Analysis of cytopathologic examination and hybrid capture in women receiving care in basic health units

Análise do exame citopatológico e captura híbrida em mulheres atendidas em unidades básicas de saúde

Deise Jaqueline Ströher[1], Thaís Delgado Brandolt Aramburu[2], Vinícius Tejada Nunes[3], Jacqueline da Costa Escobar Piccoli[4], Vanusa Manfredini[5]

ABSTRACT

Introduction: Cervical cancer is one of the most frequent cancers among Brazilian women and its relationship with the human papillomavirus (HPV) is well established. **Objective:** To analyze the presence of DNA/HPV using Hybrid Capture method for women in the city of Uruguaiana (RS). **Methods:** During the period of January to December 2015, 51 cervicovaginal samples were collected from patients who sought care at Basic Health Units in the city. After the collection, conventional and liquid-based cytological analysis was performed. **Results:** The results of the study indicate the prevalence of genital HPV infection in 5.9% of the samples; low-risk DNA/HPV was detected in 3.9% of patients of reproductive age (PIR); and 2.0% of PIR presented high-risk DNA/HPV. By stratifying the prevalence of HPV in age, we found positivity between 16 and 31 years. **Conclusion:** Conventional cytology is often inconclusive and, in such cases, using molecular biology methods that detect the DNA/HPV presence would be very useful.

Keywords: Neoplasm; Papillomaviridae; ; Molecular biology.

RESUMO

Introdução: O câncer de colo de útero é um dos mais frequentes entre as mulheres brasileiras e a sua relação com o Papilomavírus Humano (HPV) é bem estabelecida. **Objetivo:** Analisar a presença de DNA/HPV por meio do método de captura híbrida em mulheres no município de Uruguaiana (RS). **Métodos:** No período compreendido entre janeiro e dezembro de 2015, foram coletadas 51 amostras cervicovaginais de pacientes que buscaram atendimento nas Unidades Básicas de Saúde do município. Após a coleta, foi realizada a análise citológica convencional e em base líquida. **Resultados:** Os resultados encontrados no estudo indicam a prevalência de infecção genital por HPV em 5,9% das amostras analisadas, sendo DNA/HPV de baixo risco em 3,9% e DNA/HPV de alto risco em 2,0% das infecções. Ao estratificar a prevalência de HPV por faixa etária, observou-se positividade entre 16 e 31 anos. **Conclusão:** A citologia convencional pode ser, muitas vezes, inconclusiva e, nesses casos, utilizar uma metodologia de biologia molecular que detecte a presença do DNA/HPV seria muito útil.

Palavras-chave: Neoplasia; Papiloma Vírus Humano; Biologia molecular,

INTRODUCTION

The cancer of the uterine cervix, also known as cervical cancer (CC), is caused by persistent infection of certain types of human papillomavirus (HPV). CC is the third most frequent tumor in the female population and the fourth leading cause of death of women by cancer in Brazil. According to the National Cancer Institute, the estimate for 2014 was 15,590 new CC cases[1].

It is estimated that 90% of women who developed CC were exposed to HPV[2]. HPVs involved in infections of the anogenital region can be classified according to their capacity to generate malignant neoplasias in the following categories: HPV of low oncogenic risk; HPV of high oncogenic risk; and genotypes of probable high risk[3].

Currently, more than 100 different types of HPV are known and about 20 of them are tropism for the squamous epithelium of the anogenital region (colon, vulva, perineum, perianal, and anal region)[4]. The World Health Organization (WHO), in partnership with the International Agency for Research on Cancer (IARC), identified types 16 and 18 as the main etiologic agents of CC. It is estimated that 75–80% of the female population will be infected before age of 50 years[5].

The CC prevention examination was implanted in the public network in Brazil in 1999 and aims at the early detection of neoplasia and its precursor lesions by periodic cytological analysis of the smear obtained by using the Papanicolaou technique. Statistical data show that effective screening can reduce the incidence of invasive forms of CC by up to 91%. However, the incidence of the disease remains one of the highest among malignant neoplasms occurring among Brazilian women[6].

Although cytopathological examination is the most widely used method for the screening of CC precursor lesions, its vulnerability to collection errors, slide preparation, and subjectivity in interpreting results may compromise its sensitivity and specificity. New techniques have been developed with the aim of improving the accuracy of the cytopathological examination, namely cytology in liquid medium[7].

