

Prevalence of High-Risk *Human papillomavirus* Genotypes and Predictors factors for Cervical Cancer in Unimmunized Brazilian Women without Cytological Abnormalities



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Abstract

HPV is the most common sexually transmitted disease based on its oncogenic activity as high-risk (hr) types, low-risk types (lr) and types of undetermined-risk depending upon the associated diseases. The aim of this study was to evaluate the HPV infection prevalence and to identify the risk factors as demographic, behavioral and biological variables related to cervical cancer in unimmunized and sexually active women patients attended at public health institutions. A cross-sectional study conducted from 2014 to 2016 was performed at Rio de Janeiro city. All women were randomly selected and submitted to clinical and sociodemographic investigation. About 100 cervical cell scrapings were collected with a cervical cytobrush during the gynecological examination. After nucleic acid amplification directed to a conserved region of the HPV L1 gene, high-risk type specific primers (HPV16/18/31/45) were performed for genotyping. Statistical analysis was applied to analyze the risk factors associated with HPV infection in 18 sociodemographics variables obtained from informative questionnaires. This study demonstrated by the Chi-square test that of the 100 participants, 20% (95% CI: 12-28) were DNA HPV positive. HPV 18 and 45 were the most frequently detected HPV types. Most women were currently not married (56%) and married or cohabitating (44%) ($p < 0.05$). Psychosocial and psychosexual issues demonstrated that six percent of the women exhibit history of sexually transmitted diseases, except HIV, with 85% of women having sex with until to five partners ($p < 0.03$) and 15% used oral contraceptives, while 71% did not use any type of condom ($p < 0.03$). A previous history of STD was not significantly related. Cytological status was without alterations in the population investigated. This paper contributed to a better understanding about the natural history of HPV from of the pre-immunization prevalence study of the active sexually and clinical free lesions women in Brazil.

Keywords: Cervical cancer; Epidemiology; *Human papillomavirus*; Predictor factors; Prevalence

Introduction

Human papillomavirus (HPVs) are double stranded, non-enveloped DNA virus of the *Papilloma viridae* family. The mucosal HPV types have been described in the *Alpha papillomavirus* genus that infects the anogenital epithelium. HPV is the most common sexually transmitted disease based on their oncogenic activity as high-risk (hr) type, low-risk type (lr) and type of undetermined-risk depending upon the associated diseases [1-3].

Cervical cancer is the most common cancer in the developing countries. Several studies described the prevalence and age distribution of different HPV types with systematic review about the HPV vaccination programme [4] including risk populations [5] among adolescents [6] and young women with normal cytology [7]. In Scotland, the type specific prevalence of HPV was performed using three specimen types for urine (32.2%), self-taken swab (39.5%) and liquid based cytology samples (49.4%)

[8]. In England, a comparative prevalence study and odds ratio of HPV infection in the three different age groups (16-18y.o; 19-21 y.o; 22-24 y.o) were performed in the pre-immunization and post-immunization period [9].

The United States epidemiological study, using self-administered questionnaire about HPV vaccine and other characteristics, was conducted among mothers with her daughter and son between the ages of 9 and 17 years [10]. Brazilian study of HPV in oral mucosa detected 23.2% prevalence in patients free of clinical-pathological alterations [11]. DNA HPV was detected in the oral mucosa of asymptomatic men who have had over 16 sexual partners since initiation activity [12].

In Greece, HPV-16 and 51 were the most prevalence detected high risk HPV (hrHPV) types of 27.4%. Multiple HPV infections were detected in younger women (14-19 y.o) [13]. A population-

based study in Costa Rica, showed a positive association between vaginal pH and multiple HPV types infections and intraepithelial neoplasia [14]. Another study about cost-effectiveness analyses of HPV vaccination against cervical cancer and prevention of genital warts was performed in the Netherlands using epidemiological and economic parameters [15]. The economic impact of quadrivalent HPV vaccination was analyzed in Brazil also using a transmission dynamic model [16] as in Estonia [17] and Scotland [8].

