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Keywords:	HPV, Cervical cancer, risk factors, sexual behaviour, questionnaire

PREDICTORS OF HUMAN PAPILLOMA VIRUS (HPV) INFECTION IN ITALIAN WOMEN

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ABSTRACT

Background: HPV infection is a "necessary cause" of cervical cancer and it is sexually transmitted. Due to upcoming mass vaccination investigation on risk factors for infection is the basis to implement prophylactic strategy even in older women.

Objective: The aim of the study was to evaluate predictors of high-risk HPV infection in adult women.

Methods: Between 2006 and 2008, one hundred women aged >18 years, with no previous treatment for cervical lesions, were screened for high-risk HPV infection in Rome, Italy. Risk factors for HPV infection were investigated through a questionnaire including: ethnicity, religion, education, marital status, sexual behaviour, gynaecological and obstetrical history, smoking and alcohol intake.

Results: Multivariate analysis identified the "never married-separated/divorced" status (OR: 3.38; 95% CI: 1.14-10.12) as predictor of HPV infection, while having a higher age at the first sexual intercourse shows a protective effect (OR: 0.84; 95% CI: 0.71-1.00). A trend for the association between the infection and having more than three lifetime partners was also observed (OR: 2,57; 95% CI: 0.86-7.71). No significant association was found for other demographic characteristics investigated.

Conclusions: These findings provide a contribution in the knowledge of an adult population defining a "high risk" sexual behavioural profile and could be helpful to target prophylactic strategies in older woman.

Keywords

HPV, cervical cancer, epidemiology, risk factors, sexual behaviour, questionnaire.

INTRODUCTION

Cervical cancer (CC) is associated with Human Papillomavirus (HPV) infection, which is transmitted during sexual intercourse [Walboomers et al., 1999; American Cancer Society, 2008]. CC is the second most common cancer in woman worldwide and is more frequent in socio-economically deprived regions where proper screening and/or vaccination programs are not easily available [World Health Organization, 2006; Almonte et al., 2008].

Over one hundred HPV genotypes have been identified and classified into low-risk (LR) (e.g. type 6,11) and high-risk (HR) (e.g. type 16,18,31,33,45) groups, according to their potential oncogenic effects [Muñoz et al., 2006]. In the U.S.A., 75% of women aged between 14 and 50 years become infected during their lives, but the great majority of HPV genital infections are transient and are cleared within 2 years by host immune response [World Health Organization, 2006]. Persistent infections with HR-HPV is involved in the pathogenesis of cervical intraepithelial neoplasia and in the progression to cervical cancer [Snijders et al., 2006].

HPV DNA prevalence varies by continent and by age: it is high (up to 30%) in young women (<25 years) all over the world except for Asia, decreasing in the middle-age group, even though a second rise among in perimenopausal women has been recently observed [Almonte et al., 2008; Bosch et al., 2008; Smith et al., 2008].

Contracting HPV genital infection appears to be associated with increased sexual activity and thus with number of partners; other involved factors are age at the first sexual intercourse, marital status, cigarette smoking and contraceptives use [Vaccarella et al., 2006; Almonte et al., 2008; Herbert and Coffin, 2008]. However epidemiological studies investigating risk factors for HPV infection have not been entirely consistent, due to the differences in population characteristics (demographic

growth rate, sexual habits, HPV prevalence) as well as to the potential misclassification involved in the assessment of sexual history [Schlecht et al., 2001; Schroder et al., 2003].

Despite increasing effort, the burden and natural history of HPV infection is not completely understood and the upcoming mass vaccination will certainly change HPV epidemiology. Therefore baseline (i.e. pre-vaccine) epidemiological investigations on risk factors for infection should be encouraged to provide the basis to implement prophylactic strategies.

The aim of the present study was to investigate predictors of HR-HPV infection in women referred as outpatients to the "Regina Elena" National Cancer Institute and to the University "La Sapienza" in Rome, Italy, for HPV DNA detection and cervical cancer prevention.