Conventional cytology does not detect the HPV virus itself; it only detects the cellular changes caused by the virus. It presents great specificity, but it has limited sensitivity due to the variation

[1]PhD student in the Biochemistry Graduate Program of the *Universidade Federal do Pampa* – Uruguaiana (RS), Brazil.
[2]Nurse responsible for the Women's Health Sector of the Uruguaiana Municipal Health Department – Uruguaiana (RS), Brazil.
[3]Nurse from the *Universidade Federal do Pampa* – Uruguaiana (RS), Brazil.
[4]Professor at the Genomics Laboratory of the *Universidade Federal do Pampa* – Uruguaiana (RS), Brazil.
[5]Professor at the Hematology and Cytology Laboratory of the *Universidade Federal do Pampa* – Uruguaiana (RS), Brazil.

in the interpretation of the results. However, when well performed, this test is still of fundamental importance in the screening of CC and its precursor lesions[8].

Hybrid capture is a signal amplification method that uses labeled ribonucleic acid (RNA) probes for hybridization to HPV targeting deoxyribonucleic acid (DNA). The second generation of this technique, the hybrid capture version II, is being used in diagnostic laboratories in addition to cytology. This method detects viral DNA in cervicovaginal materials by means of RNA probes capable of recognizing low-risk and high-risk HPV sequences. The sensitivity of this technique is comparable to that of the polymerase chain reaction (PCR), in particular to detect high-grade lesions. This method is useful for determining viral load[8].

OBJECTIVE

To analyze the presence of DNA/HPV by means of the hybrid capture method in women receiving care at the Basic Health Units of the city of Uruguaiana (RS).

METHODS

Population Studied

The study included women of all ages and of various ethnic groups living in the city of Uruguaiana who were visiting Basic Health Units for routine gynecological examinations. The inclusion criterion was sexually active women of any age group living in the city of Uruguaiana (RS). The exclusion criteria included: women who were hysterectomized, undergoing gestational, or menstrual period, or with mental deficit that would hinder the understanding and completion of the questionnaire.

A total of 51 cervicovaginal samples were collected between January and December 2015. Cervical cytology was performed in two ways: the conventional method and cytology on a liquid basis.

The participation of women in the research was made through prior clarification and signing of the Informed Consent Form (ICF), according to resolution CN 196/96-MS and in accordance with the principles contained in the Declaration of Helsinki of the World Medical Association (1964, Reformulated in 1975, 1983, 1989, 1996 and 2000). All the objectives of this study and methodology are contained in a main study called "Elderly Women Project on the Uruguay River: Epidemiological Profile, Citomorphological of the Elderly of Uruguaiana, RS", which was evaluated and approved by the Ethics and Research Committee of the *Universidade Federal do Pampa* (CEP-Unipampa), of Rio Grande do Sul, under number 869.813.

CYTOPATHOLOGICAL EXAM

The conventional cytological smear was composed of two samples: scraped ectocervical and endocervical that were collected with Ayre spatula and endocervical brush. After collection, the material was immediately fixed with 95% alcohol. The cervicovaginal smears were sent to the Central Laboratory of Public Health (LACEN - RS).

Diagnoses were performed using the nomenclature based on the Bethesda 2001 system and the Brazilian Society of Cytopathology (SBC). The cytopathological changes were classified as atypicals of indeterminate significance of squamous and glandular cells (ASC-US / AGUS); low-grade intraepithelial lesions (LIEBG), which include CIN I; high-grade intraepithelial lesions (LIEAG), which include CIN II and CIN III; and cancer for squamous invasive carcinoma.

Tests for DNA/HPV

The hybrid capture method (DM Molecular), processed by Digene technology – a molecular hybridisation technique associated with monoclonal antibodies, allows the detection of 1 pg/mL of HPV DNA, equivalent to 0.1 copy of virus per cell. It is considered positive when RLU/PCA ratios for group A viruses (6, 11, 42, 43 and 44) and / or RLU / PCB for group B viruses (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are equal to or greater than 1.

Statistical Analysis

For the analysis of the results, the women were classified according to the age range in patients of reproductive age (PRA) and patients of non-reproductive age (PNRA). The data were plotted in the program Microsoft Office Excel and later analyzed using the program GraphPad Prisma, expressed in percentage.

RESULTS

The mean age of the women assisted during the study period was 42.7 years, with ages ranging from 16 to 86 years. **Table 1** shows the demographic, behavioral, and reproductive variables of women receiving care at the Basic Health Units of the municipality of Uruguaiana and classifies reproductive and non-reproductive age. The table shows that 66.7% of the study participants are of PRA, in the age range of 16–45 years. Patients classified as PNRA, in the age range of 46–86 years, corresponded to 33.4% of the women participants.

Regarding the marital status of the study participants, the majority of PRAs were single (35.3%) or married (41.2%). On the other hand, in the PNRA group, 41.2% stated that they did not have fixed partners, were widowed or lived without partners at that time.

Regarding the educational level, there was a higher frequency of women (35.3%) who reported having completed elementary education in the PRA group. Among the PNRAs, it is noteworthy that 11.7% are illiterate, and 11.7% have completed elementary education.

