Frequency and genotyping of human papillomavirus in women submitted to citology

Frequência e genotipagem do papilomavírus humano em mulheres submetidas à citologia oncótica

Emmanuele Pariz Silva[1], Giovanna Grünewald Vietta[2], Lisléia Golfetto[3], Marco Antonio Zonta[4], Eloisa Regina Gularte[2], Maria Elisabeth Menezes[5], Daiane Cobianchi[2]

ABSTRACT

Introduction: Among the sexually transmitted virus, the human papilloma virus (HPV) is the most prevalent and may be detected a considerable number of sexually active women. He is considered the main agent of cervical cancer. Therefore, the high-risk HPV identification can aid in the prevention of cervical lesions. **Objective:** To evaluate the occurrence of HPV infections, comparing different methodologies, as well as some risk factors and their potential association in the development of cervical cancer in women submitted to cytopathology treated in ambulatory Unit Family and Community Health (USFC) of the University of Vale do Itajaí (UNIVALI). **Methods:** 118 samples were evaluated sexually active women who sought care for screening of cervical cancer in USFC and UNIVALI. All samples were subjected to polymerase chain reaction (PCR) and the liquid and conventional cytology. However, only 64 women were subjected to hybrid capture methodology (CH2). **Results:** The prevalence of HPV was 43.22% by PCR and 25% for CH2; analysis of the results was observed association between HPV and the following variables: ethnicity ($p<0.016$), scholarity ($p<0.012$), human immunodeficiency virus (HIV) ($p<0.008$), preservative ($p<0.02$), oral contraceptives ($p<0.03$), younger age at first sexual intercourse ($p<0.07$), conventional cytology ($p<0.002$) and liquid cytology ($p<0.029$). **Conclusion:** The incidence of HPV infection is high and the high-risk HPV was primarily associated with the younger age at first sexual intercourse.
Keywords: papillomaviridae; polymerase chain reaction; sexually transmitted diseases.

RESUMO

Introdução: Dentre os vírus de transmissão sexual, o papilomavírus humano (HPV) é o mais prevalente, podendo ser detectado em considerável número de mulheres sexualmente ativas. Ele é considerado o principal agente causador do câncer do colo do útero. Portanto, a identificação do HPV de alto risco pode auxiliar na prevenção de lesões do colo uterino. **Objetivo:** Avaliar a ocorrência de infecções pelo HPV, comparando diferentes metodologias, assim como alguns fatores de risco e seu potencial de associação no desenvolvimento do câncer do colo uterino em mulheres submetidas à citopatologia atendidas nos ambulatórios da Unidade de Saúde Familiar e Comunitária (USFC) da Universidade do Vale do Itajaí (UNIVALI). **Métodos:** Foram avaliadas 118 amostras de mulheres sexualmente ativas que buscaram atendimento para rastreio do câncer cervical na USFC e da UNIVALI. Todas as amostras foram submetidas à reação em cadeia da polimerase (PCR) e às citologias líquida e convencional. Entretanto, apenas 64 mulheres foram submetidas à metodologia de captura híbrida (CH2). **Resultados:** A prevalência do HPV foi de 43,22% pela técnica de PCR e de 25% pela CH2; na análise dos resultados observou-se associação do HPV com as seguintes variáveis: etnia ($p<0,016$), escolaridade ($p<0,012$), vírus da imunodeficiência humana (HIV) ($p<0,008$), preservativo ($p<0,02$), anticoncepcional ($p<0,03$), início da atividade sexual ($p<0,07$), citologia convencional ($p<0,002$) e citologia líquida ($p<0,029$). **Conclusão:** A ocorrência de infecção pelo HPV é elevada e o HPV de alto risco foi principalmente associado ao início precoce da atividade sexual.
Palavras-chave: papilomavírus humano; reação em cadeia da polimerase; doenças sexualmente transmissíveis.

INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide. In Brazil, cervical cancer is the third most common tumor in women, only surpassed by breast and colorectal cancer, and the fourth leading cause of cancer death in women in the country. In the South region, this type of cancer is the fourth most frequent tumor in women (15.87/100,000); in Santa Catarina, the estimated rate is 14.97/100,000. For 2016, the occurrences of approximately 15,590 new cases in Brazil are estimated[1].

Persistent infection by human papillomavirus (HPV) is considered the main cause of cervical cancer[2] and the main way of acquiring the virus is through sexual intercourse[1]. It is estimated that 75–80% sexually active women will be infected by one or more types of HPV throughout their lives. However, 80% infections are transient and counteracted by the immune system without causing injury. The other 20% can progress to lesions that precede cervical cancer[2]. The relationship between HPV and carcinogenesis depends mainly on the type of virus and its persistence and integration with the host cell[3].

