

Cervical cancer screening program based on HPV testing and conventional Papanicolaou cytology for jail inmates

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ABSTRACT

Background: To assess the validity of Human Papillomavirus (HPV) testing in a group of women at high risk for developing cervical cancer, a screening intervention was applied to a population of jail inmates in Rome, Italy. This cross-sectional study provided also new insights on the risk factors and on the HPV genotype distribution. **Methods:** We have invited 350 inmates to the preliminary stage of the screening program and 98 inmates decided to participate to the study and filled out a questionnaire for the history of attendance to previous cervical screening and for the known risk factors for cervical malignancies. HPV DNA test, conventional Pap smear and HPV genotyping were performed. **Results:** The percentage of women with High Risk (HR) HPV positivity were 19.3%. The inmates with LSIL/HSIL status showed a significantly higher prevalence of HR-HPV positivity (100% vs. 16.3%; $p < 0.001$) and of multiple HPV types (60% vs. 1.2%; $p < 0.001$) compared to women with normal/ASCUS Pap smear. HPV16 was the predominant genotype in either single or multiple infections. **Conclusions:** The results indicated that HPV DNA-based approach is a strategy useful for incarcerated women which do not have the opportunity or the social and cultural environment to receive preventive care.

Keywords: Cervical Cancer Screening; HPV; Inmates

1. BACKGROUND

During the last few years, cervical cancer screening programs for women in prison have been carried out in the United States and in Canada [1-5]. Although the results of such preventive care interventions are still not complete and require further work and follow-up, these studies have pointed out the interesting profile of a jail inmate population as an high risk group for developing cervical malignancies. In fact, several socio-demographic risk factors including race/ethnicity, lower level of education and drug or alcohol abuse are responsible for the higher incidence rates of cancer in prisoner women [6,7].

Human papillomaviruses (HPVs) of the high risk types are known to play a causative role in cervical cancerogenesis [2]. HPV infection is correlated primarily with the number of sex partners, but is positively associated also with smoking and oral contraceptive use [8-10].

A number of randomized controlled trials have recently shown that HPV testing for cervical cancer screening shows a higher sensitivity compared with the conventional Papanicolaou cytology [11-13].

These studies have indicated that the prevalence of HPV infection in the general population is strictly related to the age of the women attending the screening [14], with a peak incidence of HPV infection occurring at the age of 16-20, as well as to their socioeconomic status [6].

With the aim to contribute to the assessment of the validity of the HPV DNA testing for cervical cancer screening and to increase the knowledge of the role of the risk factors and on the distribution of the HPV types, we have conducted a preventive care program by parallel HPV DNA test and conventional cytology on a popula-

tion of women prisoners in the “Rebibbia” Female Jail of Rome.

2. METHODS

This cross-sectional study was approved by the Second Faculty of Medicine of “Sapienza” University of Rome, by the Regina Elena National Cancer Institute of Rome and by the “Rebibbia” Female Jail of Rome. The protocol was designated following the recommended guidelines of the following Italian scientific societies: SICPCV (Italian Society of Colposcopy and Cervico-Vaginal Pathology) and GISCI (Italian Group of Cervical Cancer Screening), adapted in accordance with the experimental arm of the New Technologies for Cervical Cancer (NTCC) [12].

2.1. Procedure and Questionnaire

In the preliminary stage of our study, to invite the inmates to participate to the screening program we first organised three sequential meetings in the prison theatre in order to describe the value of cervical cancer screening, the role of the HPV infection and the routes of viral transmission. To explain the aims of the program and the procedure of the gynecological control we used slides with cartoons and figures to favour the comprehension from a multi ethnic population.

98 out of 350 inmate women decided to participate to the study and filled out a questionnaire by themselves. The questionnaire was written in Italian and was translated into English and Spanish. The questions included those required for the history of attendance to previous cervical screening as well as the willingness to be screened in the prison setting and those for the known risk factors for cervical malignancies such as smoke, drug/alcohol and sexual behaviour (**Table 1**). Written informed consent was obtained from all participants.

2.2. Specimen Collection

The women then underwent a gynecological examination including three different cervical samples: 1) a sample of cervical cells taken by Cervical sampler, (Digene Corporation, Gaithersburg, MD) and collect in standard transport medium for HPV DNA test; 2) an exo-endo-cervical scraping for conventional Pap Test; 3) a sample of cervical cells taken by Ayre’s Spatula and Cytobrush (Cytobrush DOC, Gardening, Genova, Italy) and collect in Transport buffer for HPV DNA genotyping.

