Journal of Clinical Virology 73 (2015) 1–7

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Daily self-sampling for high-risk human papillomavirus (HR-HPV) testing

Karin Sanner[a,*], Ingrid Wikström[a], Inger Gustavsson[b], Erik Wilander[a], Julia Hedlund Lindberg[b], Ulf Gyllensten[b], Matts Olovsson[a]

[a] Department of Women's and Children's Health, Uppsala University, SE-751 85 Uppsala, Sweden
[b] Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, SE-751 08 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 17 May 2015
Received in revised form
27 September 2015
Accepted 30 September 2015

Keywords:
Cervical cancer
Human papilloma virus (HPV)
Self-sampling
Repeated sampling
Screening

ABSTRACT

Background: Self-sampling for HPV as part of primary screening is a well-tolerated method for women not attending organized Pap smear screening and could increase coverage of cervical cancer screening.
Objective: To investigate if the prevalence of HR-HPV varies from day to day in infected women and if one single sample is reliable for detecting an ongoing infection.
Study design: This is a prospective cohort study on 12 premenopausal and 13 postmenopausal women performing daily self-sampling for HR-HPV testing. They were all HR-HPV-positive 1–3 months ago. Postmenopausal women were sampled for 28 days and premenopausal women sampled during bleeding-free days in one menstrual cycle. A possible difference in viral load between the estrogen-dominated proliferative phase and the progesterone-dominated secretory phase was analyzed.
Results and conclusions: Consistent results throughout the sampling period were observed for 19 women, with either a daily presence of HPV (14 women) or no HPV at all during the sampling period (5 women). Of 607 samples from 25 women, 596 were consistently positive or negative for HPV during the sampling period and 11 were inconsistent (2%). There was no difference in HPV copy number between the estrogen dominated proliferative or progesterone dominated secretory menstrual cycle phases.

The major finding was a high degree of consistency concerning HR-HPV positivity and negativity of HR-HPV in vaginal fluid during a sustained period of daily self-sampling. It does not appear to matter whether the sample is collected in the proliferative or secretory phase.

1. Background

Genital human papillomavirus (HPV) is a common sexually transmitted agent [1–3]. High-risk human papillomavirus (HR-HPV) is detected in most cases of cervical carcinoma worldwide [4] and is present in the vast majority of precancerous cervical lesions [5]. HR-HPV testing has an increasingly important role in cervical cancer prevention programs because of its greater sensitivity compared with cytology for the detection of precancerous lesions [6–8].

Vaginal self-sampling for analysis of HR-HPV does not result in a lower sensitivity compared with clinician-collected cervical samples when using a PCR-based analysis [9], and self- and clinician-obtained samples show highly consistent results irrespective of the method/device used for self-sampling [10–12]. By repeating an HR-HPV test within a few months after a positive primary test result, greater specificity may be achieved compared with conventional Pap smears [13]. Additional advantages of self-sampling include the fact that sampling is easy and quick to perform, and can be done in one's own private setting.

Shifting to HPV self-sampling as part of primary screening requires a test strategy that is both safe and effective. The HPV test has to show consistent results between sample occasions and indicate clinically relevant on-going infections. In previous studies it has been proposed that a number of factors, such as recent unprotected vaginal intercourse and frequent HPV sampling, might affect the amount of vaginal HR-HPV in a sample, but none of these studies has shown that the ability to detect an HR-HPV infection is hampered [14,15]. The effect of sampling during different stages of the menstrual cycle has also been addressed in several studies, with somewhat conflicting results. Some studies have shown a relationship between menstrual cycle phase [14–16] and the amount of HR-HPV detected, while others have not [17,18].

* Corresponding author.
E-mail address: Karin.Sanner@kbh.uu.se (K. Sanner).

http://dx.doi.org/10.1016/j.jcv.2015.09.016
1386-6532/

Table 1
Characteristics of women who performed repeated self-sampling for analysis of HR-HPV ($n = 25$).

		Premenopausal		Postmenopausal		All women	
		n	Percent	*n*	Percent	*n*	Percent
Stable relationship	Yes	8	70	7	54	15	60
	No	4	30	6	46	10	40
Parity	Yes	9	75	11	85	20	80
	No	3	25	2	15	5	20
Induced abortion	Yes	5	42	4	31	9	36
	No	7	58	9	69	16	64
Spontaneous abortion	Yes	2	17	2	15	4	16
	No	10	83	11	85	21	64
Lifetime sexual contacts[a]	≤3	0	0	3	23	3	12
	4–20	9	75	8	62	17	68
	>20	3	25	1	8	4	16
Smoking	No	7	58	6	46	13	52
	Yes	5	42	7	54	12	48

[a] Data not available for 1 (postmenopausal) woman.

2. Objectives

The primary aims of the current study were to determine the prevalence of HR-HPV from day to day in 12 pre- and 13 postmenopausal women, and to investigate if prevalence is influenced by hormonal changes during the menstrual cycle. Short-term fluctuations in virus detection have previously been studied [2,19,20], but to our knowledge there are no previously presented data on repeated daily sampling for HPV testing.