Currently, there are more than 200 known types of HPV, of which about 40 infect the genital tract. They are classified according to their oncogenic potential. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 are classified as having high oncogenic risk, being directly related to the development of lesions and cancer. Of these, types 16 and 18 are the main etiological agents of this type cancer[4]. On the other hand, types 6, 11, 32, 40, 42, 44, 61, and 62 are

[1]Undergraduate Student in the Biomedicine Course, Universidade do Vale do Itajaí (UNIVALI) – Itajaí (SC), Brazil.
[2]Professor in the Biomedicine Course, UNIVALI – Itajaí (SC), Brazil.
[3]Graduate Student in the Graduate Program in Pharmacy, Universidade Federal de Santa Catarina (UFSC) – Florianópolis (SC), Brazil.
[4]PhD in Infectious Diseases, Universidade Federal de São Paulo (UNIFESP). Director of Laboratório IN CITO – São Paulo (SP), Brazil.
[5]President of Instituto de Biologia Molecular Aplicada (IBIOTECNO). Director of Laboratório de Análise e Pesquisa do Gene (DNAnálise) – Florianópolis (SC), Brazil.

 DOI: 10.5533/DST-2177-8264-2015271-205

classified as having low oncogenic risk, as they are associated with benign lesions and condyloma acuminata[5].

HPV plays a central role in the etiology of most cases of cervical cancer. However, although it is a necessary cause, it is often not sufficient for the development of cervical cancer. It is recognized that other factors, such as smoking, alcohol consumption, use of oral contraceptive, use of immunosuppression drugs, number of sexual partners, early sexual activity, and other sexually transmitted diseases (STDs), modulate, jointly with the virus, the transition of the infection to malignancy[6,7].

Histologically, cervical cancer is preceded by a series of cellular changes in the original epithelium characterized by premalignant lesions. Changes can be classified, using the Bethesda system, into atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells that cannot exclude high grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (L-SIL), and high-grade squamous intraepithelial lesion (H-SIL)[8].

Papanicolaou stain method was the first way to detect changes consistent with injuries suggesting HPV infection. Even today, it is the most widely used test in screening programs for lesions that precede cervical cancer, given its scope, cost, and ease of implementation. However, it presents false-negative rates ranging from 15% to 50%. Still, over the years, the developed countries that have adopted it as a cervical cancer screening method observed a decrease in the number of cervical cancer cases[9].

To improve the sensitivity of conventional cytology (Papanicolaou), liquid-based cytology has been developed, which can be defined as a means for cell preservation that is capable of improving the quality of cell samples for analysis, as well as enabling the preservation of cell DNA. This methodology allows to automate and standardize the preparation and staining of cytological slides and facilitates molecular analysis[10].

Liquid-based cytology also allows better identification of cellular changes and a decrease in artifacts in the samples, thus reducing unsatisfactory cases. It also allows the possibility of performing additional tests, such as the molecular biology of HPV and other STDs, from the same collection[11].

Colposcopy is another test used as a strategy to detect clinical changes that may indicate possible precedent lesions of cervical cancer. The test allows the visualization of the cervix with a colposcope. It is often used to detect preinvasive diseases to prevent the development of cancer. This test is conducted in situations where the cytology detects abnormal cells, clinical examination presents alterations and in women who already underwent previous treatment for the characteristic lesions caused by HPV[12].

Over the years, the introduction of molecular biology tests with cytology (Pap smear) significantly increased the sensitivity of screening for cervical cancer[13]. Thus, screening for cervical cancer through molecular biology tests began to be considered a strategy for early screening of the virus in women. Among the methods currently available for HPV detection are hybrid capture (HCII), polymerase chain reaction (PCR), solid-phase hybridization (microrrays), and in situ hybridization[14]. Although all methods can be used for this purpose, only the HCII test, a qualitative test, is approved by the Food and Drug Administration and the Brazilian Health Surveillance Agency for the diagnosis of HPV[15].

The HCII is a method based on the hybridization of complementary RNA probes to the genomic sequences of the 18 most common types of HPV that infect the anogenital tract of sexually active men and women. These 18 types are further classified into two groups: high-risk group A (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and low-risk group B (HPV 6, 11, 42, 43, 44).

PCR is based on the specific amplification of segments of the HPV genome, and has the potential to detect very low levels of viral load in cells and tissues, even in latent infections[14].

OBJECTIVE

To evaluate the occurrence of HPV infections, comparing different methodologies, as well as some risk factors and their potential association to the development of cervical cancer in women undergoing cytopathology, treated at the clinic of the Family and Community Health Unit (USFC) of the University of Vale do Itajaí (UNIVALI).

MATERIAL AND METHODS

A total 118 sexually active women aged 16 years and above participated in this study, randomly selected, who sought outpatient treatment at the clinic of UNIVALI for routine screening for cervical cancer by Papanicolaou (Pap smear) from August 2013 to April 2014. The study was approved by the Research Ethics Committee of UNIVALI, under the protocol number 445.967. Patients who agreed to sign the informed consent were included in the study and had cervical material collected for molecular test and conventional and liquid-based cytology.