It was also found that 70.6% of the PRAs started sexual activity at an age less than or equal to 16 years and 29.4% declared that they started sexual activity after 16 years of age. In contrast, 70.6% of the PNRAs reported having had their first sexual intercourse before reaching the age of 16.

When asked about the use of contraceptives, 55.9% of the PRAs and 82.3% of the PNRAs answered that they were not using oral contraceptives. Regarding the number of partners, 91.2% of the PRAs reported having had one to two sexual partners in the last

year. In the PNRA group, 64.7% said they had not had any sexual partners in the past year.

According to the distribution of the results of the cytopathological examination shown in **Figure 1**, 47 (92%) patients presented the cytology within the limits of normality, while 4 (8%) patients presented cell alteration.

In all cases, the cytological alterations were LIEBGs. Regarding the age group, the altered results were distributed in PRA, between 26 and 32 years.

Table 2 shows the results of the viral load by the hybrid capture test. The prevalence of genital HPV infection in the analyzed samples was 5.9%, all in PRA, and in 3.9% of infections, low-risk DNA/HPV and 2.0% high-risk DNA/HPV. When stratifying the prevalence of HPV in the age group, positivity was observed between 16 and 31 years. Positive results were not observed for the PNRA test.

DISCUSSION

Studies in Latin America have detected an association between CC risk and the following sexual habits: number of partners, early sexual activity, and use of oral contraceptives, among others[9]. In the present study on behavioral factors, 91.2% of the PRAs reported having had one to two sexual partners in the year and 70.6% had started sexual activities at an age less than or equal to 16 years. On the other hand, in our study most of the PRAs (55.9%) were not using oral contraceptives.

Table 1 – Demographic, behavioral and reproductive variables of women assisted at the Basic Health Units of the municipality of Uruguaiana, classified in reproductive and non-reproductive age.

VARIABLE	PRA (16-45 years)	PNRA (46-86 years)
Age	34 (66.7%)	17 (33.4%)
Marital status		
Single	12 (35.3%)	6 (35.3%)
Married	14 (41.2%)	4 (23.5%)
Others	8 (23.5%)	7 (41.2%)
Educational level		
Illiterate	0	2 (11.7%)
Incomplete elementary	8 (23.5%)	13 (76.5%)
Complete elementary	12 (35.3%)	2 (11.7%)
Incomplete high-school	7 (20.6%)	0
Complete high-school	7 (20.6%)	0
Higher education	0	0
Onset of sexual activities		
≤16 years	24 (70.6%)	12 (70.6%)
>16 years	10 (29.4%)	5 (29.4%)
Use of contraception		
Yes	15 (44.1%)	3 (17.7%)
No	19 (55.9%)	14 (82.3%)
Number of sexual partners during the year		
None	0	11 (64.7%)
1 to 2	31 (91.2%)	5 (29.4%)
More than 2	**3 (8.8%)**	**1 (5.9%)**

PRA = patients in reproductive age
PNRA = patients in non-reproductive age

Roteli-Martins et al.[10] indicate that 76.4% of patients reported onset of sexual activity between 14 and 20 years. This scenario poses even greater risks for HPV susceptibility when the onset of sexual practice is devoid of condoms. The practice of younger women's sexual activity is greater than that of older women. This fact is confirmed in our study, which indicated that 64.7% of the PRNAs say they had not had sexual partners in the past year. It can be said that the sexuality of the elderly woman is surrounded by prejudices and failures of the information about the aging process and the changes in sexuality in different age groups, especially for older age groups[11].

One of the risk factors for HPV infection is limited level of education and low socioeconomic status. However, younger women still report a higher level of education[12]. Thus, it can be observed that the PRAs have a better level of schooling, since 35.3% of the women affirm that they have finished elementary school, unlike the PNRAs that affirm that they have not finished elementary school.

A higher level of education contributes to the reduction of the number of cases of the disease, since school attendance allow

