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HPV genotype prevalence in cytologically abnormal cervical samples from women living in south Italy

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Abstract

Human papillomavirus (HPV) infection is the commonest sexually transmitted infection, and high-risk HPV types are associated with cervical carcinogenesis. This study investigated: the HPV type-specific prevalence in 970 women with an abnormal cytological diagnosis; and the association of HPV infection and cervical disease in a subset of 626 women with a histological diagnosis. HPV-DNA was researched by nested PCR/sequencing and the INNOLiPA HPV Genotyping assay. The data were analysed by the chi-square test ($p \leq 0.05$ significant).

Overall, the HPV prevalence was 37.7%; high-risk genotypes were found in 88.5% of women and multiple-type infections in 30.9% of the HPV-positive women. The commonest types were HPV-16 (8.2%), HPV-6 (5.0%), HPV-51 (4.2%) and HPV-53 (3.6%). Among the women with histological diagnosis, HPV was evident in 19.9% of those without lesions, 65.8% of those with low-grade lesions and 100% ($p = 0.002$) of those with high-grade lesions. The commonest types were HPV-16 (in 14.7% low-grade and 42.8% high-grade lesions), HPV-31 (4.7% and 14.3%, respectively) and HPV-33 (2.0% and 14.3%, respectively). Two high-grade lesions contained exclusively one uncommon type, namely, HPV-83 and -85. This study confirmed the high prevalence of HPV infection and high-risk genotypes among women with cervical abnormalities living in Italy. These data may contribute to increasing the knowledge of HPV epidemiology and designing adequate vaccine strategies.

Keywords: Genotypes; Distribution; Cervical; Samples

1. Introduction

Genital human papillomavirus (HPV) infection is the most common sexually transmitted viral infection worldwide (Baseman and Koutsky, 2005). It is associated with various clinical conditions, ranging from asymptomatic infections to benign and malignant diseases of the genital mucosa (Clifford et al., 2003). At present, more than 140 HPV types have been identified, of which about 40 infect the genital tract (de Villiers et al., 2004). On the basis of phylogenetic and epidemiological characteristics, they are grouped as low-risk HPV (LR-HPV: e.g., HPV-6 and -11), commonly associated with low-grade cervical lesions; and high-risk HPV (HR-HPV: e.g., HPV-16 and -18), associated with high-grade lesions and cancer (Bosch and Munoz, 2002; zur Hausen, 2002).

The prevalence of HPV infection varies greatly around the world, being dependent on the target population and the severity of the disease (Baseman and Koutsky, 2005). Variations have also been reported in the distribution of the different HPV types. Even though HPV-16 is the most frequently found type in healthy women and patients with cervical cancer, viral genotypes other than HPV-16 display a heterogeneous distribution (Munoz et al., 2003; Vaccarella et al., 2006a). In light of the recent development of HPV-type-specific prophylactic vaccines (Villa et al., 2005), epidemiological studies in different geographical areas are, therefore, required in order to assess the role of HPV infections in different countries. At present, little is known about the prevalence and distribution of HPV types in Italy. Only a few studies, mainly based in northern Italy, have investigated the prevalence of cervical HPV infection in healthy women (Centurioni et al., 2005; De Francesco et al., 2005; Ronco et al.,

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2005) and in women with cytological abnormalities (Gargiulo et al., 2007; Rassu et al., 2005; Tornesello et al., 2006). Moreover, most of the studies have researched the presence of the most common HR-HPV types, without identifying other less frequent viral genotypes.

The reliable identification of HPV types is relevant to assessing the epidemiological and clinical role in HPV-related cervical disease. To date, various PCR-based HPV assays have been described and compared (Husnjak et al., 2000). Of these, one of the most sensitive is the nested PCR with the MY09/11 and GP5+/6+ primer pairs (Giovannelli et al., 2004; Strauss et al., 2000). After the amplification step, HPV typing is performed by one of several methods, such as the direct cycle sequencing or DNA hybridisation. Recently, a general PCR, with SPF10 primers, has been developed (Kleter et al., 1999), where the amplimer size is only 65 bp, whereas the amplimer sizes of GP5+/6+ and MY09/11 are 150 and 450 bp, respectively. Since the sensitivity of DNA detection by PCR is inversely related to the size of the amplimer, the SPF10 primers permit highly sensitive, broad-spectrum HPV detection (Melchers et al., 1999). After amplification, SPF10 PCR products are analysed for HPV typing by a reverse line probe hybridization assay (LiPA) with HPV type-specific probes (commercially available assay INNO-LiPA HPV Genotyping, Innogenetics). The SPF10/LiPA has also been reported as an extremely sensitive method for the simultaneous detection of multiple HPV types (Kleter et al., 1999; van Hamont et al., 2006). However, due to the wide heterogeneity of HPV types infecting the genital tract, limitations regarding the different efficiency of the various HPV PCR assays are still evident, and it has been reported that the true HPV prevalence may be underestimated if only one method is used (Qu et al., 1997).