MATERIALS AND METHODS

Population and study design

This study was part of a cooperative project conducted from April 2006 to December 2008, supported by the Italian Ministry of Health and coordinated by the "Regina Elena" National Cancer Institute. The study population were women who consecutively attended the Department of Gynaecologic Oncology of the "Regina Elena" National Cancer Institute and of the University "La Sapienza" of Rome, as outpatients for HPV DNA detection and cervical cancer prevention. Exclusion criteria were: age <18 years, positive history for cancer, previous surgical treatment for cervical lesions, presence of immunological disorders, pregnancy or cognitive impairment. Eligible patients were asked to participate and a written informed consent obtained. The study was approved by the Local Medical Ethical Committee.

At enrolment a baseline questionnaire investigating common risk factors for HPV infection and for cervical cancer was given by the physician just before the clinical examination. A gynaecologist collected clinical data and during the scheduled clinical procedures collected cervicovaginal specimens for HPV DNA analysis to be delivered to the Department of Pathology.

Questionnaire

The questionnaire, to be filled in by the patient, consisted of multiple choice questions addressing: ethnicity, religion, educational level, marital status, gynaecologic and obstetric history, sexual behaviour (age at first intercourse, lifetime number of partners, frequency of intercourse), contraceptive use, alcohol use and smoking. Knowledge about Pap test and frequency of testing was also investigated.

HPV DNA Detection and Genotyping

Cervico-vaginal specimens were collected by cytobrush (Cytyc, Rome, Italy) and Ayre spatula (Cytyc), in 20 mL of PreservCyt solution (Cytyc), stored at 4°C until the use.

HR HPV test was performed according to scientific evidence by means of the HC2 technique (Qiagen, Milan, Italy), a semi-quantitative signal-amplified hybridization assay for the chemiluminescent detection of the 13 most common HR HPV types (genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), as described by the manufacturer [Leinonen et al., 2009]. Before the HC2 testing, 4 ml of PreservCyt solution were processed by means of the HC2 Sample Conversion Kit (Qiagen). The positive cut-off (CO) value was considered the average of the positive control samples. The results were considered positive when the ratio between the specimen's light emission units (RLU) and the chosen positive CO (RLU/CO) was 1 or greater.

HPV DNA genotyping was carried out by using Linear Array HPV Genotyping Test (LA Roche Molecular System, Rome, Italy). 250 µl of PreservCyt sample were taken

for DNA extraction, as described by the manufacturer. The assay involves the amplification of a 450 bp amplicon, generated by a consensus PGMY primer set, targeting the conserved L1 region [Gravitt et al., 2000; Coutlée et al., 2002]. The detection of amplified products was achieved by the hybridization to a nylon strip, containing two levels of β-globin control probes and 37 LR and HR anogenital HPV types probes (types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6180).

Statistical analysis

For the analysis the patients were categorized as "HPV-positive" when at least one of the two basal HPV DNA tests were positive for high risk genotypes and as "HPV-negative" when it tested negative to both tests.

Descriptive statistics were computed for all variables. Continuous data were reported as the mean and standard deviation or the median and range; categorical data were represented by frequencies and percentage values. The Student's T-test was used to compare continuous variables; the Pearson's Chi-Squared and Fisher's Exact test, when appropriate, were used to detect potential differences between categorical variables. Cohen's Kappa and its relative 95% Confident Interval (95% CI) was used to assess the concordance the between HC2 and Linear Array HPV Genotyping Test in detecting the HPV status. P-values less than 0.05 were considered statistically significant.

Odds ratios (ORs) and their relative 95% CIs were computed through univariate and multivariate logistic regression models in order to evaluate the association between HPV infection and socio-demographic and behavioural variables. Data of some variables were grouped due to the small number. "Primary" and "secondary school" educational levels were grouped together. Furthermore, "marital status" was divided into two categories "never married-separated/divorced" and "married-living together-

widow", because, according to the "marriage protection hypothesis", family life is considered to be associated with healthier behaviour and because widowhood is similar to cohabitation in sociologic terms [Contoyannis and Jones, 2004; Sellors et al., 2003].