Data collection was based on a research protocol by means of a questionnaire with objective questions answered by the patient during the consultation. The variables evaluated were age (stratified every 5 years starting from the age of 15), educational level (primary, secondary, and higher education, without discriminating whether the study was complete or incomplete), ethnicity (white, black, and brown), information on disease (which is cervical cancer, which is HPV, how to prevent), Pap smear, number of sexual partners over a lifetime (up to 5, above 5), age of onset of sexual activity (≤17 years and >17 years), previous pregnancies (none, one, or more), abortion (yes or no), previous history of other STDs, use of hormonal contraceptives and smoking.

Two samples were collected from each patient, the first with an Ayre spatula and CitoBrush for conventional cytology, and the second with the collection kit SurePath™ (BD) for molecular testing and liquid-based cytology. Conventional cytology was performed in the supporting laboratory of cytopathology under a special agreement with USFC. The liquid-based cytology was performed in a fully automated way in the Cytology Diagnostics Laboratory IN CITO (SP).

The colposcopy examination was performed in 95 patients using a colposcope to visualize the cervix under bright light, with 10–40 times magnification. The colposcopic images were analyzed after the collection of Pap smear, with application of a 5% acetic acid and/or 4% Lugol solution (Schiller).

The samples were also subjected to a molecular biology study through the HCII method by Digene & Co. in Laboratório DNAnálise (SC) for the detection of HPV DNA. This method has clinical

sensitivity of 1 pg/mL, equivalent to one virus copy per cell. The test was considered positive when the test's rate of relative light units (RLU) over two positive controls was equivalent to 1 pg/ml of HPV DNA or more. According to recent studies, this cutoff value adds greater sensitivity and specificity to the test[16].

PCR was performed in the Laboratory of Molecular Biology and Mycobacteria of Universidade Federal de Santa Catarina (UFSC-LBMM), Florianópolis. The PGMY0911 primers were used for detection of HPV DNA[17], which amplify a 450 bp segment of the HPV L1 gene. As an internal control, the PCO3/PCO4 primers[18], which amplify a 110 bp segment of the gene of human β-globin, were used. These were used as controls for the presence of inhibitors in the PCR reaction[18].

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 and of the lesions that precede cervical cancer (HCII, PCR, liquid-based and conventional cytology), 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

The collection of material was performed in 118 sexually active women. The age range was 16–69 years, mean age of 40.4 years. Fifty-two women (44.1%) were aged up to 35 years and the others (66, 55.9%) were above that age. The most prevalent age group was women above 45 years (39.8%).

The prevalence of HPV DNA was 43.22% (51/118) in the sample with PCR and 35% (23/66) with HCII.

Table 1 describes the distribution of the variables studied and their association with the presence of HPV. A significant difference was observed between women that were positive and negative for HPV in relation to the variables: ethnicity (p<0.016), education (p<0.012), human immunodeficiency virus (HIV) (p<0.008), condom use (p<0.02), oral contraceptives (p<0.03), younger age at first sexual intercourse (p<0.07), conventional cytology (p<0.002), and liquid-based cytology (p<0.029).

The frequency of HPV infection measured through the PCR methodology was higher in women aged 25 years or older (47.4%) and over 45 years (53.2%) **(Figure 1)**. Regarding the education level and ethnicity, there was a higher prevalence in brown women with higher education level (**Table 1**).

Among the analyzed patients, 15 (12.7%) did not know what cervical cancer is, 101 (85.6%) did not know what HPV is, and 64 (54.2%) did not know how to prevent themselves. Of the 118 participants, 117 had undergone the Pap smear. Sexual behavior was analyzed by the onset of sexual activity and the number of partners. As for the age of onset of sexual activity, there was a higher, statistically significant prevalence in women who started sexual activity after the age of 17 years (48.8%). Regarding the number of sexual partners, although no statistical significance was observed, a higher

Table 1 – General characteristics and risk factors of the women (n=118).

