Vaginal self-sampling is an adequate means of screening HR-HPV types in women not participating in regular cervical cancer screening

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Abstract

In France, about 40% of women aged 25–65 years do not participate in regular screening and thus are at high risk (HR) of cervical cancer. Human papillomavirus (HPV) vaginal self-sampling is a valuable alternative in this population. This study aimed to assess the prevalence of HR and LR (low-risk) HPV infection in 3767 women aged >35 years from mid-socioeconomic backgrounds who carried out HPV vaginal self-sampling at home. HPV vaginal self-sampling was better accepted than the Pap-test in women aged 35–69 years who were previously non-responders to individual invitation. From the 933 self-collected swabs studied (24.7%), 62 were HPV-infected (6.6%), and 73 HPV types were found. HPV 16 was the most frequently found (43.5%), followed by 53 (23.2%), 18 (12.3%), 66 (12.3%), 31 (6.8%), 33 (5.4%) and 58 (2.7%). Ten women (16.2%) were infected by multiple HR-HPV types. Median HPV 16 load was 104.000 copies/10^6 cells and median HPV 18 load was 833 copies/10^6 cells. Six women (9.3%) harboured LR-HPV types. The 12-month follow-up of 43 HR-HPV positive women (69.3%) revealed CIN2–3 lesions in three women (6.9%), all HPV 16 infected, and harbouring an HPV 16 load >5 $\log_{10}$ copies/10^6 cells. Women harbouring HR-HPV types other than HPV 16/18 were older than women harbouring HPV 16/18 types (55 years vs. 46.9 years, p 0.0008). The high frequency of HR-HPV types in women >50 years deserves further investigation to elucidate the mechanism involved (re-infection or reactivation).

Keywords: Cervical screening, HPV self sampling, HPV genotyping, human papillomavirus, participation rate
Original Submission: 4 June 2012; **Revised Submission:** 4 September 2012; **Accepted:** 28 September 2012
Editor: L. Kaiser
Article published online: 22 October 2012
Clin Microbiol Infect 2013; **19:** E44–E50
10.1111/1469-0691.12063

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Introduction

High-risk oncogenic human papillomavirus (HR-HPV) infection is the major risk factor for the development of cervical cancer and cervical intraepithelial neoplasia (CIN) [1].

In France, 3000 new cases of cervical cancer are detected each year, leading to 1000 deaths per year [2]. Cervical cancer screening is performed on an individual basis and about 44% of the target population is not screened [3]. Cytological screening is often stopped at the age of 60 or 65 years, yet cancer incidence and mortality tend to increase from the age of 65 years [4]. According to recent studies, HPV DNA testing, in addition to cytology, could be useful for primary cervical cancer screening [5,6]. Among women aged 35 years or older, primary HPV screening with cytology triage displayed a higher specificity than conventional Pap-smear screening [7]. In addition, HPV 16 viral load correlates with the severity of cervical lesions [8–10].

HPV vaginal self-sampling appears particularly useful for women with infrequent access to gynaecological healthcare [11–13] or refusing cytological screening because of cultural barriers or reluctance to have vaginal speculum examination.

In addition, more than 50% of all cases of cervical cancer are observed among women who do not respond to an invitation to have a Pap-test [14]. Moreover, the quality of self-sampling is usually satisfactory whatever the age. By contrast, in post-menopausal women the transformation zone is scarcely visible and the performances of cervical cytology are markedly decreased [15]. Recently, a preliminary pilot study using

vaginal self-sampling for HPV testing was undertaken in two suburbs in the northern part of Marseille, where the socio-economic level is low [13]. The rate of HPV infection was 23.3%, with 14.1% of HR-HPV types, including 25% of HPV-type 16. In that study, the age of HR-HPV-infected, LR-HPV-infected and multiple HPV-infected women was 42, 48 and 55 years, respectively.

