

Human papilloma virus (HPV) genotypes prevalence in a region of South Italy (Apulia)

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Abstract

Introduction. Since human papillomavirus (HPV) is the central casual factor in cervical cancer, understanding the epidemiology and geographical area distribution of the most prevalent HPV genotypes constitutes an important step towards development of strategies of prevention.

Aim. The aim of this study was to investigate the prevalence of HPV infection and to determine HPV types distribution among 822 HPV positive women and some sexual male partners in Apulia (Italy).

Methods. HPV DNA detection and genotyping was performed by nested-PCR for the L1 region and reverse line blot hybridization allowing the specific detection of 24 HPV genotyping both high risk (HR) and low risk (LR).

Results. The most prevalent HPV genotypes were HPV 16 (35%), HPV 31 (16%) HPV 6 (9%), HPV 58 and 66 (7%), followed by HPV 33 (6%), HPV 18 and 56 (4%), HPV 70 and 45 (3%), HPV 53 and 11 (2%). Currently 1.5% of tested specimens remained unclassified. Multiple infections with at last two different high-risk HPV genotypes were observed in 10% of specimens.

Conclusions. This finding adds knowledge to HPV epidemiological investigation, and addresses further studies aimed to consider public health for identifying groups at risk for cervical cancer.

Key words

- human papillomavirus (HPV)
- DNA virus
- sexually transmitted disease
- HPV test
- Pap smear

INTRODUCTION

Human papillomavirus (HPV) is a wide family of DNA viruses that can infect basal epithelial cells of the skin or inner-lining tissues and are categorized as cutaneous types or mucosal types [1, 2].

The main areas of research include the biological characteristics of HPV, epidemiology, and related diseases, prevention strategies through appropriate barrier contraception, pap smear screening, and the potential role of vaccines [3, 4].

Cutaneous HPV types are epidermotropic and infect the keratinized surface of the skin, targeting the skin of the hands and feet; on the other hand, mucosal types infect the lining of the mouth, throat, respiratory, or anogenital tract epithelium.

As reported from WHO, HPV infection is one of the

most common sexually transmitted infections, generally asymptomatic, with the worldwide prevalence in women with normal cytology of 11.4% (11.3-11.5%; 95% CI) [1].

In fact women are more susceptible to the oncogenic effect of HPV, mostly at the genital site and on the uterine cervix [5, 6].

The anatomical characteristics of the female genital tract, mainly in the genital mucosal histology, may in part explain female vulnerability for clinical sequelae.

Epidemiological studies suggest that about 80% of infected women will have acquired genital HPV by age 50, which makes HPV infection the norm rather than the exception [7-9].

However, sexually active women less than 25 years of age consistently have the highest rates of infection [10-12].

Age-standardized HPV prevalence worldwide has been shown to fluctuate, nearly 20 times between populations, from 1.4% in Spain to 25.6% in Nigeria [6].

Based on their association with cervical cancer and precursor lesions, HPV can also be classified as high-risk (HR-HPV) and low-risk (LR-HPV) oncogenic types [10, 11].

LR-HPV types, such as HPV 6 and 11, can cause common genital warts or benign hyperproliferative lesions with very limited tendency to malignant progression, while infection with HR-HPV types, highlighting HPV 16 and 18, is associated with the occurrence of pre-malignant and malignant cervical lesions [10].

HR-HPV types are also associated with many penile, cervical, vulvar, anal, and head and neck carcinomas, and contribute to over 40% of oral cancers [11].

HPV genome, associated with histone-like proteins, is protected by a capsid formed by two late proteins, L1 and L2 [8].

The taxonomic status of HPV types, subtypes, and variants is based on the sequence of their L1 genes, which differ from each other by at least 10%, 2-10%, and 2%, respectively [12, 13].

The critical molecules for initiation and progression of cancer are the oncoproteins E5, E6, and E7, that act largely by overcoming negative growth regulation by host cell proteins and by inducing genomic instability, a hallmark of HPV associated cancers [14-16].

