprovix.

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smeared over a glass slide and fixed with 95% alcohol before transport to the laboratory. In the laboratory the slides were washed to transfer the cells into Digene's Transport Medium and HC2 was performed as described earlier.

RESULTS

High-risk HPV DNA was detected in 39% (n = 17) of the samples taken by SSD and in 43% (n = 19) of the samples taken by cytobrush. Women over 30 years of age represented 59% of all women positive for high-risk HPV (n = 10), when tests were performed using HC2 on the SSD, whereas women under 30 years represented 41% (n = 7). Consequently, 33% of women over 30 years of age participating in the study tested positive for high-risk HPV, as did 50% of women under 30 years of age.

The results of the HPV test were compared with each other. The agreement between results of self-collected samples and clinician-collected samples was good with an index of validity 0.86 and a kappa value 0.72. (kappa value > 0.70 good agreement) (Table I). HPV-testing of self-collected samples using the SSD achieved performance measures of 78.9% sensitivity and 92,0% specificity in comparison with HPV testing of samples collected using cytobrush. (11)

Women who participated in self-collecting with the SSD found it very easy (70%) or relatively easy (30%) to use; 77% of the women did not experience any pain or discomfort when taking the sample, and 23% experienced only very slight discomfort. The majority of the women (90%) judged that they would be able to take the sample themselves at home. There was a positive response to self-sampling, with 90% of the women answering that they would take the sample at home and return it for investigation.

DISCUSSION

Launching of a national screening programme for cervical cancer in Sweden in the 1960s has decreased cervical cancer prevalence by approximately 50%. However, 10–20% of women do not attend screening, and they represent 50% of the women who are later diagnosed as having cervical cancer. Further, 25% of women diagnosed as having cervical cancer have a history of normal cytology (4, 9, 10, 12, 13).

Table I. *Number of positive or negative test. Comparison of self-sampling device (SSD) and cytobrush samples with regard to results after human papilloma virus analysis (kappa 0.72)*

	Cytobrush		
SSD	Positive (%)	Negative (%)	Total (%)
Positive	15 (34)	2 (5)	17 (39)
Negative	4 (9)	23 (52)	27 (61)
Total	19 (43)	25 (57)	44 (100)

Offering non-participating women the opportunity to self-collect vaginal samples at home might decrease the number of non-attenders and result in saving lives. This study shows that the SSD sampler has about the same reliability as sampling of cervical smear with a cytobrush to detect HPV.

The study also demonstrates that self-collecting has high acceptance among women participating in the study at a gynaecological reception (90%). Self-collecting at home has not yet been offered and it remains to be seen whether it succeeds in increasing the number of women being screened for cervical cancer. Whether or not HPV tests can replace the Papanicolaou smear as a primary screening tool is still not clear, but self-collecting of dry vaginal smear requires a HPV test, since DNA endures and remains unchanged even during longer transports. Self-collecting also has the potential to expand access to screening in low-resource settings and sparsely populated areas and could decrease the costs associated with HPV testing by eliminating the need for a clinic visit for gynaecological examination. However, the usefulness of self-sampling in younger women (< 35 years) is doubtful since their infections often are transient. The idea of HPV DNA testing home-obtained samples has been presented before by Nobbehuis et al. (14).

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