The aim of this research was to evaluate the prevalence of HPV types in a cohort of women with abnormal cervical cytology in Palermo, south Italy, by means of the combined use of nPCR/direct sequencing and the SPF10 PCR/LiPA assay. A further aim was to establish the distribution and association of HPV types in a subset of histologically confirmed cervical lesions.

2. Materials and methods

2.1. Study population

The analysis involved a total of 1000 women who consecutively came into the Virology laboratory at the Department of Hygiene and Microbiology (Policlinico University of Palermo, Italy) between October 2003 and May 2005, with a request for HPV testing. These women had been attending different gynaecology outpatient clinics or private practice gynaecologists and were then referred to colposcopy and HPV testing for an abnormal Pap smear. All the women brought with them a cytological diagnosis. The cytology had been reported according to the Bethesda system, and thus classified as atypical squamous cell of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL). A subset of women ($n = 626$) provided addition information regarding the histological diagnosis subsequent to the Pap tests and this information were used to examine the relationship between HPV infections and histologically diagnosed cervical lesions. The study was approved by the informed consent of each subject participating in the study.

2.2. Sample collection and processing

Cervical cells were obtained with the combined use of an Ayre's spatula and an endocervical cytobrush, and placed into 20 ml of PreservCyt Solution (Cytyc). The cells were washed with phosphate-buffered saline, pelleted by centrifugation at 13,000 rpm per 5′, the pellet resuspended in 200–500 μl of digestion solution (10 mM Tris–HCl, pH 8.3, 0.45% NP40, 0.45% Tween 20) with 200 μg/ml proteinase K and incubated at 60 °C for 2 h. The proteinase K was inactivated at 95 °C for 10′. Clinical samples were checked for DNA by standard amplification of the human β-globin, as described elsewhere (Giovannelli et al., 2004). In brief, PCR for β-globin was performed in a final reaction volume of 50 μl, containing 10 μl of isolated DNA, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mmol/l $MgCl_2$, 0.001% gelatin, 200 μM each deoxynucleoside triphosphate, 50 nM of each primer PC04 and GH20, 1 U of AmpliTaq Gold™ polymerase (Applied Biosystems). The PCR conditions were as follows: preheating for 8 min at 95 °C was followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C and a final extension of 5 min at 72 °C. Ten microlitres of a crude digest of β-globin positive specimens were used for HPV testing.

2.3. nPCR amplification and direct sequencing

The amplifications were carried out in a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany), and all PCR products analysed by electrophoresis in 8% polyacrylamide gel. Amplification controls were: a blank control, a negative control (human Wi-38 cell line) and a positive control (SiHa and HeLa dilutions corresponding to 100 HPV-16 and -18 DNA copies). The nPCR with MY09/11 and GP05+/GP06+ primers was performed as described elsewhere (Giovannelli et al., 2004). The HPV genotyping procedure was based on direct sequencing of amplification fragments. The amplification products were purified by Microcon YM-100 Filter Devices (Amicon, Millipore), and about 5 ng of nPCR product was added to 4 μl of Terminator Ready Reaction mix, 4 μl of 0.8 μM primer, and deionised water to a final volume of 20 μl. The cycle incubator program consisted of 25 cycles of 30 s at 96 °C, 45 s at 45 °C and 1 min at 60 °C. The purification of reaction mixtures was performed by Centrisep Spin Columns (Princeton Separations, NJ) and the mixture was analysed on an ABI Prism 310 Genetic Analyzer, (PerkinElmer Applied Biosystem). Alignments were obtained from the on-line BLAST server (Giovannelli et al., 2004); HPV genotypes were considered as low- or high-risk, according to two recently published HPV classifications (de Villiers et al., 2004; Munoz et al., 2003).

2.4. SPF10 PCR/LiPA assay

In some samples, infection with multiple HPV types was suspected due to the presence of numerous ambiguous and/or