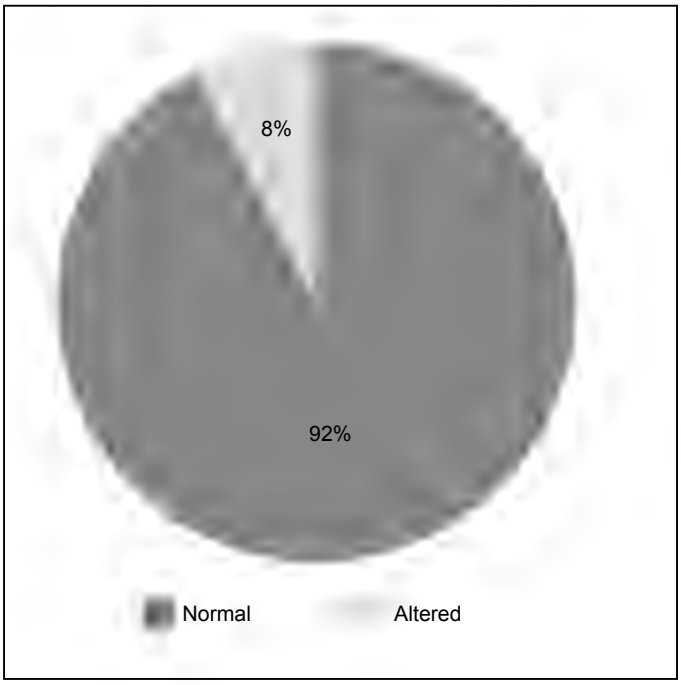

Figure 1 – Percentage of normal and altered cytopathologic reports of patients who underwent gynecological cytopathology in Basic Health Units of the city of Uruguaiana.

Table 2 – Results obtained in the cytology on liquid basis in relation to viral load by the hybrid capture test for oncogenic and non-oncogenic DNA/HPV.

DNA/HPV	n	Viral load	% of patients
RLU/PCA ≥ 1pg/mL Non-oncogenic	1 1	1.02 pg/mL 1.00 pg/mL	3.9%
RLU/PCB ≥ 1pg/mL Oncogenic	1	1.01 pg/mL	2.0%
Total	**3**		**5.9%**

students to reach a level of knowledge capable of influencing preventive measures when one has a better understanding about the disease[11]. However, in the present study, the highest prevalence of cases of HPV infection is still found in PRAs that have a higher level of schooling when compared to PNRAs.

Regarding the marital status of the women participating in the study, one can see a higher percentage of PRAs who declare themselves married or living with a partner. In this view, a study by Rama et al.[13] indicate that sexual activity of women with a stable union predisposes them to the virus. Indeed, it is understood that these women believe to live within a standard of reliability and safety in relation to the their partner and therefore do not recognize the need for STD prevention, which in turn makes them more vulnerable to HPV infection and other sexually transmitted diseases.

The literature indicates that a higher incidence of CC affects women between the ages 40 and 60 years, and that the cancer is not common in women under 20 years of age. However, 70% of cases of CC could be related to HPV, with the highest prevalence of HPV contamination in young women between the ages of 15 and 25, the period of sexual initiation. In this study, the prevalence of genital HPV infection was in the age range of 16–31 years. According to Roteli-Martins et al.[10], adolescents who are sexually active have the highest rates of infection, incidents, and prevalence.

The prevalence of genital HPV infection in the present study was 8.8% for both viral types (oncogenic and non-oncogenic). Data from the literature indicate a general prevalence of cervical infection by HPV ranging from 13.7 to 54.3%. For women with normal cytology, the prevalence of HPV infection in the cervix ranges from 10.4 to 24.5%[14]. The divergence between the literature data and the findings of this study may be related to the sample number and a high number of PNRAs.

Studies show that the highest prevalence of genital HPV infection is found in women under 25 years of age, with a progressive linear decrease after this age and reaching values below 5% after 55 years[13]. The fact that we did not have any positive results from HPV infection in PNRA in our study may be related to changes in sexual habits, which would make women less exposed. On the other hand, it is true that there is an increase in the number of STD/AIDS cases among the elderly.

According to Castle et al.[15], 15% of the women who underwent Pap smears may present a negative cytological result with positive hybrid capture for high-risk HPV. In this study, we observed this profile in 2.9% of the analyzed women.

When we compared the results of the positive hybrid capture DNA/HPV test with the conventional cytology of the study patients, we found that the result of conventional cytology is negative. Results such as these, discordant between cytological and molecular methods, may occur due to the observer's difficulty to interpret the slide, problems in the collection or even because of the high sensitivity of the molecular method.

CC is a highly preventable disease, with preventative measures including early detection and treatment of precursor lesions. Currently, in addition to conventional cytology and DNA/HPV detection several biomarkers are being studied. In this sense the evolution and improvement of new molecular techniques that allow for the evaluation of cellular alterations, and the simultaneous analysis of these markers may contribute to a significant decrease in the number of patients who develop CC.

CONCLUSION

Conventional colpo-cytology may be inconclusive and, in such cases, using a methodology that detects the presence of DNA/HPV would be very useful. Hybrid capture has been widely used in the routine of clinical analysis laboratories because it is faster to obtain the results as opposed to more complex methodologies, such as PCR, for example.

Conflict of Interest

The authors declare no conflict of interest.

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Address for correspondence:
VANUSA MANFREDINI
Laboratório de Hematologia e Citologia Clínica
Universidade Federal do Pampa
BR-472, km 585
Uruguaiana (RS), Brasil
CEP: 97500-970
E-mail: vanusamanfredini@unipampa.edu.br

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