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Short communication

Prevalence and genotypes identification of human papillomavirus infection in a population of South Italy

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Abstract

Background: A limited number of human papillomavirus (HPV) types account for the majority of invasive cervical cancer cases.
Objectives: To assess, in a southern Italian region, where HPV infection had not yet been investigated, the prevalence of type-specific HPV infection.
Study design: Multiplex PCR was used to test cervical specimens from 871 asymptomatic women.
Results: The HPV infection rate was 23.1%, with the highest prevalence being observed in women aged 20–30 years (32.6%). Type 16 was the most frequent HPV type detected either in mono-infected (39.8%) or in multi-infected (46.3%) women.
Conclusions: The HPV infection rate was higher than reported from other Italian areas. Our results further emphasise the importance of vaccinations to immunize females before they acquire HPV infection.

Keywords: HPV infection; Cervical cancer; HPV prevalence; Age

1. Introduction

Over 40 of 100 human papillomavirus (HPV) genotypes infect the epithelial and mucosal lining of the anogenital tract (Schiffman and Castle, 2003). On the basis of epidemiologic evidence certain genotypes of HPV are recognized as the etiological cause of cervical cancer (Munoz et al., 2006), and HPV viral DNA is identified in more than 90% of cervical carcinomas by PCR (Bosch et al., 1995; Karlsen et al., 1996; Walboomers and Meijer, 1997). Those genotypes can be further classified into high-risk types (namely, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and low-risk types (namely, 6 and 11), according to their capacity to cause cervical cancer (Munoz et al., 2003).

The purpose of the present work was that to define the prevalence of HPV infection in a selected area of the Apulia region, Southern Italy, where it had not been previously investigated. The investigation used multiplex PCR to detect HPV DNA in specimens collected from asymptomatic women.

2. Materials and methods

2.1. Study population and collection of specimens

A total of 871 women referred to the Gynaecology Outpatient Department, Ospedali Riuniti, Foggia, Apulia, Italy, were recruited from May 2005 to June 2007. Colposcopy was performed and cervical scrapes were obtained from 838 women. In addition, 33 routine cervical samples collected in a liquid-based cytology medium (ThinPrep®) were processed.

2.2. DNA isolation and multiplex PCR assay

Extraction of total DNA was carried out with the QIAamp DNA Minikit (QIAGEN, Hilden, Germany) according to

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Table 1
Base-pairs products generated by multiple PCR and corresponding HPV genotypes

Genotype	Base-pairs (bp)
PCR-mix-1 "16–35"	
HPV 16	325
HPV 31	520
HPV 33	227
HPV 35	280
PCR-mix-1 "18–59"	
HPV 18	425
HPV 39	340
HPV 45	475
HPV 59	395
PCR-mix-1 "52–66"	
HPV 52	360
HPV 56	325
HPV 58	240
HPV 66	304
PCR-mix-1 "6,11"	
HPV 6	260
HPV 11	425

the manufacturer's instructions. PCR assay for simultaneous amplification of two HPV genes, E1, and E2, used the amplification kit "HPV Low and High Risk Typing", purchased from Nuclear Laser Medicine s.r.l., Settala, Milano, Italy. In order to selectively amplify the high-risk genotypes and genotypes 6 and 11 we used four primer cocktails, namely PCR-mix-1, "16–35", "18–59", "52–66", "6–11", each containing two to four type-specific primers (Table 1). PCRs were carried out according to the manufacturer's instructions.

Multiplex PCR products were electrophoresed on 2% agarose gel and after ethidium bromide staining were readily identified on the basis of the corresponding length of the amplicons (Table 1). The amplification procedure was validated by the simultaneous detection of a 723-bp product of the human beta-globin gene (Saiki et al., 1986).

2.3. Cytology

The Papanicolau (pap) screen was used to detect cytological changes in the cervix of high-risk HPV women.

Table 2
Absolute frequencies of the single- and multiple-HPV genotypes detected by multiplex PCR among 202 HPV-positive women

HPV single genotype	No. of single HPV genotype positive
16	59
18	4
31	11
33	5
39	2
45	2
52	2
56	19
58	5
59	7
6	30
11	2

HPV multiple genotypes	No. of multiple HPV genotypes positive
16–18; 16–31; 16–52; 31–39; 33–6; 45–31; 45–39; 45–52; 52–66; 56–59 = 1 each	10
16–56	6
31–52	3
31–58	2
33–56	2
6–11–16; 6–16–18; 6–52; 6–16–45; 6–58–56 = 1 each	5
6–16	12
6–31	2
6–56	4
6–58	2
11–56	2
11–16; 11–18; 11–31; 11–33–56 = 1 each	4

3. Results

HPV DNA was detected in 23.1% (202 of 871) of women. HPV infection was due to a single genotype in 73.2% and to multiple genotypes in 26.7% of infected women; two genotypes were detected in 24.2% and three genotypes were detected in 2.4% of infected women (Table 2). HPV 16 was the most common genotype being detected in 39.8% (59 of 148) of the single genotype HPV-positive women, and in 46.3% (25 of 54) of the multiple genotypes HPV-positive women, respectively. Genotypes 6 and 11, were detected in 21.6% (32 of 148) of the single genotype-infected

Table 3
Prevalence (%) of LR-, HR-, and LR/HR-HPV-infected women

Age (years) (no. of subjects)	No. of HPV-infected women (%)	No. of LR-HPV-infected women (%)	No. of HR-HPV-infected women (%)	No. of LR/HR-HPV-infected women (%)
20–30 (242)	79 (32.64)	18 (7.31)	40 (16.52)	21 (8.53)
31–40 (278)	61 (21.94)	4 (1.41)	37 (13.30)	20 (7.09)
41–50 (173)	22 (12.71)	4 (2.31)	16 (9.24)	2 (1.15)
>50 (67)	10 (14.92)	1 (1.49)	8 (11.94)	1 (1.49)
nd (111)	30 (27.02)	6 (5.40)	14 (12.61)	10 (8.54)

nd, not determined. LR-HPV, low-risk-HPV genotypes; HR-HPV, high-risk-HPV genotypes; LR/HR-HPV, low-risk/high-risk-HPV genotypes.