3. Study design

This study is a prospective cohort study where 25 recently HR-HPV-positive women performed repeated daily self-sampling for HPV typing. All women had performed self-sampling 1–3 months ago, either with the Qvintip method [21] or the same test as in this current study, and were found to be positive for HR-HPV. Both postmenopausal and premenopausal women were asked to participate in the study, and all of them had at least one positive HR-HPV test result before they were included. The postmenopausal women collected samples for 28 consecutive days while the premenopausal women collected samples each day from the first to the last bleeding-free day over one menstrual cycle. Most (20/25) of the participating women had not attended an organized Pap-smear program for the last 6 years. One of the premenopausal women (ID no. 11) had amenorrhea as a result of having a levonorgestrel-releasing intrauterine device (LNG-IUD) and another (ID no. 7) used oral contraceptive pills (desogestrel continuously), while none of the others used any hormonal contraception or other hormonal treatment. One of the postmenopausal women (ID no. 23) used hormone replacement therapy (HRT). The majority of the women reported a stable relationship with a male partner. There was a trend towards a higher number of lifetime sexual contacts among the premenopausal women. The frequency of smokers (48%) was high compared with that in the general population, which is about 22% for Swedish women at this age (Table 1).

The sampling period for the premenopausal women was divided into estrogen-dominated proliferative phase and the progesterone-dominated secretory phase in order to test whether the level of vaginal HR-HPV differed between these two phases of the menstrual cycle. Samples obtained from the first bleeding-free day until the day before expected ovulation were assigned to the estrogen-dominated period. The day of ovulation was estimated to take place 14 days before the start of the next bleeding. Samples obtained from day 6 after ovulation until three days before the first day of next bleeding belonged to the progesterone-dominated period. The woman using a levonorgestrel-releasing intrauterine device (LNG-IUD) and the woman using oral contraceptive pills (desogestrel continuously) were not included in this part of the study.

The women were instructed to perform vaginal self-sampling using a Rovers® Viba-brush (Rovers Viba-brush; Rover Medical Devices B.V., Oss, the Netherlands) as previously described [22]. Briefly, all women received written instructions describing the sampling procedure, and those who had not performed self-sampling before also received verbal instructions. The participants were instructed to insert the brush approximately 5–10 cm into the vagina and gently turn it once. The brush was then removed and the sample applied to an indicating FTA elute micro card ™ (Whatman, Inc., Clifton, New Jersey, art. no. WB120411) by placing the brush in the middle of the application area and rolling it one full circle across that area. The sample was then air-dried before a cover was folded over the sampling area for protection during storage and transport.

The FTA micro-elute cards were numbered according to the day in the cycle and sent to the HPV-lab, Department of Immunology, Genetics and Pathology, Uppsala University, for analysis of HPV. The samples were analyzed using a real-time PCR-based assay. This assay detects and quantifies the following 12HR-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 as earlier described [11,23]. The HPV test (denoted hpVIR) was developed as a research tool [23] and has a similar clinical sensitivity and specificity as Hybrid Capture 2 (HC2) [24]. The method of dry collection of cervical epithelial cells has been evaluated by a comparison of the DNA collected by cytobrush and by the FTA micro elute card, and the FTA card has been shown to represents a suitable medium for collection of cervical cell samples [11]. We have previously compared self-sampling and physician collected sampling using the FTA card and shown that the Viba-brush and the FTA micro-elute card are suitable for self-sampling of vaginal cells and subsequent HPV typing [22].

The limit of detection (LOD) of the real-time PCR assay used in hpVIR is a single starting target DNA molecule. However, there are several reasons why such a low number is not feasible to quantitate accurately. The limit of quantification of the hpVIR assays for HPV and the HMBS single copy gene is 10 copies per assay. This limit was set based on generating standard curves using dilution series with known amounts of target molecules. In order to determine if a sample contains sufficient amount of biological material for an HPV test to be considered informative, we used a threshold of 10 copies of the HMBS house-keeping gene per PCR. For the HPV test itself, the sample had to contain a minimum of 10HPV copies to be considered as positive. The hpVIR test is subjected to yearly quality controls by participation in the WHO test panel, and has scored 100% in this yearly evaluation. Since only one HPV test has been used in the present study, we cannot generalize the results to HPV tests with different levels of sensitivity.

During the period when daily samples were collected, the women were asked to complete a diary with information on vaginal intercourse, barrier protection, genital or systemic infection, vaginal discharge or bleeding.

Cervical biopsy samples for histology were obtained from every woman in the study before repeating daily sampling. Women with abnormalities in their histological results and positive for HR-HPV, were treated by means of cervical conization, performed after the sampling period (data not shown).

Wilcoxon's matched pair signed rank test was used to determine if the menstrual phases had different HR-HPV levels. Spearman's rank correlation coefficients were used to estimate the associations between the number of virus copies and the sampling day for each