More than 200 HPV genotypes and several HPV types associated with particular diseases as oral lesions (Heck's disease, oropharyngeal carcinoma, laryngeal papillomas), anogenital warts (Bowenoid papulosis, Buschike-Lowenstein tumor), Epidermodysplasia verruciformis (plane warts, Pityriasis-like plaques, squamous cell carcinomas of sun-exposed skin) have been described [18]. Previous studies investigated the most efficient and cost effective cervical cancer screening program. The Brazilian Immunization Program recommended the vaccine to be administered at 9 to 11 years of age before most adolescents became sexually active. HPV vaccination is the best preventive strategy against cervical cancer, cervical intraepithelial neoplasia and genital warts. Licensed HPV vaccines are bivalent (Cervarix®) against HPV16/18 (Glaxo Smith Kline Biologicals S.A.), quadrivalent (Gardasil®) target HPV16/18/6/11 (*Sanofi Pasteur/ Merck Sharp & Dohme- MSD*) or nonavalent vaccines (Gardasil-9®) target HPV16/18/31/33/45/52/58/6/11 and have been applied in many countries [19].

Several potential benefits of vaccination have been associated in HPV related diseases as cancer anus-genital, head and neck cancer, colorectal tumors, cancer penile and recurring respiratory papillomatosis. The aim of this study was to evaluate the HPV infection prevalence and to identify the risk factors as demographic, behavioral and biological variables related with the cervical cancer in unimmunized and sexually active women patients attended at public health institution.

Material and Methods

Epidemiological study

A cross-sectional study conducted from 2014 to 2016 was performed at the Manguinhos Complex community in Rio de Janeiro city. This study was approved by Sérgio Arouca Public Health National School Committee (number 686.868). The participants, who spontaneously accessed the ambulatory of gynecology attending in a Public Family Health Program, were randomly selected and interviewed. All women were submitted to clinical and socio-demographic investigation.

Questionnaire

The responders without a previous history of immunosuppressive disease, not pregnant and sexually active regardless of age were included in the cohort. The women who had been vaccinated against HPV and have had intraepithelial

lesions or neoplasia were not considered eligible for enrolment in this study. HPV educational leaflet was developed to facilitate the participation of invited women. About 18 demographic and socio-economics characteristics were used in the questionnaire. The profile was measured based on biological variables age, ethnic group, familiar income, education, marital status, number of partners, alcohol use, tobacco smoking, abortion, sexual transmitted diseases (STD), oral anti-contraceptive and condom use of the female population.

Molecular screening

About 100 cervical cell scrapings were collected with a cervical cytobrush from the ecto- and endo-cervix of the uterus during the gynecological examination. The clinical samples were immersed in 10mM Tris buffer, and stored at -20 °C. DNA extraction was performed using Qiagen DNA extraction Kit. In order to evaluate the viral DNA quality, swab samples were performed by β -globin PCR primers (PC04/GH20). The presence of DNA-HPV was detected by PCR amplification (450bp) using consensus primers (MY09/MY11) [20] and Nested PCR (GP5+/GP6+) [3]. After nucleic acid amplification directed to a conserved region of the HPV L1 gene, high-risk type specific primers (HPV16/18/31/45) were performed for genotyping. In addition, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay patterns of L1 PCR products were used for genotyping including four restriction endonucleases (PstI, HaeIII, DdeI and RsaI).

Statistical analysis

Statistical analysis using the Chi-square test was applied in order to analyze the risk factors associated with HPV infection in 18 socio-demographic variables obtained from informative questionnaires. The data were stored in an Excel spreadsheet and the association between nominal variables and the positive outcome for HPV was performed by Fisher's exact test or χ^2 . To determine the correlation between the methods of diagnosis of HPV (PCR and Nested PCR) the Kappa test was tested. Thus, the low correlation attributed for Kappa values were between 0.00 and 0.20, fair correlation had values between 0.21 and 0.40, moderate correlation was between 0.41 and 0.60, good correlation had values between 0.61 and 0.80, and excellent correlation had values between 0.81 and 1.00.

