Single and multiple human papillomavirus infections in cervical abnormalities in Portuguese women

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Abstract

Persistent infection with high-risk (HR) human papillomavirus (HPV) types is necessary for cervical cancer development. However, little is known about the influence of multiple HPV infections on cervical lesion risk. The aim of this study was to evaluate the frequency of single and multiple HPV infections in Portuguese women, and to assess the frequency of multiple infections in cervical intraepithelial neoplasia (CIN). HPV prevalence, type-specific prevalence and extent of multiple infections were assessed in 1057 cervical samples. The Clinical Array HPV assay was used to detect 35 HPV types. According to histological diagnosis, 425 samples were normal, 375 were CIN1, and 257 were CIN2+. HPV status was studied in relation to age and lesion severity. The prevalence of HPV infection was 52.7%; 25.4%, 67.2% and 76.7% were positive for any HPV type in the normal, CIN1 and CIN2+ cases, respectively. Among HPV-positive cases, 32.0% were associated with multiple infections. Among multiple infections, 96.1% harboured HR HPV types and 38.2% HR–low risk (LR) HPV types. Overall, 33 different HPV types (18 HR and 15 LR) were detected. HR HPV types (44.1%) were significantly more prevalent than LR HPV types (8.6%). The most frequent genotype was HPV 16 (25.5%), followed by HPV 31, 53, 66, 58, and 51. Multiple infections showed a significant increase (p 0.005) according to severity of neoplasia, particularly for HR–HR HPV infections (p 0.003). No association between age and multiple HPV infections was observed (p 0.812). However, multiple HR HPV infections were more frequent in women under 30 years of age (35.3%).

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Introduction

Cervical cancer is the third most common malignancy of the female genital system, and is the second most common cancer in women worldwide [1].

Clinical and subclinical human papillomavirus (HPV) infections are the most common sexually transmitted infections in the world. On the basis of molecular epidemiological evidence, HPV types can be classified into high-risk (HR) types, which are frequently associated with the development of premalignant and malignant epithelial lesions of the cervix, and low-risk (LR) types, which are found mainly in condylomata acuminata (genital warts) and benign lesions, including a large proportion of low-grade squamous intraepithelial lesions [2,3].

Portugal has a relatively high incidence rate for cervical cancer (13.5/100 000) and one of the highest mortality rates (4.5/100 000) in the European Union [4], mainly because of a limited population-based screening programme for cervical cancer.

Persistent HR HPV infection has been well established as the central cause of cervical cancer [2,5,6]. For this reason, interest has increased in the detection of HPV DNA for primary cervical screening [7–9]. The development of new commercial assays has improved the quality of HPV detection and genotyping, and revealed the presence of multiple HPV infections in women with and without cytological abnormalities. To date, 20–50% of HPV-positive women have been reported to be infected with multiple HPV types [10–12]. Co-infection with more than one HPV type has been observed more frequently among young women and among those with cytological abnormalities or an impaired immune response [6,13–21].

However, the clinical importance of co-infection with multiple HPV types remains a controversial area of investigation. In some studies, these infections have been associated with persistent infections and with a higher risk for cervical intraepithelial neoplasia (CIN) [14,16,18,19,21,22], whereas other studies have reported no increased risk for CIN or cervical cancer development in women infected with multiple HPV types, as compared with women with single-type infections [6,13,15,20].

In Portugal, limited data are available concerning the distribution of HPV genotypes as single or multiple infections. Therefore, the main purpose of this study was to evaluate the frequency of single and multiple HPV infections in Portuguese women, and to assess the frequency of multiple infections in CIN.

The identification of multiple HPV types in different grades of cervical neoplasia may provide important information about the impact of single/multiple HPV type-specific associations as a risk factor for the persistence and progression of cervical neoplasia, and could be helpful for appropriate cervical cancer prevention strategies.

Materials and Methods

Clinical samples

This study was carried between January 2006 and December 2008 in the Lisbon area and in the southern region of Portugal. The study population comprised 1500 sexually active women, attending primary healthcare clinics of the National Health Service and gynaecological outpatient clinics, who were referred to the National Institute of Health for opportunistic screening and for evaluation of HPV-associated lesions. Although not representative of the general population, the studied group includes a high proportion of women at risk for cervical cancer and a broad range of outcomes. Women who were pregnant, had undergone hysterectomy or had been treated for CIN within the last 12 months were excluded.

Cervical cell samples were obtained with a cytobrush during clinical examination for cytological analyses (Pap smears). The residual exfoliated cells were stored at −20°C until DNA extraction. The final diagnosis of the women with abnormal cytology was made by histological evaluation on colposcopically directed biopsies. Outcome was defined as normal (normal cytology and/or negative biopsy), CIN1 or CIN2 or worse (CIN2+) on cytological/histological examination [23]. Both diagnoses (cytological and histological) were confirmed by experienced pathologists.

All women provided voluntary written informed consent for the tests and answered a questionnaire. The study protocol was approved by the sponsored competent ethical review committees.

HPV DNA detection

DNA was extracted manually from all cervical specimens. Cell suspensions were digested with 100 μg/mL proteinase K for 3 h at 56°C, and purified by spin-column chromatography. Nucleic acids were resuspended in a final volume of 100 μL and stored at −20°C until use for PCR amplification. Genotyping was performed by using the Clinical Array HPV Assay (now CLART HPV 2; Genomica, Madrid, Spain), according to the manufacturer's procedures. This methodology uses biotinylated primers that amplify a 450-bp fragment within the HPV L1 region. Co-amplification of an 892-bp region of the cystic fibrosis transmembrane conductance regulator gene and a 1202-bp fragment of a transformed plasmid provides a control to ensure DNA extraction adequacy and PCR efficiency. Amplicons are detected by hybridization in a low-density microarray containing triplicate DNA probes specific to 35 types (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89). Semiquantitative results can be obtained in an automatic reader.

In this study, HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82 were considered as HR HPV (including probable and possible HR types), and HPV 6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 85 and 89 as LR HPV (including unclassified types) [2,24].

Statistical analysis

To determine the prevalence of HR HPV and LR HPV types, cases were counted more than once if they harboured a multiple infection with a mixture of both. The prevalence of individual HPV types was determined as they appeared in single or in multiple infections. Multiple HPV infection was defined as two or more HPV types detected. For multiple HPV infections, the proportion was assessed in relation to severity of lesion, and compared with data from women harbouring a single HPV infection. Data were analysed using the chi-squared test, and, when appropriate, Fisher's exact test to calculate all p-values. The ORs, together with 95% CIs, were computed with the use of 2×2 contingency tables. All analyses were performed with SPSS version 17.0 software. Differences were considered to be statistically significant when p-values were <0.05.

Results

Of the 1500 selected women, 443 were excluded because they were not eligible or were considered to be inadequate