The number of lifetime sexual partners was categorized into up to three and more than three according to the median value. The multivariate logistic regression model was developed using stepwise regression (forward selection) with predictive variables that were significant in the univariate analyses. The enter and remove limits were p = 0.10 and p = 0.15, respectively

The data were analysed by using the SPSS version 11.5 (SPSS, Inc., Chicago, IL).

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RESULTS

Ninety-four out of the 100 consecutive eligible women submitted to the HPV DNA detection and genotyping tests agreed to complete the questionnaire.

According to the results of detection and genotyping HPV DNA tests 54 (57%) women were classified as "HR-HPV-positive" and 40 (43%) as "HPV-negative". The agreement between the two tests was 91% (Cohen's K: 0.83 95% CI[0.63 -1.00]).

Overall, twenty-five different genotypes were identified: the most common high-risk types were 16 (28%), 31 (16%), 51 and 52 (14%) (Figure 1). No single low-risk infection was found. Multiple HPV types were detected in 26 (50%) out of the 52 positive women at the Linear Array HPV Genotyping Test. Co-infection by two viruses were found in 25% of the positive cases, by three viruses in 15% and by more than three viruses in 10%. Co-infection by three or more genotypes were more frequent in young (>35 years) woman (33% vs 9%).

Table I shows the characteristics of the population. All except one were Caucasian and the great majority (90%) belonged to the Christian faith (data not shown). The "positive" women were slightly younger but the mean age values did not differ significantly in the two groups. The "positive" women were more frequently "never married-separated/divorced", reported a lower age at their first sexual intercourse (FSI) (17,9 vs 20.3 years), a higher number of lifetime partners (5.3 vs 3.0), smoking habits and alcohol consumption. No differences in educational level, number of pregnancies and of childbirths, frequency of intercourse, and use of estroprogestinic as well as condoms were observed. Almost all women reported to have knowledge of the Pap test and to undergo it regularly or occasionally.

At univariate analysis marital status, age at the FSI, lifetime numbers of sexual partners, past or current smoking habits, years of smoking, number of cigarettes, and regular use of alcohol were all factors predicting HPV infection (Tab.II). Multivariate analysis confirmed that the "never married-separated/divorced" status and a lower age at the first sexual intercourse were independent factors predicting HR-HPV infection (Tab.II). A trend for the association between the infection and having more than three lifetimes partners was also observed. No association was found for other variables included in the logistic regression model.

DISCUSSION

Cervical cancer is the second most common cancer in women worldwide and the estimate of the attributable fraction due to HPV infection range from 90% to 98% [Bosch and de Sanjosé, 2003]. HPV 16 and 18 are the most frequent types associated with CC and two prophylactic vaccines against these high-risk variants have been

recently introduced in several countries mainly in young women (up to 26 years)[Ault and the FUTURE II Study Group, 2007; Harper and Paavonen, 2008].

This study describes the predictors of HR-HPV infection in a sample of Italian women. The results highlight the behavioural characteristics that identify a "high-risk" profile for HPV infection, providing useful information to widen future prophylactic strategies also in older age-classes.

The high compliance obtained (94%) confirmed the practicable features of the questionnaire employed and showed a positive attitude towards cervical cancer prevention of these women who, once informed about the aim of the study, agreed to disclose information on their sexual behaviour.

The results of the multivariate analysis showed that having a "never married-separated/divorced" status and an early age at the FSI are independent risk factors for HR-HPV infection. A trend, although not significant probably for the small size of the population, was also observed for having more than three lifetime partners. These findings are in agreement with previous studies showing that having a "never married" or a "separated/divorced" status, an early age at the FSI or a higher "sexual age" (i.e. years of being sexually active) are all factors associated with HPV infection [Almonte et al., 2008; Sellors et al., 2000; Lenselink et al., 2008; Tonon et al., 1999]. According to other authors age at the FSI is more likely to be a predictor of the total number of partners in a lifetime than a factor independently associated with the infection [Hildesheim et al., 2001; Burchell et al., 2006].

