Scand J Infect Dis 36: 456–459, 2004

Taylor & Francis
healthsciences

The Prevalence of the Human Papillomavirus in Cervix and Vagina in Low-risk and High-risk Populations

MARC BAAY[1], VERONIQUE VERHOEVEN[2], KRISTIEN WOUTERS[3], FILIP LARDON[1], PIERRE VAN DAMME[4], DIRK AVONTS[2], ERIC VAN MARCK[5], PAUL VAN ROYEN[2] and JAN B. VERMORKEN[1]

From the [1]Department of Medical Oncology, [2]Centre for General Practice, [3]Health House for Prostitutes in Antwerp, [4]Department of Epidemiology and Social Medicine, [5]Department of Pathology, University of Antwerp, Antwerp, Belgium

Concordance of HPV between vagina and cervix may be influenced by sample taking and by differences in flow of cervical epithelial cells. To investigate the latter aspect, from 96 women visiting their general practitioner, and 63 sex workers visiting a STI clinic, both vaginal and cervical samples for HPV detection were obtained by the doctor to standardize sample taking. To identify factors that may influence the flow of cervical epithelial cells to the vagina, a questionnaire on intimate hygiene was obtained. The overall HPV prevalence was 22.8%; 14.3% in the general population (14.3% in the cervix, 11.9% in the vagina), compared with 34.4% in sex workers (31.1% in the cervix, 27.9% in the vagina). There was excellent agreement between HPV prevalence in vaginal and cervical samples. The overall agreement was 94.5% (kappa = 0.83, 95% CI: 0.77–0.89); in the general population agreement reached 97.6%, compared with 90.0% in sex workers. Vaginal infection may influence concordance, but for validation of this finding larger studies are necessary. The high concordance found between HPV prevalence in vagina and cervix warrants further study of the applicability of self-sampling to improve coverage rates by attracting women who would otherwise not obtain a pap test.

M. Baay, Department of Medical Oncology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium (Tel. +32 38202576, fax. +32 38202248, e-mail. Marc.Baay@ua.ac.be)

INTRODUCTION

Introduction of mass screening for cervical cancer by pap smear in the industrialized world has led to a reduction in incidence and mortality. However, not all women participate in screening, and women who have never been screened are at an increased risk of developing invasive cervical cancer (1). Both health service-related problems, e.g. the absence of a female screener, and patient-centred problems, especially fear of embarrassment or pain, are reasons for non-participation (2, 3). The pap smear, although not in the medical sense of that word, is, in a woman's perception an invasive sampling method. Consequently, cervical cancer screening could be more efficient if a less invasive test were available. Human papillomavirus (HPV) is a common genital sexually transmitted infection (STI), and persistent high-risk HPV infection is strongly linked to cervical cancer (4). Recently, several investigations have shown that self-sampled vaginal material can be used for HPV detection. In these studies a good correlation was found between self-sampled vaginal material and a cervical sample taken by a professional (5–9). A previous investigation on Chlamydia trachomatis screening has shown that the threshold for self-sampling is low in general practice, and that most women prefer this to sampling by a professional (10).

Potentially, concordance of HPV between vagina and cervix may be influenced by sample taking and by differences in flow of cervical epithelial cells. By having the sample taken by a professional we hoped to obtain the highest concordance possible, by standardization of the sample taking. The prevalence of HPV in the cervix as well as the vagina was investigated in a low-risk group of women (women visiting their general practitioner (GP) for cervical cancer screening) and a high-risk group of women (sex workers visiting a sexually transmitted infection (STI) clinic for prevention of STI and hepatitis B vaccination). Also, using a questionnaire, we aimed to identify factors that might influence the flow of cervical epithelial cells to the vagina.

MATERIALS AND METHODS

Study group

Between October 2001 and March 2003, 159 women were enrolled in this study. Of these women, 96 visited their general practitioner (GP) for a routine pap smear, whereas 63 sex workers visited a STI clinic for a regular check-up. All women were given written information about the study and provided oral consent to participate in the study. The study protocol was approved by the medical ethical board of Antwerp University. The GP or STI doctor first took a vaginal swab using a polyurethane-tipped swab (Culturette EZ, Becton Dickinson) and then a cervical sample using a Cervex-Brush (Rovers, Oss, The Netherlands). In the STI clinic separate brushes were taken for cytology and HPV detection. Finally, a questionnaire was completed by the doctor, in consultation with the patient.