The present study aimed to assess the prevalence of HR- and LR-HPV infection as well as HPV 16 or 18 load in women aged 35 years and older from two mid-socioeconomic cities around Marseille: Vitrolles and Marignane. These two cities have a relatively good healthcare system, but we tested women aged 35–65 years who did not attend regular cytological screenings for cervical cancer (referred to hereafter as non-attending). For this reason, self-collected vaginal swabs were considered valuable tools for detecting HPV infection, especially oncogenic HPV infection. Another objective was to evaluate the compliance with follow-up in this target population.

Methods

Study population: the target population was composed of women aged 25–69 years living in two cities around Marseille (Vitrolles and Marignane) without a Pap-smear recorded in the National Insurance Registry for more than 2 years. A total of 17 330 women were invited to have a Pap-smear by individual mailing, free of charge in a local medical analysis laboratory. The number of women participating in self-sampling HPV tests was, however, limited by the financial support for the study (i.e. 4000 tests).Therefore, 9334 women aged 35–69 years without a Pap-test for more than 2 years (national insurance listing) who had not responded to a first invitation to undergo cytology were randomized into two groups: 4934 women receiving a second invitation to have a Pap-test and 4400 women receiving a proposal to perform HPV self-sampling at home.

The small unbalance between the two groups was due to the limited financial support for self-tests. After exclusion of 633 women for refusal or recent cytological screening, 3767 women remained in the HPV self-sampling arm. The flowchart of the study design is shown in Fig. 1.

Cervical cytology analysis

Among the 4934 women who received a second invitation to have a Pap-test, 4314 women were eligible for Pap-test (reasons for ineligibility were refusal, hysterectomy and pregnancy). Cervical cells were obtained using a cervical brush for conventional cytological slides.

Cytology was carried out at private cytology laboratories, and cytological diagnosis was carried out according to The French National Agency of Health consensus guidelines on the quality control of cervical smear screening [16] and formulated according to the 2001 Bethesda classification [17].Those carrying out cervical cytology analyses were blinded to the result of HPV genotyping.

Vaginal self-sampling and virological analysis

Flocked swabs (Copan Diagnostics, Brescia, Italy) were used as self-sampling devices. The women sampled their vaginal fluid using UTM tubes containing universal transport medium. The collected material was returned via mail in a prepaid envelope to the Department of Virology of the Timone Hospital (Marseille, France) and was stored at −20°C until use. After thawing, 250 μL of vaginal cell suspension was used for DNA purification using the QIAampDNA Mini Kit (QIAGEN, Courtaboeuf, France) modified as follows: samples were incubated at 56°C for 2 h in lysis solution containing proteinase K. The DNA was eluted in 100 μL of elution buffer and stored at −20°C until use. HPV genotyping was assessed by PCR (MY09/ MY11 primers), sequencing, phylogenetic analysis, and cloning if necessary, as described [13]. Quantitations of HPV 16 and 18 positive samples were performed using the quantitative duplex real-time PCR method, as reported [8]. HPV viral load was expressed as the number of HPV copies per million cells.

To follow-up abnormal tests, the virology and cytology laboratories communicated their results to the management centre 'ARCADES' in charge of sending them to the women with recommendations and to their referent physicians. A reminder regarding the management of abnormal results was also sent to these practitioners (gynaecologycal examination and colposcopy in the case of abnormal Pap-test and gynaecological examination and Pap-test if HPV-HR positive). The collection of follow-up data for abnormal tests was also monitored (mailing and telephone call) by the ARCADES centre after 3, 6 and 12 months.

Ethical approval

Ethical approval was given by the ethical committee of 'Sud Mediterranée 2 and Marseille 2' for all randomized studies on HPV testing in the Bouches du Rhône area performed by the ARCADES association.

Statistical analysis

Statistical analysis was performed using the SPSS (version 12.0) and EPI INFO 6 softwares. A Yates's corrected chi-squared test was used to detect differences between groups. A descriptive analysis of the frequency and the distribution of the various