Variables	PCR-HPV (-) n (%)	PCR-HPV (+) n (%)	p-value*
Ethnicity			
White (n=102)	60 (58.8)	42 (41.2)	0.016
Black (n=12)	6 (50.0)	6 (50.0)	
Brown (n=4)	1 (25.0)	3 (75.0)	
Education level			
Primary (n=53)	33 (62.3)	20 (37.7)	0.012
Secondary (n=58)	32 (55.2)	26 (44.8)	
Superior (n=7)	2 (28.6)	5 (71.4)	
HIV			
No (n=109)	63 (57.8)	46 (42.2)	0.008
Yes (n=9)	4 (44.4)	5 (55.6)	
Smoker			
No (n=101)	58 (57.4)	43 (42.6)	0.465
Yes (n=17)	9 (52.9)	8 (47.1)	
Oral contraceptive			
No (n=77)	41 (53.2)	36 (46.8)	0.003
Yes (n=41)	26 (63.4)	15 (36.6)	
Conventional cytology			
Normal (n=116)	67 (57.8)	49 (42.2)	0.002
L-SIL (n=2)	0 (0)	2 (100.0)	
Liquid-based cytology			
Normal (n=106)	61 (57.5)	45 (42.5)	
L-SIL (n=8)	5 (62.5)	3 (37.5)	0.029
H-SIL (n=3)	1 (33.3)	2 (66.7)	
ASC-US (n=1)	0 (0)	1 (100.0)	
Onset of sexual activity			
≤17 years (n=75)	45 (60.0)	30 (40.0)	0. 07
>17 years (n=43)	22 (51.2)	21 (48.8)	
Pregnancy			
None (n=23)	11 (47.8)	12 (52.2)	0.232
One or more (n=95)	56 (58.9)	39 (41.1)	
Miscarriage			
No (n=84)	48 (57.1)	36 (42.9)	0.530
Yes (n=34)	19 (55.9)	15 (44.1)	
Number of partners			
Up to five partners (n=102)	58 (56.9)	44 (43.1)	0.585
More than five partners (n=16)	9 (56.3)	7 (43.7)	

PCR: polymerase chain reaction; HPV: human papillomavirus; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion; L-SIL, low-grade squamous intraepithelial lesion; H-SIL, high-grade squamous intraepithelial lesion.

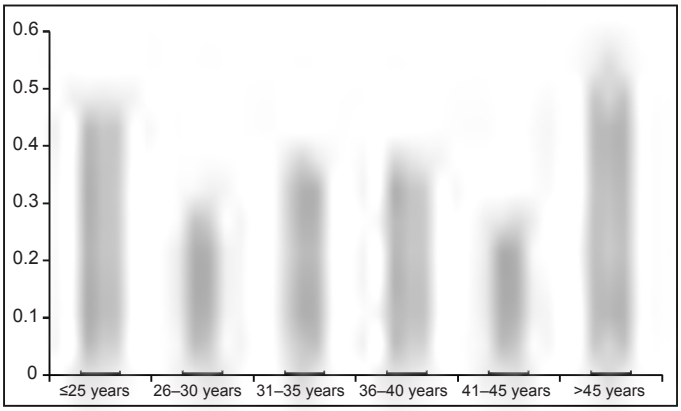

Figure 1 – Percentage distribution by age of the positive cases for polymerase chain reaction of human papillomavirus.

frequency of HPV was observed in women who had more than five partners (43.7%) (**Table 1**).

In relation to parity and abortion, no cases showed statistical significance. However, there was a higher prevalence of HPV in women who have suffered a miscarriage (44.1%) (**Table 1**).

In the assessment of STD history, we observed 55.6% positivity for HPV and HIV, with statistical significance (**Table 1**), and high prevalence of high-risk HPV in patients with STD history (83.3%) (**Table 2**).

Regarding the prevalence of HPV among patients who used oral contraceptives, 46.8% do not use oral contraceptives (**Table 1**). When analyzing only the positive cases for HPV, there was a higher frequency of low-risk HPV in contraceptive users (40%). For high-risk HPV, the frequency was higher in patients who do not use contraceptives, but without statistical significance (**Table 2**).

In relation to smoking, female smokers had a higher prevalence of HPV (47.1%) than nonsmokers (42.6%), but not significantly (**Table 1**).

When observed separately through the HCII technique, high- and low-risk HPV distribution according to age of onset of sexual activity was of 78.6% and 21.4% for women who initiated sexual activity before the age of 17 years, respectively. Regarding the number of sexual partners, between the low-risk and high-risk HPV groups, it is noted that in both categories the prevalence of the high-risk group was higher than the low-risk with statistical significance. There was also a higher prevalence of high-risk HPV (75%) in patients who have suffered at least one miscarriage (**Table 2**).

Of the 118 women, only 95 underwent colposcopy, which was positive in 47.4% of women. Twenty-eight samples (29.5%) were positive for acetic acid, and 35 (36.8%) were positive for the Schiller Test.

Regarding the conventional cytology, the frequency of HPV positivity was 100% for women with abnormal cytology (L-SIL and ASC-US) and 22.2% for women with normal cytology. In the liquid-based cytology, positivity for HPV was shown in 42.2% women with normal cytology, 37.5% women for L-SIL, 66.7% women for H-SIL, and 100% women for ASC-US (**Table 1**).

Regarding the degree of concordance between cytology techniques, there is 0.85% (one sample) of agreement between positive samples for both methodologies and 88.98% (105 samples) between the negative. Discrepancy was observed in 9.32% (11 samples), in which liquid-based cytology was positive and conventional cytology was negative. In one sample (0.85%), the liquid-based cytology was negative and conventional cytology was positive. According to the Kappa association test, there is fair correlation between the techniques (κ=0.224) (**Table 3**).