Results

In order to verify HPV infection, this cross-sectional design study demonstrated by using the Chi-square test that of the 100 participants, 20% (95% CI: 12-28) were DNA HPV positive. HPV 18 and 45 were the most frequently detected HPV types. Of these, 8% were amplified by HPV18/45 indicating co-infection (5% HPV18 and 3% HPV18/45); and 12% were undetermined types. HPV16 and 31 types were not detected to none of the samples tested. A group of 100 women was divided into six aged groups (≤ 25 y.o [15%, 95% CI]; 26 to 30 y.o [15%, 95% CI]; 31 to 35 y.o [25%, 95% CI]; 36 to 40 y.o [5%, 95% CI]; 41 to 45 y.o [5%, 95%

CI]; ≥45 y.o [35%, 95% CI]). Distribution among racial/ethnic groups was representative when 47% of women were white and 53% were black and on the race/ethnicities. About 16% reported being smokers and 3% drug users. Of all the participating, 27% used alcoholic, 40% reported having had at least one abortion, 15% used oral contraceptives, while 71% did not use any type of condom (p<0.03). Most women were currently not married

(56%) and married or cohabitating (44%) (p<0.05). Psychosocial and psychosexual issues demonstrated that six percent of the women exhibit history of sexually transmitted diseases, except HIV, with 85% of women having sex with until to five partners (p<0.03) by chi-squared tests and 14% started sexual activity under the age 17 (Table 1 & 2) (Figure 1 & 2).

Table 1: General characteristics and risk factors of the women (n=100).

Variables	PCR-HPV (-)	PCR-HPV (+)	p-value*
Ethnicity (100) White (n=48) Not white (n=52)	40% (40) 40% (40)	40% (40) 40% (40)	0.42
Marital status (100) Unmarried (n=44) Married (n=56)	39% (39) 41% (41)	5% (5) 15% (15)	0.05
Smoker (100) No(n=84) Yes (n=16)	67% (67) 13% (13)	17% (17) 3% (3)	0.89 13% (3)
Oral contraceptive (100) No(n=85) Yes (n=15)	69% (69) 11% (11)	16% (16) 4% (4)	0.48
Codom use (100) No(n=71) Yes (n=29)	53% (53) 27% (27)	18% (18) 2% (2)	0,05
Education (100) ≤ 8 years (n=47) >8 years (n=53)	42% (42) 38% (38)	5% (5) 15% (15)	0.02
Onset of sexual activity (100) ≤17 years (n=65) >17 years (n=35)	51% (51) 29% (29)	14% (14) 6% (6)	0.60
Pregnancy (100) None (n=16) One or more (n=84)	13% (13) 61% (67)	3% (3) 17% (17)	0.89
Miscarriage (100) No(n=60) Yes (n=40)	48% (48) 32% (32)	12% (12) 8% (8)	0.10
Number of partners (100) Up to five partners (n=85) More than five partners (n=15)	71% (71) 9% (9)	14% (14) 6% (6)	0.03

Table 2: Risk factors of the women through nested PCR method (n=100).

Variables	nPCR-HPV (-)	nPCR-HPV (+)	p-value*
Ethnicity (100) White (n=48) Not white (n=52)	40% (40) 41% (41)	8% (8) 11% (11)	0.61
Marital status (100) Unmarried (n=44) Married (n=56)	39% (39) 42% (42)	5% (5) 14% (14)	0.08
Smoker (100) No(n=84) Yes (n=16)	66% (66) 15% (15)	18% (18) 1% (1)	0.2
Oral contraceptive (100) No(n=85) Yes (n=15)	68% (68) 13% (13)	17% (17) 2% (2)	0.72
Codom use (100) No(n=71) Yes (n=29)	56% (56) 25% (25)	15% (15) 4% (4)	0,57
Education (100) ≤ 8 years (n=47) >8 years (n=53)	42% (42) 39% (39)	5% (5) 14% (14)	0.04
Onset of sexual activity (100) ≤17 years (n=65) >17 years (n=35)	55% (55) 26% (26)	10% (10) 9% (9)	0.28
Pregnancy (100) None (n=16) One or more (n=84)	13% (13) 68% (68)	3% (3) 16% (16)	0.10
Miscarriage (100) No(n=60) Yes (n=40)	46% (46) 35% (35)	14% (14) 5% (5)	0.20
Number of partners (100) Up to five partners (n=85) More than five partners (n=15)	68% (68) 13% (13)	17% (17) 2% (2)	0.72