Sexual activity with changing partners represents an additional well-known predictor of infection [Almonte et al., 2008; Herbert and Coffin, 2008; Vaccarella et al., 2006; Chan et al., 2009; Sellors et al., 2000; Sellors et al., 2003; Lenselink et al., 2008; Shields et al., 2004; Venuti et al., 1994]. A pooled analysis of the IARC HPV surveys of

11,337 women of nine countries (including areas Latino America, South-Eastern Asia, Spain and Africa) reported a significant increased risk of HPV infection (any type) for women with two or more lifetime partners vs one (OR: 1.86; 95%CI: 1.63-2.11) [Vaccarella et al., 2006]. In several case-control and cross sectional studies conducted in countries at different HPV prevalence the lifetime number and the number of sexual partners in the last 6-12 months were strongly associated with the presence of the infection [Almonte et al., 2008; Lenselink et al., 2008; Winer et al., 2003].

In this study the multivariate analysis showed no association between the risk of infection and the use of oral contraceptives, the use of condom, smoking and alcohol intake. The findings are consistent with the results of other studies, however no conclusive data are available [Lenselink et al., 2008; Sellors et al., 2003; Sellors et al., 2000; Winer et al., 2003]. In fact, the protective effect of a condom against HPV infection is not well established and does not seem to be as strong as for other sexual transmitted infections because of its transmission by skin-to-skin contact [Vaccarella et al., 2006; Burchell et al., 2006]. Data on the role of smoking is still debated and some studies show a positive association with current or past smoking habits [Sellors et al., 2000; Winer et al., 2003; Chan et al., 2009]. Smoking has been independently associated with an increased risk of cervical cancer and therefore has an acting role as a HPV co-factor in the cancerogenesis process [International Collaboration of Epidemiological studies of Cervical Cancer, 2007].

The study was not aimed to assess the prevalence of HPV infection. The high prevalence of the HR-HPV DNA infection observed in this population is probably due to the sample selection among patients referred by their general practitioners to two qualified Institutions for Research for HPV detection and cervical cancer screening, and could represent a limitation of the study. Other limitations are the small size of the

sample that does not allow to investigate the role of a specific HR-HPV type, and, as highlighted by other authors, the potential misclassification involved in reporting sexual history due to the recall biases or to intentional under-reported number of partners [Schroder et al., 2003; Schlecht et al., 2001]. Finally, previous infections in HPV negative patients were not assessed by serologic markers taking into account the low sensibility of the test as well as the scant relevance of the transient infections in the carcinogenesis [Snijders et al., 2006; Schiffman and Castle, 2003]

Epidemiology of HPV infection is going to chance because of upcoming mass vaccination. HPV vaccine trials have established that both vaccines (the bivalent against 16,18 and the quadrivalent against 16,18,6,11) produce a potent immunological response and are associated with an efficacy rate close to 100% for at least 5 years in naïve women [Villa et al., 2005; Villa et al., 2006; Harper et al., 2004; Harper et al., 2006; Ault and the FUTURE II Study Group, 2007; Paavonen et al., 2009]. National regulatory agencies (i.e. Food and Drug Administration, European Medicines Agency) have approved the two commercial products (Gardasil™ and Cervarix™) in most countries for use in young women (up to 26 years). Furthermore, because of the evidence of immunobridging also in women up to the age of 55 years, in selected regions there are approvals for women aged >26 years [Harper and Paavonen, 2008; Schwarz et al., 2009; Castellsagué et al., 2009].

Taking into account the safety and efficacy data available, increasing interventions to immunize women of different ages are expected.

CONCLUSIONS

Prevalence of HR-HPV infection is high in this selected population and is mainly associated to sexual behaviour. These findings contribute to the knowledge of an

adult population in defining a “high risk” behavioural profile and could be helpful to target prophylactic strategies in older woman.

Conflict of interest statement

The authors have no conflict of interest to disclose.

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Table I. Characteristics of the 94 patients according to HPV DNA tests results.