According to the results found, we evaluated the correlation and/or non-correlation between the samples analyzed. Between the HCII and PCR techniques, there are 17.2% (11 samples) correlation between the samples identified as positive and 35.9% (23 samples) correlation between the negative. Non-correlation was found in 28.1% (18 samples) samples, in which HC2 was negative, whereas the PCR showed to be positive, and 18.8% (12 samples) showed positive HCII and negative PCR. This demonstrated that there is low correlation between the techniques, according to the Kappa association test (0.037) (**Table 4**).

DISCUSSION

Because of the strong association of HPV with the appearance of cervical lesions, there are a great number of studies comparing methods used for screening cervical cancer, linking it with the main risk factors.

According to population estimates, in the general female population, the prevalence of HPV infection varies from 2% to 44%. This wide variation is due to the difference in the mean age of the populations studied and the sensitivity of the methods used to detect HPV infection[7].

These results showed a high prevalence of HPV positivity through both PCR (43.22%) and the HCII methods (35%) in the sample.

Table 2 – Distribution of positive cases for human papillomavirus (high- and low-risk) through the hybrid capture method, according to the risk factors (n=23).

Variables	Low-risk HPV	High-risk HPV	p-value*
	n (%)	n (%)	
STD			
No (n=11)	5 (45.5)	6 (54.5)	0.020
Yes (n=12)	2 (16.7)	10 (83.3)	
Oral contraceptive			
No (n=13)	3 (23.1)	10 (76.9)	0.54
Yes (n=10)	4 (40.0)	6 (60.0)	
Onset of sexual activity			
≤17 years (n=14)	3 (21.4)	11 (78.6)	0.239
>17 years (n=9)	4 (44.0)	5 (55.6)	
Miscarriage			
No (n=19)	6 (31.6)	13 (68.4)	0.085
Yes (n=4)	1 (25.0)	3 (75)	
Number of partners			
Up to five partners (n=18)	5 (27.8)	13 (72.2)	0.053
More than five partners (n=5)	2 (40.0)	3 (60.0)	

STD: sexually transmitted diseases; HPV: human papillomavirus.

Table 3 – Comparison of correlating and non-correlating results between conventional cytology and liquid-based cytology techniques.

	Negative conventional cytology	Positive conventional cytology	Total
	n (%)	n (%)	n (%)
Negative liquid-based cytology	105 (88.98)	1 (0.85)	106 (89.80)
Positive liquid-based cytology	11 (9.32)	1 (0.85)	12 (10.20)
Total	116 (98.30)	2 (1.70)	118 (100)

Table 4 – Comparison of correlating and non-correlating results between the hybrid capture and polymerase chain reaction techniques.

	Negative PCR	Positive PCR	Total
	n (%)	n (%)	n (%)
Negative HCII	23 (35.9)	18 (28.1)	41 (64.1)
Positive HCII	12 (18.8)	11 (17.2)	23 (35.9)
Total	35 (54.7)	29 (45.3)	64 (100)

PCR: polymerase chain reaction; HCII: hybrid capture.

These data corroborate those in the literature, which shows high prevalence and incidence of HPV, according to the population studied and the diagnostic method[7].

Dunne et al.[19] found a higher prevalence in women aged between 20 and 24 years. In another study, the authors observed that there is a peak in women aged below 25 years and that after that age, the prevalence declines gradually[20]. In the sample studied, the prevalence of HPV peaked in patients aged below 25 years, and then there was a new peak in patients older than 45 years. More recent epidemiological studies have described a second peak in the prevalence of HPV infection in the Americas and Africa in older women, aged around 45 years or more[20]. Since this is a cross-sectional study, it is not possible to say whether the prevalence of HPV in women over 45 years is due to the persistence of a previously acquired infection or due to reinfection.

Several sociodemographic and behavioral factors are described as risk factors for cervical cancer. Several authors point to a higher risk for cervical lesions in less educated women[21,22]. In our study, however, we found a higher prevalence of this infection in women with superior education (71.4%), followed by women with secondary education (44.8%). However, Adam et al.[21] found no association between the level of education and HPV infection. These findings demonstrate the difficulty in analyzing an isolated variable, since there are probably a combination of risk factors.

There also seems to be a relationship between early onset of sexual activity and a higher risk of acquiring HPV infection, possibly due to the increase in the exposure time to the virus[23]. In the samples analyzed, the highest prevalence was found among women who initiated sexual activity after the age of 17 years. However, when analyzed separately through the HCII method, there was evidence of a higher prevalence of high-risk group among patients with early onset of sexual activity under the age of 17 years (78.6%).

For Fedrizzi et al.[7], the high number of sexual partners is one of the main risk factors for HPV infection. The relationship between the number of sexual partners and the risk of HPV infection is found in several studies[22,23]. Our prevalence of HPV infection was 43.7% for women with more than five partners. However, it had no statistical significance. When the high- and low-risk HPV groups were analyzed separately, the prevalence of high-risk HPV was higher than the low-risk both in women with few as in those with a high number of sexual partners, with statistically significant results.