When immunocompetence is weakened and/or the virus belongs to one of the more aggressive oncogenic subtypes, cancer may finally occur [17].

Most HPV infections are "cleared" by the immune system and do not result in clinical diseases [13].

The diagnosis of HPV infection and the clinical consequences can be made following an abnormal smear test or HPV testing.

Cervical HPV-related lesions are typically asymptomatic, but in the case of invasive diseases, some symptoms like atypical vaginal blood losses, smelly vaginal discharge, urinary or anorectal symptoms, and weight loss could arise as consequences of malignant proliferation and cancer.

For genital warts, visual inspection is normally sufficient for diagnosis.

In this study, we investigated the most prevalent genotypes in a large group of population living in Apulia Region (South-East Italy), composed of infected women and their partners.

MATERIALS AND METHODS

In this study whose duration was from January 2008 to December 2014, a total of 822 Apulian HPV positive women and some sexual male partners were included in the study. The mean age of the patients was 42.3 years (range, 25 to 65 years).

In details, we analyzed 768 women and 54 men, partners of infected women.

Informed consent was obtained from all participants at the time of sampling.

For DNA based study cells were collected by Ayer's spatula scraping from both female cervix and male urethra.

Then the spatula were dipped in sterile phosphate buffer saline (PBS pH 8.0) and 200 µl of this solution were centrifuged (1000 g × 10 min). The cell pellet was re-suspended in 400 µl of sterile PBS buffer and preserved at 4 °C for further analyses.

HPV DNA detection and genotyping was performed by NESTED-PCR for the L1 region and Reverse Line Blot Hybridization allowing the specific detection of 24 HPV genotyping both high risk (HR) and low risk (LR) [18, 19].

DNA extraction and purification was performed by automated QIAcube System (QIAGEN, Germany) following manufacturer protocols.

Then HPV DNA detection was done by NESTED-polymerase chain reaction (PCR) amplification with the use of the AMPLIQUALITY HPV-HS Bio Kit (AB ANALITICA, Italy) following manufacturer instructions.

Briefly, the method consists of a first amplification of the viral genome L1 region of the was amplified, followed by a second NESTED amplification with biotinylated primers.

To assess the quality of extracted DNA, thiosulfate sulfurtransferase (TST) gene region (202 bp) was amplified at the first amplification; PCR products were analyzed using 3% agarose gel electrophoresis with ethidium bromide staining to visualize the DNA under ultraviolet light: poor or no TST amplification indicated a lack of sufficient cellular material for PCR or the presence of PCR inhibitors.

Reverse Line Blot Hybridization was completed with AMPLIQUALITY HPV-TYPE Kit (AB ANALITICA, Italy).

Multiple HPV infection was defined as two or more HPV types.

When determining the prevalence of HR-HPV and LR-HPV types, men were counted more than once if they harboured multiple infections.

RESULTS AND DISCUSSION

In the present study, HPV prevalence was expressed as percentage of HPV positive samples against all HPV tested cases.

HPV DNA was detected in 477 (58%) of the 822 patients by NESTED-PCR.

The most prevalent HPV genotypes were HPV 16 (35%), HPV 31 (16%) HPV 6 (9%), HPV 58 and 66 (7%), followed by HPV 33 (6%), HPV 18 and 56 (4%), HPV 70 and 45 (3%), HPV 53 and 11 (2%).

The HR-HPV subtype was found in the 85% of HPV-positive samples.

Multiple infections with at least two different HR-HPV genotypes were observed in 10% of specimens, and currently only the 1.5% of tested specimens remained unclassified.

This may reflect the frequent exposure of these patients to multiple HPV genotypes due to unprotected sexual contacts.

The prevalence of each HPV genotype is depicted in Figure 1.

The methods used in the present study have been extensively validated.

