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Epidemiology and genotype distribution of human papillomavirus (HPV) in women of Sardinia (Italy)

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ABSTRACT

The human papillomavirus (HPV) infection is necessary for the development of cervical cancer. Our study aims to evaluate the rate of HPV circulation in our population, to identify the prevalent genotypes and to establish correlation with cervical abnormalities. Furthermore, the awareness of women about HPV issues was investigated.

This study included 864 women attending the Oncologic Prevention Service for their routine Pap test screening or the Health Promotion Mother-Child Service for counselling about sexual activity, from July 2006 to September 2007. All the participants gave their informed consent to be enrolled in the study and were invited to fill in a questionnaire about the socio-cultural state, sexual activity and awareness about HPV. The women samples were tested for HPV-DNA and HPV genotypes: any type of HPV-DNA was detected in 31.0% of the women; single or multiple infections sustained by HPV-16 or HPV-18 represented 43.5% of all HPV infections, accounting for infections in 11.8% of the recruited women. The HPV and high-risk HPV (HR-HPV) prevalence significantly declined in women older than 46 years. The Pap test result was available in 490 women; 48.1% of the Pap test positive women had also an HPV infection and among these 22.7% were infected by HPV-16 and/or HPV-18 genotype, while 51.9% (94/181) were HPV negative. The analysis by binary logistic regression showed that genotype 16 and/or 18 is a risk factor for the Pap positive test with a odds ratio (OR) of 2.9 (95% C.I. 1.4–5.9) and 3.6 (95% C.I. 1.58–8.42) respectively, while age is a protective factor (OR 0.97, C.I. 95% 0.96–0.99); furthermore, the mean age at the first sexual intercourse and the mean number of partners since the beginning of sexual activity, were statistically associated with the risk of HPV infection. More than half of women were aware about HPV, its sexual transmission and of its correlation with cervix cancer.

Our findings evidenced that HPV infection is frequent in women aged 18–46 years in Sardinia and particularly that 16 and 18 HPV genotypes are detectable in more than 40% of the infected women. The proportion of women informed about HPV issues is sufficient to guarantee an aware approach to HPV vaccination.

1. Introduction

Human papillomavirus (HPV), a non-enveloped, double-stranded DNA virus, belonging to the Papillomaviridae family, that primarily infects the epithelium and induces benign and malignant lesions of the genital mucosa, is necessary for the development of cervical cancer; the association between HPV and cervical cancer is unique as no other major human cancer is dependent on a single factor for its development [1–3].

Over 120 HPV types have been identified and approximately 40 types infect the mucosal epithelium of the genital tract. Of these, 16 types have been classified as "high-risk" (HR-HPV) as they are associated with the malignant progression of cervical tumors and with other genital and head–neck malignancies [4–6]. HPV types 16 and 18 account for approximately 70% of cervical cancer cases worldwide with other high-risk types such as HPV-45, HPV-31, HPV-33 and HPV-52 accounting for the remaining cervical malignancies [7]. HPV "low-risk" types (LR-HPV), mainly HPV-6 and HPV-11, are

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Table 1
Age specific numbers and proportion with respect to the whole sample of 864 women.

Age classes (years)	No.	%
18–24	277 (271)	32.1
25–35	235 (229)	27.2
35–45	205 (203)	23.7
≥46	147 (146)	17.0

Values in parenthesis represent number of specimens whose HPV-DNA analysis was performed.

rarely detected in high-grade cervical lesions but cause the majority of anogenital warts [8,9].

Virtually all epidemiological studies provide evidence that genital HPV infection is very common in young sexually active women with prevalences as high as 76–80% [10,11]. In most cases (70–90%) HPV infection is a transient and self-limited infection and the virus is cleared by the host innate immune response [2,12]. The clearance of high-risk HPV types may require up to 14 months, a period longer than required by low-risk HPV types (5–6 months) [11,13,14]. However, in some cases the immune response fails to clear the infection and a persistent infection is established. In subjects persistently infected by high-risk HPV types there is a risk of progression to high-grade cervical intraepithelial neoplasia (CIN) and invasive cancer [2,15–19].

The link between HPV and cervical cancer has given impetus to the development of prophylactic vaccines against the most common HPV types; in parallel interest has raised to determine age specific burden of the infection and to identify the major genotypes supporting infection in different countries.

In recent Italian studies the prevalence of HR-HPV genotypes among women has been as high as 7–26% with a decreasing trend in older classes [20,21].

This study aims to evaluate the prevalence of HPV infection in a population of South Sardinia, to identify the prevalent genotypes and to establish correlation with cervical abnormalities. Furthermore, the awareness of women about HPV issues was investigated.

