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Type-specific persistence and associated risk factors of human papillomavirus infections in women living in central Italy

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ABSTRACT

Objective: We examined persistence and clearance of human papillomavirus (HPV) infections and risk factors associated with persistence in 79 women based on the results of two sequential tests performed over 12–24 months.

Study design: Between February 2008 and August 2009, 398 women aged 18–63 years were examined for presence of cervical HPV infection by cervical scrape specimen and PCR. Detection was performed using Linear Array (LA) HPV Genotyping Test. All women were interviewed, and a short questionnaire was administered to collect information on socio-demographic characteristics, sexual and reproductive history, smoking habits, oral contraceptive use, history of sexually transmitted diseases, and *Chlamydia trachomatis* or *Mycoplasma* spp. infections. Pearson's χ^2 test was used to verify the association between all independent variables with the response variable.

Results: Initially, high risk-HPV (HR-HPV) and low risk-HPV (LR-HPV) infection was detected in 69.6% and 30.4% of the women, respectively, whilst multiple infections occurred in 53.2%. HPV 16 was the most common (20.2%) high-risk type, followed by 52, 31 and 53. At follow-up, HR-HPV infection was detected in 50.6% of the women; among these, 67.5% had persistent infection, while 12.5% acquired other high-risk types, and 20.0% of those positive for LR-HPV at entry had a new HR-HPV infection. Multiple infections were detected in 38.0% of the women. HPV 16 and 31 were the most frequent types, followed by HPV 73. Type-specific HR-HPV persistence was found in 49.1% of women. HPV 31, 39 and 73 were the most frequently persistent types, whilst HPV 16 was the least persistent.

No significant age difference between women with persistence or clearance was found. The highest HR-HPV persistence occurred in the 22–27 years old group, whereas clearance increased in women aged 28–33 years. No significant association between persistent HR-HPV infection and oral contraceptive use, smoking habits and history of sexually transmitted disease was detected both at entry and follow-up study. The association between *C. trachomatis* or *Mycoplasma* spp. and HPV persistence could not be investigated because of the low detection rate of these microorganisms.

Conclusions: The persistence of HR-HPV infection level was similar to that reported elsewhere, and HPV 31, 39 and 73 showed the highest likelihood of persistence, partially in agreement with other studies. The clinical relevance of the low persistence of HPV 16 and other HR-HPV is unknown. Persistent HR-HPV infection in women aged 22–27 years was in agreement with other authors. To the best of our knowledge, this is the first report on persistence of HR-HPV infections in Italy in a general population, although we examined a small sample in a short follow-up time.

1. Introduction

Human papillomavirus (HPV) infections are among the most common sexually transmitted infections worldwide, and the most important cause of cervical cancer. Genital infection is usually transient, leading to no visible lesions or low-grade lesions that often regress spontaneously. The persistence of high-risk HPV (HR-HPV) infection is a key factor for the development of cervical cancer [1,2], and detection of viral persistence can be used to identify women at greatest risk of cervical precancer [3]. Although there is a wide variation in definitions of HPV persistence and there is no consensus regarding what exactly constitutes a "persistent infection", persistence is often defined in term of duration of a type-specific infection [1,4]. Moreover, HPV persistence is most commonly defined as two or more HPV positive time points; consecutive HPV positive visits are generally required for persistence, and the median time of the testing interval is 6 months [4].

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The reasons why most HPV infections resolve without further disease whereas some persist with an elevated risk of precancer are unknown. Studies that examine the natural history of HPV infections, however, will aid in identifying associations between HPV type, persistence and progression to precancer, and risk factors associated with viral persistence. Additional risk factors implicated in viral persistence and etiology of cervical cancer include smoking habits, high parity, long term oral contraceptive use, and coinfection with other sexually transmitted diseases [5].

The aim of this study was to investigate persistence and clearance of cervical HPV infection and risk factors associated with persistence in women who had type-specific HPV testing based on results from two sequential tests performed over 12–24 months.

2. Materials and methods

2.1. Study population

Molise (central Italy) is the second smallest Italian region, with a population of about 97,500 women aged 18–63 years (total population: 321,000). Between February 2008 and August 2009, 398 women aged 18–63 years who consecutively visited the Molise Region main hospitals (Campobasso, Isernia, and Termoli) for routine Pap smear were examined for the presence of HPV infection. Women with cervical lesions were not included in this study. The initial results of this prevalence study have already been reported [6]. HPV was detected in specimens from 129 women, who were telephoned and invited to participate in a follow-up study, consisting of a second cervical scrape specimen collection 12–24 months from first recruitment, using the same procedure as the initial examination. All patients were advised about the methods and objectives of the research and signed an informed consent.