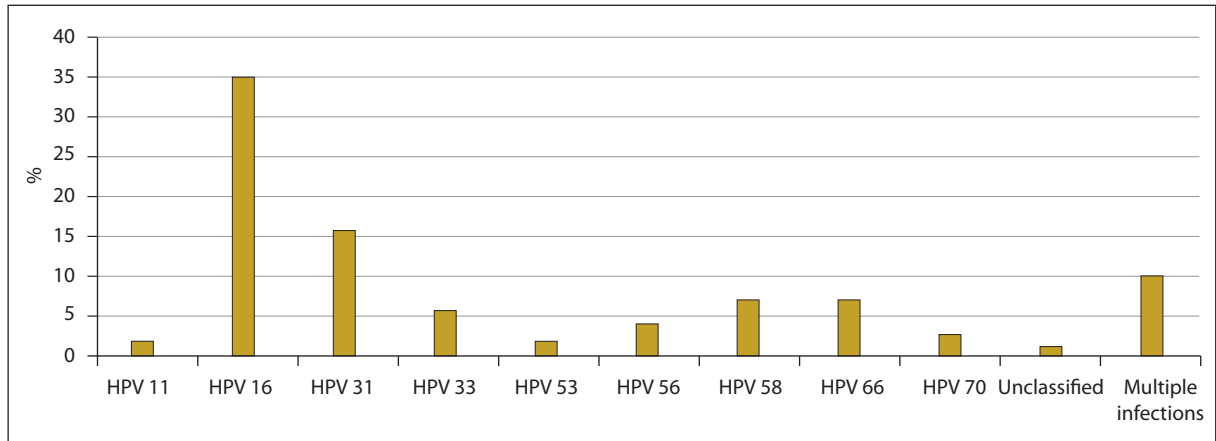


Figure 1
Distribution as percentage of the most prevalent HPV genotypes in the selected population.

All negative controls and positive controls for PCR amplification, HPV DNA detection, and genotyping yielded the appropriate results. Taken together, it is unlikely that the results of the present study are due to lack of reliability of the methods used.

Furthermore in our study also males partners were tested. A specific issue to be dealt with when counseling women which HPV infections relates to “who infected whom” [20-22].

The evaluation and treatment of male sexual partners of women with clinical or subclinical infection (genital warts or abnormal Pap smear results) is also not known to have a clinical benefit [21].

This question becomes more painful when oncogenic HPV strains are etiologically related to precancerous lesions that can progress to cervical or vulvar cancer [22, 23].

Studies assessing the carrier or infected status of partners of HPV-infected women indicate that subclinical lesions are far more common than diagnosed by simple visual genital examination [20, 21].

Most of these lesions are subclinical (*i.e.*, only visible after acetowhite staining and/or with HPV DNAtest of the partner).

They are often associated with the presence of HR-HPV, indicating that male sexual partners of women with cervical intraepithelial neoplasia might constitute a reservoir for HR- HPV [24].

Nowadays, are available two prophylactic vaccines for HPV; a bivalent vaccine Cervarix which induces protective immunity against HPV 16 and 18; and a quadrivalent vaccine, Gardasil, inducing protective immunity against HPV 6, 11, 16, and 18 [5, 25].

An open debate remains the cost effectiveness of universal HPV vaccination.

There is also unpublished evidence that these vac-

cines reduce both the incidence and virulence of genital cancers in men.

Few countries have recommended male vaccination: USA, Canada, Austria, and Australia.

A recent review of the economic literature has concluded that universal vaccination may not be cost-effective [26].

However, the conclusions drawing may be inaccurate for other authors.

According to the European Center for Disease Prevention and Control [27], future evaluation by the decision-makers in various countries of the results obtained by the next generation of intervention programs will focus on the critical issues that still exist.

Clearly, more research is required. It is necessary to expand this study to a larger number of patients, in order to better evaluate genital HPV types distribution among women and men in this Region.

Authors' contributions statement

DDV supervised and coordinated the research and has given final approval of the version to be published. FP, FI, GD were involved in the acquisition of the data. CF coordinated the manuscript. AB was involved in drafting and revisiting the manuscript and bibliographic research. FMC and RM were responsible for the statistical elaboration.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationship with other people or organizations that could inappropriately bias conduction and findings of this study.

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