2. Materials and methods

2.1. Study population

This study included 864 women attending the Oncologic Prevention Service for their routine Pap test screening or the Health Promotion Mother-Child Service for counselling about sexual activity from July 2006 to September 2007. Both services are part of the n. 8 Cagliari Health Service District. The size of age classes among the women enrolled in the study is reported in Table 1.

At the time of their visit all the participants were informed about the study and its purpose and gave their informed consent. They were then invited to fill in a self administered questionnaire including data items about education, employment, lifetime number of male sexual partners, age at first sexual intercourse, history of sexually transmitted diseases, contraceptives methods, smoking; some questions were also addressed to assess knowledge of women about HPV and their attitudes about anti-HPV vaccine.

2.2. Sample preparation

Cervical specimens were collected with Cervex-brush and suspended in a 20 ml preservation solution, called Liqui-Prep, made by a mixture dilute of denaturated ethanol (20%). The tube was vortexed to remove all the material from the cervex; 1–2 ml of preservation solution was then centrifuged at 2500 rpm for 10 min, the supernatant was removed and discarded, the pellet obtained was stored at −80 °C until DNA extraction.

The DNA extraction was performed by adding *x* μl (range 20–100 μl according to the pellet size) of digestion buffer with proteinase K and by incubating at 55 °C O/N. After denaturation of proteinase at 95–100 °C PCR started.

2.3. PCR

An aliquot of crude lysate was used for the PCR.

The following primers derived from region L1 of the viral genome, forward 5′-CTTTCAGGGCAATAATGA-3′, reverse 5′-TGGTAGCTGGATTGTAGC-3′ were used for the amplification in the following 25 μl reaction mixture: 10× PCR buffer, 0.2 μM of each primer, 200 μM of each dNTP (dATP, dCTP, dGTP, and dTTP), 2.5 units of Taq polymerase (PerkinElmer/Cetus), 3 μl of sample DNA, double distilled water. The amplification was performed in a DNA Thermal Cycler (PerkinElmer/Cetus Instruments), using the following program set: 95 °C for 2 min, 5 cycles 94 °C 30 s, 50 °C for 1 min, 72 °C for 1 min and 40 cycles 94 °C 30 s, 45 °C for 1 min, 72 °C for 30 s plus an additional 5 min at 72 °C. Separate rooms were used for: preparation of DNA template, preparation and storage of reagents, setting up the amplification reaction. All reagents used in PCR were prepared, aliquoted and stored in an area free from PCR-amplified products. Amplification of a single copy human gene (β-globin), as control of DNA suitability for amplification, was performed in each reaction tube; we also used a positive and negative control in each reaction. Crude lysates that did not yield a β-globin product were extracted with phenol/chloroform and the extracted DNA re-amplified with the same β-globin primers. Samples that remained negative for β-globin were considered not amplifiable and excluded from this study.

2.4. Analysis of the amplification products

Mixture were analysed by electrophoresis in 2% agarose gel stained by ethidium bromide and visualized by UV light. As molecular weight marker we used a ladder Phi-X 174 digested with Hae-III. HPV genotyping was done by PCR-reverse hybridization. Briefly, a segment of the L1 region was amplified with GP5+/GP6+ consensus primers and labelled during PCR through the incorporation of Dig-11-dUTP (Roche Applied Science, Mannheim, Germany). Labeled amplicons were then hybridized to a panel of 24 type specific probes (high-risk: HPV-16, 18, 26, 30, 31, 33, 34, 35, 45, 51, 52, 56, 58, 59, 68 and 73; low-risk: HPV-6, 11, 40, 42, 43, 44, 54 and 70), previously immobilized to the surface of NucleoLink wells (NUNC, Denmark) and detected with a POD-conjugated anti-digoxigenin antibody (Roche Applied Science, Mannheim, Germany) and the tetra methyl benzidine (TMB) substrate (Sigma–Aldrich, Milan).

2.5. Statistical analysis

The statistical analysis of the data was obtained by the SPSS Statistical Software v. 15.0 (SPSS Inc., Chicago, IL). All data are reported as the mean value, median or frequencies and odds ratios (ORs) point estimates and their 95% confidence intervals (95% CI) were computed by LRM procedure for binary data to estimate the association between each covariate levels and HPV infection while adjusting for the effect of other variables retained in the model. Statistical significance was accepted if the *p* value was 0.05 or less.

3. Results

Among the 849 women eligible for the DNA analysis (15 samples were not amplified and then were excluded from the data