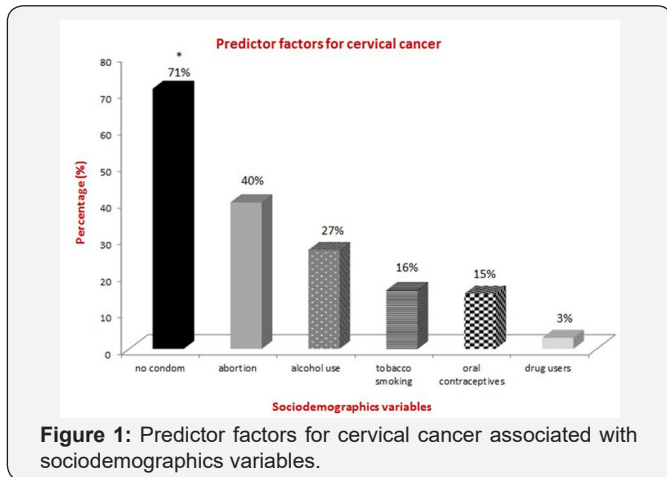


Figure 1: Predictor factors for cervical cancer associated with sociodemographics variables.

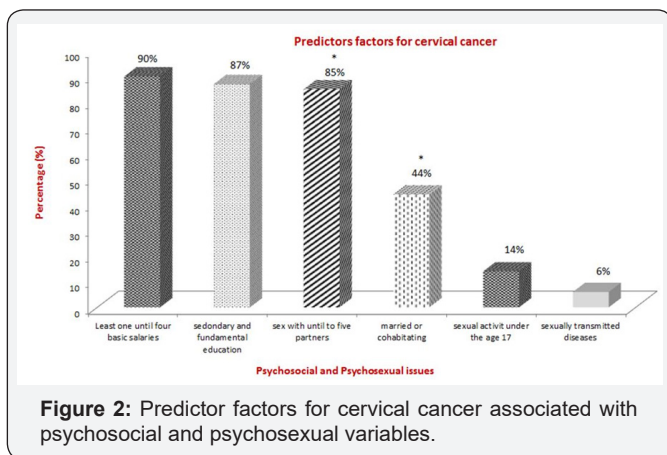


Figure 2: Predictor factors for cervical cancer associated with psychosocial and psychosexual variables.

About the employment status of all the participating women, 90% reported having had at least one until four basic salaries. Only 5% women have had higher education. The most women (87%) have had secondary and fundamental education. Comparing the genotypes, 37.5% were predominately over 45 years old, 62.5% of respondents were HPV18 positive and married and/or of cohabitation matrimonial status, 62.5% don't smoke; all of them don't use drugs and alcohol, four patients (50%) have monthly income between one to four minimum wages, 37.5% started sexual activity before the age of 17 years and 50% had less than five sexual partners (Figure 3).

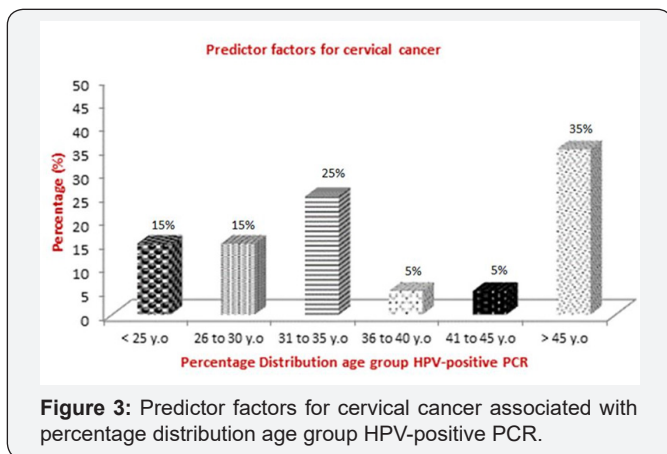


Figure 3: Predictor factors for cervical cancer associated with percentage distribution age group HPV-positive PCR.