2.3. HPV Detection

HPV DNA test was performed by Hybrid Capture II hybridization assay (HC2 Digene Corporation, Gaithersburg, Maryland USA), according to the manufacturer’s instructions. HC2 test, was used with the “high-risk” probes designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 positivity. A cervical smear was prepared for conventional Pap test and classified according to the Bethesda System 2001.

Table 1. Questions for the inmates enrolled into the screening program.

Characteristics
Patient Code
Age
Race/ethnicity (White, Latino/Hispanic, African, other)
Marital Status (Unmarried without current male partner, unmarried with current male partner, married, widowed, separated-divorced)
Questions
How many sexual partners did you have in your life?
Which was the age of your first sexual intercourse?
Did you ever practice oral sex?
Did you ever practice anal sex?
Do you smoke? If yes, how many cigarettes/day?
Do you drink? If yes, how much?
Do you use contraceptive methods? If yes, you use condom?
Do you have carried out a PAP smear during the last three years?

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HPV genotyping test was performed using a line probe assay for all cervical samples which were positive at the HC2 test. Total DNA was extracted QIAamp® DNA extraction (Qiagen, Hilden, Germany) and appropriate spin columns according to the manufacturer’s protocol. The INNO-LiPA HPV Genotyping Extra is a line probe assay designed for the identification of 28 different genotypes of the Human Papillomavirus by detection of specific sequences in the L1 region of the HPV genome. INNO-LiPA identified HPV types 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74 (INNO-LiPA; Innogenetics Gent, Belgium).

2.4. Cytological and Histological Diagnosis

All the lesions cytologically diagnosed, underwent colposcopic evaluation. The high grade lesions were immediately treated, excisional procedures were performed by a loop electrosurgical excisional procedure (LEEP), or traditional cone-biopsy, in the day surgery of Regina Elena National Cancer Institute of Rome. The histological diagnosis for all treated cases was CIN III according with cytology and colposcopic examination.

2.5. Statistical Methods

Logistic regression analysis was performed to model the association between sociodemographic characteristics, sexual and health behaviours and human papillomavirus (HPV) status or cervical cytology data. Odds Ratios (ORs) was calculated for each potential risk factors. Chi square and Fisher's exact test was used to compare categorical variables and a P for trend was calculated for ordinal categories.

3. RESULTS

As shown in **Table 2**, the epidemiological characteristics of the jail inmates corresponded to those described for a high risk population to develop cervical cancer. The sexual behaviour of the inmate group was also at higher risk because of the increased number of partners and the earlier sex intercourse. In fact, the logistic regression analysis showed that the OR for HR-HPV positivity was significantly associated with lifetime number of sex partners (OR = 2.00 for 5-9 partners; OR = 13.3 for >10 partners; $p = 0.036$).

Cervical samples from the 98 inmate participants to the screening were analyzed by HPV DNA test using the Hybrid Capture 2 hybridization assay to detect HR-HPV positivity and by conventional cytology. As shown in **Table 3**, the inmates with LSIL/HSIL status showed a significantly higher prevalence of HR-HPV positivity (100% vs. 16.3%; $p < 0.001$). In addition, the percentage of subjects positive for both HPV DNA testing and cytology was comparable to that reported in other screening programs [12,15], although a high prevalence of high grade pre-neoplastic lesions (HSIL/LSIL) was found among the inmates (**Table 2**). However, the superior sensitivity of the HPV testing compared to cytology was shown by the high percentage of cases positive for HPV DNA but negative at the cytological exam (14.3%) in agreement with several recent studies and new guidelines for cervical cancer prevention [15,16]. The OR for LSIL/HSIL status was significantly associated with the lifetime number of sex partners (OR = 1.8 for 5-9 partners; OR = 4.1 for > 10 partners; $p = 0.033$) and for the age of first sexual intercourse (OR = 2.0 for age < 15 years; $p < 0.05$) but not for none Pap smear performed during the past 3 years (OR = 0.8; $p = 0.78$).