Sample preparation and HPV detection

After vigorous vortexing, the cell suspension was centrifuged for 5 min at 3000 rpm. The cell pellet was resuspended in 0.5 ml TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and frozen at -80°C for 1h. After thawing, 100 µl of the suspension was taken, boiled for 10 min and centrifuged (5 min, 12,000 rpm). Isolation of DNA was confirmed by β-globin PCR using primers PC03/04 (11). In

 ISSN 0036-5548 DOI: 10.1080/00365540410020677

β-globin-negative cases, the remaining material was incubated overnight at 37°C in the presence of 0.2 μg/ml proteinase K. After boiling (10 min) and centrifugation the β-globin PCR was repeated.

Material positive for the β-globin PCR was subjected to the GP5+/6+HPV PCR (12). Detection of PCR products was performed in an enzyme immunoassay (EIA) format as described by Jacobs et al. (13). After detection of HPV with a high-risk (HR) HPV probe cocktail, typing analysis was performed by EIA using the probes for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, separately. The cut-off value for HPV positivity was calculated as the mean plus 3 times the standard deviation (SD) of all samples in the plate. Outliers were excluded and the mean +3SD was recalculated. This process was repeated until no further outliers were found. The final mean +3SD was taken as the cut-off value (14).

Questionnaire

A simple questionnaire asked for the use of tampons, vaginal showers, lubricants, condoms, vaginal cream, spermicidal cream and/or other intimate products. Also, the women were asked whether they had a vaginal infection and/or had used antibiotics during the last month before their visit. Finally, the phase of the menstrual cycle was noted (as d since last menstruation).

Statistical analysis

For comparison of the GP population and the STI clinic population the χ^2 test was used. The correlation of HPV in the vagina and the cervix was determined with an unweighted kappa statistic to determine the percentage of correlation beyond that expected by chance. To relate the HPV prevalence in the vagina and the cervix with practices of intimate hygiene, the Fisher's exact test was used. A p-value < 0.05 was considered statistically significant.

RESULTS

Eight cervical samples were lost due to logistic problems, and 6 samples tested negative in the β-globin PCR. Therefore, in 145 women HPV detection was complete. The mean age of the women was 31.5 y in the GP population (95% CI: 29.7–32.9 y), and 28 y in the STI clinic population (95% CI: 25.9–30.1 y) and this difference was statistically significant ($p = 0.01$). Combined HPV prevalence (cervix and/or vagina) in this study was 22.8%; 14.3% in the general population, compared with 34.4% in sex workers ($p < 0.01$, Table I). The overall agreement between HPV prevalence in vaginal samples and in cervical samples was 94.5% (kappa = 0.83, 95% CI 0.77–0.89); in the general population agreement reached 97.6%, compared with 90.0% for the STI clinic population. The HPV prevalence in the cervix was slightly higher than in the vagina (21.4% vs 18.6%, respectively). In all but 1 positive case (CHA 135) at least 1 HPV type was present in both sites (Table II). Multiple infections occurred frequently, both in vagina (16 of 27 HPV positive women, 59%) and in cervix (18 of 31 HPV positive women, 58%). The use of condoms was higher in the HPV positive group than the HPV negative women (Fisher's exact test, $p < 0.01$). Practice of intimate hygiene (use of tampons, condoms etc.) as a possible factor in HPV discordant samples did not give significant results (Table III). Only self-reported recent vaginal infections seemed to give more discordance (negative in vagina, positive in cervix). However, because of the good agreement, i.e. low number of discordant results, as well as the low number of women with vaginal infections in this study, the difference with respect to the presence of vaginal infections did not reach significance (Fisher's exact test, $p = 0.25$). Finally, there was a difference in the self-report of

Table I. *Statistics of the HPV prevalence in vagina and cervix in low-risk and high-risk populations*

	Overall *n* (%)	STI clinic *n* (%)	GP *n* (%)
No. of women	145	61	84
HPV prevalence	33 (22.8)	21 (34.4)*	12 (14.3)*
Prevalence in Cx	31 (21.4)	19 (31.1)	12 (14.3)
Prevalence in Va	27 (18.6)	17 (27.9)	10 (11.9)
Overall agreement	94.5%	90.0%	97.6%
HPV+agreement	75.8%	71.4%	83.3%
Kappa value	0.83	0.76	0.90
95%CI	0.77–0.89	0.65–0.87	0.83–0.96

* HPV prevalence STI clinic vs GP patients, χ^2 $p < 0.01$.
STI = sexually transmitted infection, GP = general practitioner, Cx = cervix, Va = vagina, HPV+agreement = agreement in samples with HPV in vagina and/or cervix, CI = confidence interval.

