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Prevalence and distribution of single and multiple HPV infections in cytologically abnormal cervical samples from Italian women

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Abstract

The prevalence of single and multiple HPV infections was assessed over a cohort of 213 women with cytological abnormalities and its association with cervical neoplasia established. Roche linear array HPV genotyping test was used to identify HPV genotypes. The most prevalent HPV genotypes in cervical cancer samples were HPV16 (61.2%), HPV52 (16.1%), HPV18 (12.9%) and HPV 31 (9.6%). Multiple HR and LR HPV infections, comprising between two and 5+ HPV types, were identified in 49.7% of samples, with a significantly lower number in severe dysplasia and cervical cancer samples ($p < 0.05$).

These results seem to indicate that detection of multiple HPV infection with HR-HPV types is not significantly better as a predictor of cervical cancer than single HR-HPV infection, though further longitudinal studies are needed to better clarify the relevance of these infections to the progression of cervical neoplasia.

Keywords: HPV; Cervical cancer; Genotypes

1. Introduction

After breast cancer, cervical cancer is the second most important worldwide cause of cancer-related mortality in women (Boyle et al., 2003). Persistent infections with human papillomavirus (HPV) are identified as a necessary, although not exclusive risk factor in the development of cervical carcinoma. Of the many HPV types, some are known to pose a greater oncogenic risk, because of the epidemiologic associations between individual HPV types and premalignant or invasive cervical lesions (Lorincz et al., 1992; Sasagawa et al., 2001; Bosch et al., 2002) and due to the type-specific prevalence of HPV in invasive cancer (Walboomers et al., 1999; Bosch et al., 1995).

Among women infected with HPV, concurrent infection with more than one HPV type is observed in about 20–50% of infected subjects (Liaw et al., 2001; Franco et al., 1999).

In particular, coinfection with multiple HPV types has been observed more frequently among younger women (Molano et al., 2002; Castellsague et al., 2001) and patients with cytological abnormalities (Ho et al., 1998; Herrero et al., 2000). HPV coinfections also occur more often among women with HIV infections (Palefsky et al., 1999; Levi et al., 2002). In some studies, these infections have been associated with a higher risk of cervical intraepithelial neoplasia (Sasagawa et al., 2001; van der Graaf et al., 2002). Other studies report no increased risk of cervical intraepithelial neoplasia or cervical cancer among women with multiple infections, as compared to women with single HPV infections (Herrero et al., 2000; Bosch et al., 2002).

Vaccination against HR-HPV types is a primary prevention measure. However, the antibody response conferred by vaccines appears to be type specific (Unckell et al., 1997; White et al., 1998), so that vaccinated subjects would be protected only against the HPV types targeted by the vaccine.

An important issue, therefore, is the composition of vaccines and in particular the number of HPV types against which they offer protection. Clinical trials evaluating the vaccine's efficacy include a quadrivalent vaccine that consists of recombinant

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viral-like particles (VLPs) of HPV 6, 11, 16, 18 (Speck and Tyring, 2006; Ljubojevic, 2006).

Vaccination policies increase concerns about the consequences of removing certain genotypes from human populations. In fact, their removal by type-specific vaccination could result in positive selective pressures on untargeted genotypes, thus increasing their prevalence or decreasing their frequency as a result of cross-type immunity (Da Silva et al., 2001).

Since little is known about the occurrence of infections with multiple HPV types and their dynamics across various types, studying the occurrence of coinfection with multiple HPV types and the interactions/association between individual types may shed new light in this area.

In this study the extent of multiple HPV infection was investigated retrospectively in women with abnormal cytology and then compared with histological diagnosis. The results will provide baseline data for a subsequent longitudinal study that will target the impact of single/multiple HPV infections and HPV type-specific persistence on the progression of cervical neoplasia.

2. Materials and methods

2.1. Population

In the present study, cervical scrape specimens were obtained from women aged 19–70 years (median age 35.6) who visited Brescia's main hospital (Spedali Civili) for routine cervical screening between May 2005 and May 2006. An average of 3900 women were screened during this period. Of these, 3500 were considered eligible for the study if they fulfilled the following criteria: (a) were not currently pregnant and were at least 2 months post partum, (b) had an intact uterus and no current referral for hysterectomy, (c) had never been treated for SILs, and (d) had no history of chronic illness (e.g. renal failure, diabetes, cancer or gastrointestinal malabsorption). We included all patients with a PAP smear classified as abnormal according to the Bethesda System (TBS, 1991). A total of 213 women out of 3500 (6.08%) met these criteria and were enrolled in the study. They were all referred for colposcopic examination and HPV testing. All patients provided informed consent for the tests.

2.2. Citology

Cytological classification was performed by experienced cytopathologists and reported according to TBS 1991, scoring atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), low grade squamous intra-epithelial lesion (LSIL), high-grade intra-epithelial lesions (HSIL) and invasive carcinoma.

2.3. Colposcopy

Colposcopic examination of the cervix was performed on all patients by several experienced colposcopists, using an agreed protocol. Lesions in the transformation zone (TZ) were assessed by applying a 5% acetic acid and iodine solution under 8×12 magnification. If colposcopy proved unsatisfactory, further exploration of the endocervix was carried out under $20\times$ magnification using a Koogan speculum. International IFCPC nomenclature was used to classify the colposcopic pattern as either: normal; abnormal TZ (ATZ), with minor changes suggesting low grade CIN (CIN1); ATZ with major changes suggesting CIN 2–3; or cancer.

2.4. Biopsy and histology

All of the 213 women underwent punch biopsy. The pathological diagnosis of cervical lesions was performed according to a five-grade framework: CIN I (grade I cervical intraepithelial neoplasia, characterised by condilomatous lesions and/or light dysplasia); CIN II (moderate dysplasia); CIN III (severe dysplasia); carcinoma *in situ*; invasive carcinoma under the Bethesda model. Histological diagnoses were used as reference standard.

2.5. HPV DNA detection

2.5.1. Specimen preparation

Specimens were collected into 20 ml of PreservCyt LBC media (ThinPrepliquid PAP vial; Cytyc Corporation, US). For isolation of nucleic acid, 250 μl of material were used with a Total Nucleic Acid isolation kit (Roche) as described by the manufacturer. Nucleic acid was resuspended in a final volume of 100 μl; 10 μl were used for PCR analysis.

2.5.2. PCR amplification of HPV DNA

The LINEAR ARRAY HPV genotyping test (Roche Molecular Systems, Inc., Branchburg, NJ, USA) employs biotinylated primers to define a sequence of nucleotides within the polymorphic L1 region of the HPV genome, which is approximately 450 bp long. A pool of HPV primers is used to amplify HPV DNA from 37 HPV genotypes, including 13 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). An additional primer pair targets the human β-globin gene as a control for cell adequacy, extraction and amplification. Amplification was performed following the manufacturer's instructions.

Following PCR amplification, the hybridization reaction and detection were performed according to the manufacturer's instructions.

2.6. Statistical analysis

All statistical analyses were performed using a χ^2-test. All p values < 0.05 were considered statistically significant.

3. Results

3.1. Cytological diagnosis

The mean age of the 213 women included in the study was 35.5 years (range 19–70). Of these, 41 (19.2%) patients had an ASCUS lesion, 96 (45%) a LSIL and 55 (25.8%) a HSIL. Three patients (1.4%) had a carcinoma *in situ*, one patient (0.46%) had an invasive cervical cancer and 17 samples (7.9%) were