Regarding the distribution of the HR-HPV types in the high risk inmate group and the amount of single and multiple infections, the genotyping assay on the 19 HPV DNA positive samples showed the much higher prevalence of HPV16 compared to the other types in either single or multiple infections (total HPV16 prevalence: 65%; single prevalence: 35%; multiple prevalence: 30%;

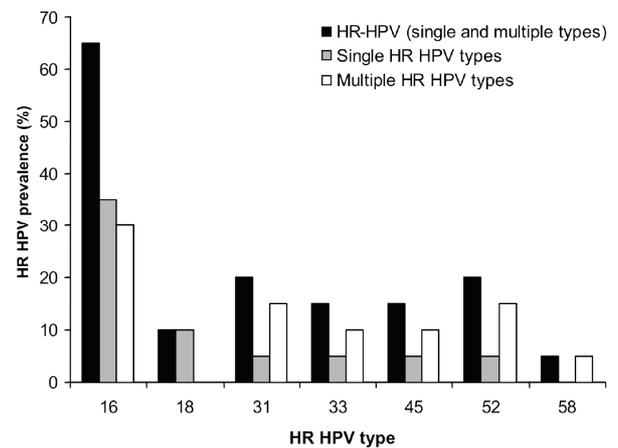


Figure 1. Overview of HR HPV type distribution in the total number of positive cases. HR HPV genotype distribution in order of predominance: HPV 16 (n = 13; 65%), HPV 31 (n = 4; 20%), HPV 52 (n = 4; 20%), HPV 33 (n = 3; 15%), HPV 45 (n = 3; 15%), HPV 18 (n = 2; 10%), HPV 58 (n = 1; 5%). Single HR HPV type distribution: HPV 16 (n = 7; 35%), HPV 18 (n = 2; 10%), HPV 31 (n = 1; 5%), HPV 33 (n = 1; 5%), HPV 45 (n = 1; 5%), HPV 52 (n = 1; 5%). Multiple HR HPV type distribution: HPV 16 (n = 6; 30%), HPV 31 (n = 3; 15%), HPV 52 (n = 3; 15%), HPV 33 (n = 2; 10%), HPV 45 (n = 2; 10%), HPV 58 (n = 1; 5%).

Figure 1), confirming that, as suggested, HPV16 is the most persistent and aggressive HR HPV type [17] and is able to promote secondary infections or co-infections with other genotypes [11]. Moreover, the inmates with LSIL/HSIL status showed a significantly higher prevalence of multiple HPV types (60% vs. 1.2%; $p < 0.001$) respect to women with normal/ASCUS Pap smear (**Table 2**).

4. DISCUSSION AND CONCLUSIONS

Although the main objective of our study was to assess the validity of the HPV DNA test in a high-risk population, our program was designed also to train the participant inmates about the value of cervical cancer prevention, because only a very low percentage of the incarcerated women has attended for cytological Pap-test screening previously. The number of participants to our study out of the total prisoners (approximately 37%) revealed a general interest to the prevention strategy.

The results of our screening appear to confirm the role of the risk factors taken into account in our study, which characterize the prison population, on the development of higher grade lesions. In fact, although the number of preneoplastic lesions diagnosed by our intervention was comparable to those resulting from larger screenings on the general populations [11,12], the increased percentage of high grade lesions among the inmates confirm the

Table 2. Association between the sociodemographic characteristics, sexual and health behaviours and the HPV status in the jail inmates.

Category	Subjects total no	HPVDNA + no (%)	OR (95% CI)	P for trend (chi square)
Age				0.0046
< 26 years	12	6 (50)	Reference	
26-35 years	25	4 (16)	0.19 (0.04-0.90)	
36-45 years	42	9 (21.4)	0.273 (0.07-1.05)	
> 45 years	19	0	-	
Race/ethnicity				0.21
White	74	16 (21.6)	Reference	
Latino/Hispanic	11	2 (18.1)	0.71 (0.14-3.58)	
African	13	1 (7.7)	0.35 (0.04-2.99)	
Marital status				0.96
Unmarried without current male partner	30	6 (20)	1.25 (0.37-4.18)	
Unmarried with current male partner	8	2 (25)	1.66 (0.27-10.02)	
Married	42	7 (16.7)	Reference	
Widowed	8	2 (25)	1.66 (0.27-10.02)	
Separated-divorced	10	2 (20)	1.25 (0.21-7.18)	
Lifetime no of sex partners				0.033
0-1	24	3 (12.5)	0.83 (0.18-3.68)	
2-4	41	6 (14.6)	Reference	
5-9	21	5 (23.8)	1.82 (0.48-6.86)	
Over 10	12	5 (41.6)	4.16 (0.98-17.54)	
Age of first sexual intercourse				0.34
< 15 years	26	7 (26.9)	2.00 (0.65-6.15)	
15-19 years	58	9 (15.5)	Reference	
> 19 years	11	2 (18.1)	1.21 (0.22-6.55)	
Oral intercourse				0.34
Never	64	14 (21.8)	Reference	
Ever	34	5 (14.7)	0.61 (0.20-1.88)	
Anal intercourse				0.99
Never	81	16 (19.7)	Reference	
Ever	17	3 (17.6)	0.87 (0.22-3.39)	
Tobacco consumption				0.60
Non smokers	23	5 (21.7)	Reference	
1-10 cigarettes	24	6 (25)	1.2 (0.31-4.65)	
> 10 cigarettes	51	8 (15.6)	0.67 (0.19-2.32)	
Alcohol consumption				0.65
Never	67	14 (20.8)	Reference	
Mild	23	3 (13.0)	0.56 (0.14-2.18)	
Abuse	8	2 (25)	1.26 (0.22-6.94)	
Condom use				0.57
Frequent	20	3 (15)	Reference	
Infrequent or never	78	16 (20.5)	1.46 (0.38-5.61)	
Pap smear during the past 3 years				0.51
Yes	15	2 (13.6)	Reference	
No	83	17 (20.4)	1.67 (0.34-8.13)	