Table II. *Type-specific prevalence of HPV in vagina and cervix*

Pat. no.	HPV in Va	HPV in Cx
cha004	52	52
cha017	31	31
cha020	31	31+51
cha027	33+58+66	66
cha032	16	16
cha050	58	58
cha051	0	16
cha052	33+58	33+58
cha057	0	31+35
cha063	0	18+45
cha064	16+33+35	33+35
cha067	58	58
cha071	0	18+66
cha076	33+35+58	35
cha078	45	45
cha085	16	16
cha087	45	45
cha088	18+56	18+52+56
cha092	33+35	33+35
cha093	31+35	0
cha108	33+35	33+35
cha112	16+35+59	16+35+59
cha114	18+59	18+59
cha118	16+18+52	16+18
cha122	18+31+35	31+35
cha124	51+56+58	56+58
cha126	0	58
cha127	35+45	0
cha128	35+51	33+35+51+59
cha135	45	33+35
cha152	0	31+39
cha154	56+66	56+66
cha157	16	16

Cx = cervix, Va = vagina.

Table III. *Practice of intimate hygiene as a possible factor in HPV discordant results*

HPV in vagina and/or cervix	VA+/Cx+ ($n=24$)	%	Va−/CX+ ($n=5$)	%	Va+/Cx− ($n=2$)	%
Tampons	9	37,5	2	40,0	1	50,0
Condoms	18	75,0	3	60,0	2	100,0
Vaginal shower	4	16,7	1	20,0	0	0,0
Lubricants	3	12,5	0	0,0	1	50,0
Vaginal cream	2	8,3	1	20,0	0	0,0
Spermicidal cream	0	0,0	0	0,0	0	0,0
Other intimate products	1	4,2	0	0,0	0	0,0
Antibiotics	3	12,5	0	0,0	0	0,0
Vaginal infections	7	29,2*	3	60,0*	0	0,0

Cx = cervix, Va = vagina.
* Fisher's exact test, $p = 0.25$.

the number of d since last menstruation. Samples from women who were HPV positive in the vagina and HPV negative in the cervix were taken at an earlier stage in their menstrual cycle (7 d, SD 1.4), compared to samples from all other women (17.7 d, SD 9.2). However, the first group consisted of only 2 women.

DISCUSSION

In this study we have shown in 2 populations at different levels of risk for acquiring HPV infections that there is a very high concordance between vaginal and cervical HPV prevalence when a polyurethane-tipped swab is used as a tool for taking vaginal swabs by a professional. Sampling was carried out by a professional as a means to standardize sample taking. However, whether this standardization has an impact on concordance is unclear. Only direct comparison of self-sampled vaginal material and vaginal material obtained by a professional can clarify this issue. This has not been done, since it was not a primary aim of this study. The high concordance found compares well with a number of studies using various devices for self-sampling (5–9), although the use of cotton-tipped swabs is less desirable (15). Condom use was higher in the HPV positive group than in HPV negative women. Condom use is generally associated with more risky sexual activity, as is witnessed by significantly more frequent condom use in the STI clinic population. Sex workers are at higher risk for HPV as well as for other STI (16). On the other hand, this finding is in line with the suggestion that condom use does not protect against HPV infection (17). Finally, the difference in HPV prevalence between the sex workers and the general population found in this study is at least partly due to the significantly different age of the 2 groups. It has been shown previously that HPV prevalence is age related (18), and this is also the case in sex workers (19).

Of all possible factors investigated, only (self-reported) vaginal infections seemed to give more discordance. Indeed, it is possible that a vaginal infection speeds up the discharge of (HPV-infected) cervical cells, thereby shortening the period of time these cells are present in the vagina. However, a larger number of women will need to be sampled to substantiate this finding. We are currently performing a larger-sized field study to validate the impact of vaginal infection on cervical and vaginal concordance. There was also a difference in the self-report of the number of d since last menstruation. Samples from the 2 women who were HPV positive in the vagina and HPV negative in the cervix were taken at an earlier stage in their menstrual cycle compared to samples from all other women. A recent study has shown that there are strong fluctuations in the prevalence of HPV in the cervix during a single menstrual cycle, with the highest rate of HPV positivity in the follicular phase (7th to 11th d) (20). If the prevalence of HPV in the vagina also fluctuates, this may have a serious impact on the efficacy and guidelines of self-sampling. Further studies are necessary to investigate the optimal time for self-sampling during the menstrual cycle.