Seventy-nine women (61.2%) completed the survey and were tested for HPV: the mean follow-up time was 20 months (range 9–28 months). Fifty women (38.8%) were lost to follow-up, represented by 24 women with cervical lesions, and another 26 who decided to have further examinations independently. A comparison of the main socio-demographic characteristics (mean age, smoking habits, oral contraceptive use, pregnancy history) and clinical features (HR-HPV types, LR-HPV, past infections) between the enrolled ($n = 79$) women and those lost to follow-up ($n = 50$) was performed, in order to exclude any selection bias. No significant differences between the two groups were found (χ^2 test, $P > 0.05$).

At the time of collection of the cervical scrape specimen, all women were interviewed in person by trained female nurses, and a short questionnaire was administered to collect information on socio-demographic characteristics, sexual and reproductive history (lifetime number of sexual partners, parity), smoking habits, oral contraceptive use and history of sexually transmitted diseases.

2.2. Defining HPV persistence

Conceptually, HPV persistence is defined as the length of time during which an individual is infected with HPV (i.e., the duration the infection persists). Because a woman can become infected with one or more types of HPV, persistence is often defined in terms of the duration of a type-specific infection [7]. Persistent type-specific HPV infection was defined as the detection of the same HR-HPV type at both examinations (first study and follow-up study). Clearance was defined as the proportion of women who were initially HR-HPV positive (at entry), but the same HR-HPV type was not found at the follow-up.

2.3. Detection and genotyping of HPV

Specimens were collected using a cytobrush and suspended in PreservCyt collection medium (Hologic Inc., Marlborough, MA) following the manufacturer's instructions. HPV was detected using the Linear Array (LA) HPV Genotyping Test (Roche Molecular Systems Inc., Branchburg, NJ, USA), following the manufacturer's instructions as previously described [6]. This test utilizes amplification of target DNA by PCR and nucleic acid hybridization for the detection of thirty-seven anogenital HPV genotypes.

2.4. Detection of Chlamydia trachomatis and Mycoplasma spp.

DNA extracted for research on HPV at baseline and at follow-up was also used for detection of *Chlamydia trachomatis* and *Mycoplasma* spp.

C. trachomatis was detected by PCR using NLO and NRO primers [8], which amplify the 1128 bp fragment of the *omp*1 (outer membrane protein 1) gene. Additionally, the presence of *Chlamydia* was assessed by using the Chlam-T (AB Analitica, Padova, Italy) kit, based on nested PCR to identify cryptic plasmid DNA, which is highly conserved in all serotypes of *C. trachomatis*. Moreover, a forward primer, My-ins, and two reverse primers MGSO and UGSO, were used to amplify an approximately 520 bp region of 16S rRNA gene of mycoplasmas and ureaplasmas [9], respectively.

2.5. Statistical analysis

For our purpose, in this study HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were grouped as high-risk, including probable/possible carcinogens HPV types 26, 53, 66, 67, 68, 70, 73, and 82 [10]; HPV types 6, 11, 40, 42, 54, 55, 61, 62, 64, 69, 71, 72, 81, 83, 84, IS39 and CP6108 were grouped as low-risk (including those with unknown risk).

Descriptive statistics were used for the analysis of persistence and clearance of type-specific HPV infection. Particularly, Pearson's χ^2 test was used to verify the association between all independent variables and the response variable. Univariate survival analysis was based on the Kaplan–Meier method with log-rank test statistics to estimate the median time to HPV persistence and clearance among women who were HPV positive at baseline, compared with women who had persistent HR-HPV infection until the end of follow-up. P values less than 0.05 were regarded as statistically significant. Statistical analyses were performed using SPSS software for Windows (version 20.0).

3. Results

3.1. Baseline characteristics at entry and risk factors

The baseline characteristics of all 79 women in the study are described in Table 1. Mean age was 33.1 years (range 22–53). Initially, HR-HPV infection was detected in 55 women (69.6%), while LR-HPV infection was found in 24 women (30.4%). The mean age of the HR-HPV positive women was 32.2 years (range 22–51). Multiple HPV infections were detected in 53.2% (42/79), and almost half of the multiple infections consisted of two HPV types (19/42, 45.2%), while the remainder had from three to seven HPV types. There was no significant association between oral contraceptive use, history of sexually transmitted disease, *Mycoplasma* and *Chlamydia* infections and HR-HPV detection. Smoking was more common in women with HR-HPV but this was not statistical significant. The number of lifetime sexual partners >1 and multiple infections significantly correlated with HR-HPV presence ($P < 0.005$) while a negative association was found with pregnancy ($P < 0.05$). Fifteen HR-HPV types were detected in the study