Table 3. Detection of HR-HPV DNA and accordance with cervical cytology in the inmates attending for the screening.

Characteristics	Overall no = 98	Normal/ASCUS Pap smear no = 86	LSIL/HSIL Pap smear no = 5	Unsatisfactory no = 7	P value
HR-HPV Positive	19 (19.4)	14 (16.3)	5 (100)*	0	< 0.001
Prevalence of multiple HPV types	4 (4.1)	1 (1.2)	3 (60)	0	< 0.001

P values are from Fisher's exact test; ASCUS, atypical squamous cells of undetermined significance; LSIL, low grade squamous intra-epithelial lesions; HSIL, high grade squamous intra-epithelial lesions; * Pap smear: 1 LSIL, 4 HSIL.

validity of the risk factors analyzed through our questionnaire.

Moreover, it is well known that most HPV infections spontaneously regress: however, if the infection is persistent, the risk of cervical cancer substantially increases [18]. In natural or organized screening programs, about 80% of the detected low grade lesions spontaneously regressed [18,19], while in a population as the jail inmates that never attended to a screening program or that had not more than a Pap test during the life the lesions are believed to persist and progress to high grade.

Our study was designed evaluating all the cytological scrapings after HR-HPV testing and our results are in accordance with those obtained by the experimental arm of the New Technologies for Cervical Cancer (NTCC) [12]. Since in HPV DNA by HC2 screening strategies it is possible to increase the length of follow up respect to Pap-test based programs (3-5 years if HPV DNA test is negative twice) [20], our approach will be very useful for incarcerated women which do not have the opportunity or the social and cultural environment to receive preventive care.

The results on the distribution of the HR-HPV genotypes among the inmates revealed the much higher prevalence of HPV16 compared to the other types in either single or multiple infections, as expected [11,17]. It is currently known that more than 40% of HR HPV positive cases display multiple viral types [21], although the possible impact of these co-infections on the progression of cervical malignancies has not been determined yet. It is also well recognized that HPV16 represents the predominant genotype [19]. In contrast, the very low percentage of cases positive for HPV18 (10%) in our population is somehow surprising, although Clifford et al. have reported heterogeneity in the prevalence of HPV18 in the general population from 11 countries [22]. However, it is known that HPV18 is under-represented in high grade lesions at the time of diagnosis and that the HPV18 associated lesions rapidly progress [23].

We found a much higher rate of unsatisfactory Pap-smears (7.1%) compared to that reported by Prandi et al. (1.99%) in a screened population in Italy [24], possibly due to the more frequent occurrence of vaginal/cervical infections in inmate women. Because one out of the

7 unsatisfactory cytological tests was HR- HPV positive, we may conclude that HPV DNA testing allows to do not repeat an unsatisfactory cervical sample [20], further assessing the value of the addition of HPV DNA testing for screening of a high-risk population.

Finally, in groups of women do not attending to cervical cancer screenings, such as the population studied in this program, the potential benefit of HPV vaccines are enormous. In fact, it is now well recognized that important additional effects of the vaccines are the cross protections against infections and diseases caused by 16/18 related HPV types, specially against HPV31 and HPV45, which are the other genotypes causally attributed to cervical cancer [25].

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