HPV infection associated with other sexually transmitted agents has been related to the development of cervical cancer[23]. In a study with Brazilian women, Cavalcanti et al.[9] reported a significant contribution of STDs in the development of cervical lesions, suggesting that they could act as cofactors in the activation of cellular transformation mechanisms or decreased local immunity in the genital tract. In this study, there was a high prevalence of high-risk HPV (83.3%) in women with a history of STDs, as well as a high frequency of HPV in women with HIV (55.6%).

Studies linking the use of oral contraceptives to the risk of cervical cancer are still controversial. It is known that HPV is responsive in vitro to the use of steroids, and that they affect and stimulate the transforming activity of viral oncogenes.

There is evidence that prolonged use of oral contraceptives, for more than 10 years, would increase the risk twice for cervical cancer, but this relationship does not seem to be present for HPV infection[7].

Noronha et al.[24] observed the contrary, women using oral contraceptives had lower risk of cervical neoplasia. In the study population, it was observed that only a small proportion of women surveyed admitted to use oral contraceptives, and the prevalence of HPV found was close to that in the group that denied using this means of contraception (46.8%).

Smoking is considered one of the most important risk factors for cervical cancer. Most of the studies that show the association of this variable with cervical cancer takes into account the duration of smoking and number of cigarettes smoked per day[25]. Moreover, according to Geller et al.[26], the prevalence of HPV in smokers is due to several mechanisms, such as the presence of carcinogenic metabolites from tobacco in cervical secretions, immunosuppression leading to viral persistence, and genomic damage (from genotoxins) to the cell.

Cavalcanti et al.[9] also found that women smokers had a higher risk of developing cervical cancer. Fedrizzi et al.[7], however, found no relationship between smoking and positivity for HPV. In our study, the prevalence of HPV DNA was higher in female smokers (47.1%) when compared to nonsmokers (42.6%), but without statistical association.

Studies comparing the two cytology techniques, conventional and liquid-based, whether with simultaneous collection or with the collection of either technique performed in different comparable patient populations, often present controversial conclusions.

There are several studies, conducted in several countries, pointing to liquid-based cytology as the most sensitive procedure for the detection of ASC-US, L-SIL and H-SIL, with greater suitability of samples and fewer unsatisfactory smears[27]. In this study, there was a similar prevalence of HPV in normal cytology results. However, for L-SIL conventional cytology had a higher frequency of HPV (100%), diverging from the literature. This could be due to liquid-based cytology having been collected after conventional cytology. Studies show that there is an increase of 64.4% in H-SIL detection in BD Sure Path™ blades. Regarding H-SIL and ASC-US, liquid-based cytology showed a higher prevalence of HPV, 66.7% and 100% respectively, confirming the data in the literature.

According to Abulafia et al.[28], the percentage of correlation between the two cytology methods is 92%. These authors also reported a higher sensitivity (76%) in liquid-based cytology than in conventional cytology, which had 68%. The liquid-based method was also more specific (86%) than the conventional method (79%) with specificity ranging from 80% to 90%. In this study, of the 118 samples analyzed by the conventional and liquid-based cytology methods, there was a correlation of 0.85%; according to the Kappa test, this correlation is fair. The samples that were negative for conventional cytology and positive for liquid-based cytology may be false-negatives, probably due to higher rates of errors in the collection and fixation on the blade, because the subject who collects the material has a greater importance in conventional cytology, often determining the quality of the test.

According to Cavalcanti et al.[9] and Jordão et al.[29], it is known that cytology is a method in which the diagnosis is somewhat subjective, and there is significant inter and intra-observer variation in cytological diagnosis, especially in L-SIL. However, the recognition of some non-classical signs in smears, such as bi or multinucleation, perinuclear halo, light dyskeratosis, and hyperchromatic nucleus,

could increase the number of cases of HPV infection diagnosed by cytology. This would be very important, since it has been found that a considerable number of negative swabs in patients showing signs that suggest virus infection, whose diagnosis is confirmed through other methods, such as, for example, biopsy and molecular techniques.

Several studies have demonstrated high correlation between the HCII and PCR techniques, reaching 76.5–90%[30]. To Saini et al.[31], PCR was more sensitive (81.8%) compared with HCII (36.4%) in detecting HPV, though HCII's specificity was much higher (96.6%) than PCR's (58.6%).

In our study, the 64 samples analyzed by the HCII and PCR methods for HPV detection, we demonstrated that HCII and PCR were in correlation in 11 samples, in which, according to the Kappa test, this correlation is low. In a study by Nomelini et al.[30], HCII showed 47.5% of positivity for high-risk HPV in the sample, whereas PCR diagnosed 87.5% of positive cases showing poor correlation between them ($\kappa<0.4$). It is believed that the failure to detect the positive samples through HCII is due to low viral load in samples, occasionally making them false-positives and false-negatives.