Variable	HR-Positive N* (%)	Negative N* (%)	p-value
N of patients	54 (57)	40 (43)	
Age			
mean (sd)	35 (10.2)	39 (11.2)	0.112
Education			0.755
Primary-Secondary school	15 (60)	10 (40)	
High school	27 (54)	23 (46)	
University	12 (63)	7 (37)	
Marital status			**<0.0001**
Married-Living togheter-Widow	13 (33)	26 (67)	
Never married-Separeted/Divorced	40 (74)	14 (26)	
Age at first sexual intercourse			**0.003**
mean (sd)	17.9 (2.4)	20.3 (4.9)	
Number of partners			**0.004**
mean (sd)	5.3 (3.8)	3.0 (3.3)	
Number of pregnancies			0.209
mean (sd)	1 (1.5)	1.3 (1.3)	
Number of childbirth			0.080
media (sd)	0.6 (0.8)	1 (1.2)	
Estroprogestinic contraceptive use			0.128
Yes	33 (63)	19 (37)	
No	18 (47)	20 (53)	
Condom contraceptive use			0.172
Yes	14 (70)	6 (30)	
No	37 (53)	33(47)	
N°sexual intercourse			0.105
Mean (sd) per months	7.9 (6.5)	5.7 (5.7)	
Smoking			**0.043**
Yes	33 (67)	16 (33)	
No	21 (47)	24 (53)	
Age at first cigarette			0.072
media (sd)	16.8 (2.8)	18.5 (3.0)	
Years of smoking			**0.008**
media (sd)	9.7 (11.1)	4.3 (7.2)	
Number of smoked cigarettes			**0.014**
media (sd)	7.3 (7.7)	3.6 (6.1)	
Alcohol consumption			**0.026**
Yes (regular and occasional drinkers)	23 (26)	8 (74)	
No	31 (50)	31 (50)	
Pap Test knowledge			0.178
Yes	54 (59)	38 (41)	
No	0 (0)	2 (100)	
Pap test frequency			0.930
Every 1-2 years	44 (58)	32 (42)	
Occasionally	7 (58)	5 (42)	
Never	3 (50)	3 (50)	

* the sum may not add to the total because of missing values.

Table II. Predictors of HR-HPV infection in women: univariate and multivariate logistic regression analysis.

Variables	Univariate OR	95% CI	p-value	Multivariate OR	95% CI	p-value
Age*	0,97	0,93-1,00	0,116			
Education						
Primary-Secondary	0,87	0,26-2,99	0,831			
High School	0,69	0,23-2,03	0,494			
University	1,00	-	-			
Marital Status						
Married/Living together/Widow	1,00	-	<0,0001	1,00	-	0,029
Never married-Separated/Divorced	5,71	2,32-14,08		3,38	1,14-10,12	
Age at first sexual intercourse*	0,82	0,71-0,95	0,007	0,84	0,71-1,00	0,055
Number of partners			<0,0001			
≤ 3	1,00	-		1,00	-	0,092
> 3	5,32	2,09-13,95		2,57	0,86-7,71	
Smoking						
No	1,00	-	0,044	1,00	-	0,131
Yes	2,36	1,02-5,44		2,24	0,79-6,39	
Alchol consumption			0,029			
No	1,00	-		1,00	-	0,117
Yes (regular and occasional drinkers)	2,88	1,12-7,41		2,56	0,79-8,33	
Estroprogestinic contraceptive use			0,13			
Yes	1,93	0,82-4,52				
No	1,00	-				
Condom use						
Yes	0,48	0,17-1,39	0,178			
No	1,00	-				

* considered as continuos variables

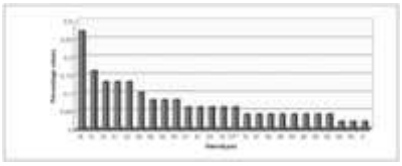

Fig. 1. Distribution of HPV genotypes detected by Linear Array Genotyping Test in the Italian women.
* CP6108

8x3mm (600 x 600 DPI)