Twenty five percent of the patients have had multiple infections (HPV18/45) and were unmarried. The 3% who have had the both types of HPV infection had more than five sexual partners. A highly significant factor associated with HPV infection was who did not use the condom and had an income between one to four minimum wages, both with 87.5%. A previous history of STD was not significantly related. Cytological status was without alterations in the population investigated.

According to the results, we evaluated the correlation and/or non-correlation between the samples analyzed. Between the PCR and nested PCR techniques, there are 12% (12 samples) correlation between the samples identified as positive and 73% (73 samples) correlation between the negative. Non-correlation was found in 7% (7 samples) samples of negative PCR, whereas the nested PCR showed to be positive, and 8% (8 samples) showed positive PCR and negative nPCR. This demonstrates that there is a moderate correlation between the techniques according to the Kappa association test ($k:0.52$). PCR-RFLP techniques demonstrated some limitations (Table 3).

Table 3: Comparison of correlating and non-correlating results between the PCR and Nested PCR techniques.

	Negative nPCR	Positive nPCR	Total
Negative PCR	73 (73%)	7 (7%)	80 (80%)
Positive PCR	8 (8%)	12 (12%)	20 (20%)
Total	81 (81%)	19(19%)	100 (100%)

Discussion

Our study analyzed only pre-immunization women at six different age groups compared with Mesheret al. (2014). The high-risk HPV prevalence of our study was 20% detected only in swab samples collected with a cervical cytobrush when compared with specimen types of Scotland women from urine, self-taken swab and liquid based cytology samples [18].

Italian women with normal cytology were DNA positive also of HPV by PCR amplification [7] when compared with our results in unimmunized Brazilian women without cytological damage. Similar to Rahman et al. [10] in a United States study, our study analyzed some different variables about demographic and social behaviors also. Our cross-sectional study detected 20% prevalence in cervical swab samples similar to the HPV prevalence of Tristão et al. [11] but not associated with the oral mucosa.

We analyzed several variables stratified by six age groups, but the vaginal pH was not included in our study. Semi-structured interviews were made by school nurses about HPV vaccine implementation [21]. There were no significant associations between vaginal pH and detection of HPV at the post-menopausal age ranges (55-64 years old) in a Costa Rica study. Over 65 y.o, strong association between a vaginal pH >5.0 and carcinogenic HPV positivity was reported by Clarke et al. [14]. Co-infections were also detected in younger women

in our study, but the HPV16 was not detected as in the Greece population by Argyri et al. [13].

Percentages of cervical cancer screening, mortality and hysterectomyed population were excluded as disease model parameters in our study differently when compared with Kawai et al. [16] analyzing the health and economic impact of HPV vaccination in Brazil. Cost-effectiveness of the bivalent and quadrivalent HPV vaccine associated with the vaccine price per dose, duration of protection by years, relative risk reduction of unvaccinated women and discount rate health effects was described by Westra et al. [15].

Conclusion

Our study estimated the prevalence of HPV in unvaccinated active women in the Manguinhos Habitation Complex, Rio de Janeiro, Brazil. This epidemiological study showed a strongly association among the presence of DNA HPV genital types with risk factors to cervical cancer in unimmunized women and of free clinical lesions. Socio-demographic characteristics as behavior changes and covariables associations related to sexual health were significantly relevant in women without cytological abnormalities. The data from this study recorded the prevalence of high-risk HPV genotypes.

This paper contributed to a better understanding about the natural history of HPV from a pre-immunization prevalence study of active sexually and free lesions clinical women in Brazil. These results showed the importance of the cofactors for progression and persistence of HPV infection and may be useful to future epidemiological surveillance reports, educational intervention and evaluation of new clinical and subclinical cases of cervical intraepithelial neoplasia and genital warts. A large challenge to public health is the cancer prevention in populations from developing countries with high risk exposure associated with the history of other STDs in different geographic regions. So, public health programs should to encourage the vaccination emphasizing the effectiveness of the vaccine as part of general HPV vaccine recommendations.

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