The samples that tested positive for the HCII and negative for the PCR techniques can be false-positive results for HCII, probably due to cross-reactivity with HPV types not detected by PCR primers, although this cannot be affirmed. Some studies have attributed these false-positive results to cross reactions with high- and low-risk probe and to the need that some samples remain near the cutoff (1 RLU)[32]. According to the standardization of the HCII test by the manufacturer (Digene), samples with RLU>1 pg/mL should be considered positive. However, some studies show that the test would be more specific if the cutoff value was around 15.56 pg/mL, making it ideal for the detection of lesions, thus reducing the possibility of false-positive results, especially in samples with viral load <100 pg/mL[15].

We also believe that PCR may have been negative due to amplification failure (inefficiency of primers) or even due to mistakes in the extraction. Lonky et al.[33] demonstrated that HCII was negative in 25% cases in which the PCR detected positive results. In situations in which the PCR was positive and the HCII was negative, we considered the PCR results because the technique is performed with primers designed for detection of high- and low-risk HPV.

CONCLUSION

This study shows that HPV DNA tests (both HCII and PCR) show higher sensitivity than the conventional and liquid-based cytology for the detection of HPV. However, if used alone, it has a lower specificity than cytology collection.

Thus, tests for the detection of HPV should be used in a complementary manner to cytology in the early detection of cervical cancer, as well as in the stratification of the risk of development of premalignant lesions. Importantly, health education has a great contribution to the field of prevention through information campaigns about cervical cancer and its risk factors. Adhesion to the monitoring programs, associated with the efficacy of diagnostic methods, is key to the success of new strategies to fight cervical cancer.

Conflict of interests

The authors report no conflict of interests.

Financial support

Pró-Reitoria de Pós-Graduação, Pesquisa e Cultura da UNIVALI (ProPPEC) (SC), Laboratório IN CITO (SP) and Laboratório DNAnálise (SC).

REFERENCES

1. Brasil. Ministério da Saúde. Instituto Nacional de Câncer. Estimativa 2012: incidência do câncer no Brasil. Rio de Janeiro: INCA. [acesso 2015 Mar 04]. Disponível em: <http://www.inca.gov.br/estimativa/2014/estimativa20091201.pdf>.
2. Alba A, Cararach M, Rodrígues-Cerdeira C. The Human Papillomavirus (HPV) in Human Pathology: Description, Pathogenesis, Oncogenic Role, Epidemiology and Detection Techniques. The Open Dermatology Journal. 2009;3:90-102.
3. Mendonça VG, Guimarães MJB, Filho JLL, Mendonça CG, Martins DGB, Crovella S, et al. Infecção cervical por papilomavírushumano: genotipagem viral e fatores de risco para lesão intraepitelial de alto grau e câncer de colo do útero. Rev Bras Ginecol Obstet. 2010;10:476-85.
4. Einstein M, Schiller J, Viscidi R, Strickler H, Coursaget P, Tan T, et al. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis. 2009;9:347-56.
5. Bernard HU, Burk RD, Chen Z, Van DK, Zur HH, De Villier EM. Classification of papillomaviruses (PVs) basedon 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9.
6. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human Papillomavirus Testing in the Prevention of Cervical Cancer. J Natl Cancer Inst. 2011;103(5):368-83.
7. Fedrizzi EM, Schlup CG, Menezes ME, Campos MO. Human Papillomavirus (HPV) infection in women of Florianopolis 2008 Santa Catarina, Brazil. DST – J Bras Doenças Sex Transm, 2008; 2: 73-79.
8. Solomon D, Nayar R. The Bethesda System for Reporting Cervical Citology: Definitions, Criteria, and Explanatory Notes. 2. ed. New York: Springer-Verlag; 2004. p. 191.
9. Cavalcanti SM, Zardo LG, Passos MR, Oliveira LH. Epidemiological aspects of Human papillomavirus infection and cervical cancer in Brazil. J Infect. 2000;1:80-7.
10. Stabile SAB, Evangelista DHR, Talamonte VH, Lippi UG, Lopes RGC. Estudo comparativo dos resultados obtidos pela citologia oncótica cérvico-vaginal convencional e pela citologia em meio líquido. Einsten. 2012;4:466-72.
11. Consolaro MEL, Engler MSS. Citologia clínica cérvico-vaginal: texto e atlas. São Paulo: Editora Roca; 2012.
12. Stofler WCEM, Nunes DR, Schneider CJI. Avaliação do desempenho da citologia colposcopia comparadas com a histopatologia no rastreamento e diagnóstico das lesões do colo uterino. Arquivos Catarinenses de Medicina. 2011;40(3).
13. Wright TC, Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, et al. Interim guidance for the use of Human Papillomavirus DNA testing as an adjunct to Cervical Cytology for Screening. Obstet Gynecol. 2004;103:304-9.
14. Rodrigues AD, Cantarelli VV, Frantz MA, Pilger DA, Pereira FS. Comparação das técnicas de captura de híbridos e PCR para a detecção de HPV em amostras clínicas. J Bras Patol Med Lab. 2009;45:457-46.
15. Tulio S, Pereira LA, Neves FB, Pinto AP. Relação entre a carga viral de HPV oncogênico determinada pelo método de captura híbrida e o diagnóstico citológico de lesões de alto grau. J Bras Patol Med Lab. 2007;43:31-5.
16. Zampirolo JA, Merlin JC, Menezes ME. Prevalência de HPV de baixo e alto risco pela técnica de biologia molecular (Captura Híbrida II®) em Santa Catarina. Rev Bras Anal Clin. 2007; 39:265-268.
17. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, Hildesheim A, et al. Improved Amplification of Genital Human Papillomaviruses. J Clin Microbiol. 2000;38:357-61.
18. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1998;239:487-91.