Although the overall agreement in this study was high, there was still better agreement between HPV prevalence in vagina and in cervix in the low-risk GP population than in the high-risk STI clinic population. This was not only caused by a higher number of women negative in both cervix and vagina in the low-risk population, but was also true for the subgroup of HPV positive women (83.3% vs 71.4%). It would seem, therefore, that screening by self-sampling is more appropriate in low-risk populations. However, self-sampling is aimed to include women who presently do not participate in screening, and this is by definition a population at higher risk for cervical cancer. The 90% concordance we found in the high-risk population still suggests that self-sampling would be appropriate and feasible in such a population.

ACKNOWLEDGEMENTS

This study was financed in part by a research grant of the Fund for Scientific Research–Flanders (Belgium) to JBV. We are grateful to all women and doctors who participated in this study. We also like to thank Greet Pattyn, Hilde Lambrechts and Vanessa Deschoolmeester for excellent technical assistance and Aziza el Hachimi for sample logistics.

REFERENCES

1. Macgregor JE, Campbell MK, Mann EM, Swanson KY. Screening for cervical intraepithelial neoplasia in north east Scotland shows fall in incidence and mortality from invasive cancer with concomitant rise in preinvasive disease. Br Med J 1994; 308: 1407–11.
2. Summers A, Fullard B. Improving the coverage and quality of cervical screening: women's views. J Public Health Med 1995; 17: 277–81.
3. Campbell H, MacDonald S, McKiernan M. Promotion of cervical screening uptake by health visitor follow-up of women who repeatedly failed to attend. J Public Health Med 1996; 18: 94–7.
4. Franco EL, Duarte Franco E, Ferenczy A. Cervical cancer: epidemiology, prevention and the role of human papillomavirus infection. Can Med Assoc J 2001; 164: 1017–25.
5. Hillemanns P, Kimmig R, Huttemann U, Dannecker C, Thaler CJ. Screening for cervical neoplasia by self-assessment for human papillomavirus DNA. Lancet 1999; 354: 1970.
6. Wright TC, Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytological screening to detect cervical cancer. JAMA 2000; 283: 81–6.
7. Dzuba IG, Diaz EY, Allen B, Leonard YF, Ponce ECL, Shah KV, et al. The acceptability of self-collected samples for HPV testing vs the pap test as alternatives in cervical cancer screening. J Womens Health Gender Based Med 2002; 11: 265–74.
8. Harper DM, Noll WW, Belloni DR, Cole BF. Randomized clinical trial of PCR-determined human papillomavirus detection methods: self-sampling vs clinician-directed-biological concordance and women's preferences. Am J Obstet Gynecol 2002; 186: 365–73.
9. Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P, et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. Can Med Assoc J 2000; 163: 513–8.
10. Verhoeven V, Avonts D, Meheus A, Leven M, Goossens H, van Royen P. Performance of the recommendations of a British advisory group for screening for chlamydia in a sample of women in general practice. J Med Screen 2003; 10: 14–5.
11. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988; 239: 487–91.
12. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995; 76: 1057–62.
13. Jacobs MV, van den Brule AJ, Snijders PJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A non-radioactive PCR enzyme-immunoassay enables a rapid identification of HPV 16 and 18 in cervical scrapes after GP5+/6+PCR. J Med Virol 1996; 49: 223–9.
14. Muller M, Viscidi RP, Sun Y, Guerrero E, Hill PM, Shah F, et al. Antibodies to HPV-16 E6 and E7 proteins as markers for HPV-16-associated invasive cervical cancer. Virology 1992; 187: 508–14.
15. Lorenzato FR, Singer A, Ho L, Santos LC, Batista RD, Lubambo TM, Terry G. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. Am J Obstet Gynecol 2002; 186: 962–8.
16. Mak R, van Renterghem L, Cuvelier C. Cervical smears and human papillomavirus typing in sex workers. Sex Transm Infect 2004; 80: 118–20.
17. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? Sex Transm Dis 2002; 29: 725–35.
18. Melkert PW, Hopman E, van den Brule AJ, Risse EK, van Diest PJ, Bleker OP, et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. Int J Cancer 1993; 53: 919–23.
19. Kjaer SK, Svare EI, Worm AM, Walboomers JM, Meijer CJ, van den Brule AJ. Human papillomavirus infection in Danish female sex workers: decreasing prevalence with age despite continuously high sexual activity. Sex Transm Dis 2000; 27: 438–45.
20. Van Ham MAPC, Melchers WJG, Hanselaar AGJM, Bekkers RLM, Boonstra H, Massuger LFAG. Fluctuations in prevalence of cervical human papillomavirus in women frequently sampled during a single menstrual cycle. Br J Cancer 2002; 87: 373–6.

Submitted December 29, 2003; accepted April 15, 2004