19. Dunne EF, Unger ER, Sternberg M, Mcquillan G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. JAMA, 2007; 297:813–819.
20. Bruni L, Diaz M, Castellague X, Ferrer E, Bosh FX, De Sanjosé S. Cervical human papillomavírus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. Braz J Infect Dis. 2010;12:1789-99.
21. Adam E, Berkova Z, Daxnerova Z, Icenogle J, Reeves WC, Kaufman RH. Papillomavirus detection: demographic and behavioral characteristics influencing the identification of cervical disease. Am J Obstet Gynecol. 2000; 2:257-64.
22. Khan MJ, Partridge EE, Wang SS, Schiffman M. Socioeconomic status and the risk of cervical intraepithelial neoplasia grade 3 among oncogenic Human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer. 2005;104:61-70.
23. Fedrizzi EM, Laureano JK, Schlup CG, Menezes ME, Campos MO. Human Papillomavirus (HPV) infection in HPV positive women of Florianópolis, Santa Catarina. DST – J Bras Doenças Sex Transm. 2011;4:205-9.
24. Noronha VL, Noronha R, Carmona B, Macedo LA, Cruz EM, Naum C, et al. Papilomavirus Humano (HPV) em mulheres com citologia oncótica dentro dos limites da normalidade. DST – J Bras Doenças Sex Transm. 2005;1:49-55.
25. Noronha V, Cruz EM, Naum-Pinho C, Mell W, Noronha R, Silveira I, et al. Papilomavirus Humano em Mulheres submtidas à Colpocitologia Oncótica. DST – J Bras Doenças Sex Transm. 2006;2:130-6.
26. Geller M, Aboim E, Campos CD. Papilomavirus humano – fatores de risco, carcinogenese, resposta imune e tratamento. J Bras Med. 2008;94(3):43-6.
27. Rahimi S, Carnovale-Scalzo C, Marani C, Renzi C, Malvasi A, et al. Comparison of conventional Papanicolau smears and fluid-based, thin-layer cytology with colposcopic biopsy control in central Italy: a consecutive sampling study of 461 cases. Diagn Cythopathol. 2008;37:1-3.
28. Abulafia O, Pezzullo JC, Sherer DM. Performance of ThinPrep liquid-based cervical cytology in comparison with conventionally prepared Papanicolaou smears: a quantitative survey. Gynecol Oncol. 2003;1:137-44.
29. Jordão AV, Ruggeri LS, Chiucheta GIR, Iva S, Consolaro M. Importância da aplicação de critérios morfológicos não-clássicos para o diagnóstico citológico de papilomavírus humano. JBPML. 2003;39:81-9.
30. Nomelini RS, Barcelos ACM, Michelin MA, Adad SJ, Murta EFC. Utilization of human papillomavirus testing for cervical cancer prevention in a university hospital. Cad Saúde Pública. 2007;23.
31. Saini R, Shen TH, Othman NH, Santhanam J, Othman N, Tang TH. Evaluation of polymerase chain reaction (PCR) method and hybrid capture II (HCII) assay for the detection of human papillomavirus in cervical scrapings. Med J Malaysia. 2007;62:206-9.
32. Castle PE, Schiffman M, Burk RD, Wacholder S, Hildesheim A, Herrero R, et al. Restricted cross-reactivity of hybrid capture 2 with nononcogenic human papillomavirus types. Cancer Epidemiol Biomarkers Prev. 2002;11:1394-9.
33. Lonky NM. Triage of atypical squamous cells of undetermined significance with hybrid capture II: colposcopy and histologic human papillomavirus correlation. Obstet Gynecol. 2003;101:481-9.

Address for correspondence:
EMMANUELE PARIZ SILVA
Rua Pedro Collere, 180 – Vila Izabel
Curitiba (PR), Brasil
CEP: 80320-320
Cel: +55 (41) 9688-1425
+1 (786) 702 7745
E-mail: manuzinh@hotmail.com

Received on: 14.04.2015
Approved on: 28.04.2015