# Prevalence of infection with high-risk HPV in women using hybrid capture conducting prevention of cervical cancer in Southern Brazil

## *Prevalência de infecção por HPV de alto risco em mulheres utilizando captura híbrida na prevenção do câncer do colo do útero no Sul do Brasil*

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**ABSTRACT**

**Introduction:** High-risk Human Papillomavirus (HR HPV) infection is known to be linked to cervical cancer, with molecular biology tests being an important tool in diagnosis. **Objective:** This study is aimed to quantify the prevalence of HPV infection in women from the Southern part of the State of Rio Grande do Sul, Brazil, correlating factors associated with the development of precursor lesions and of cervical cancer. **Methods:** 643 women were enrolled in the study, by filling out a standardized questionnaire and undergoing cytology, colposcopy, and HR HPV Hybrid Capture 2 (HC2) tests. **Results:** Most patients were aged between 20 to 39 years (70.6%), this decreased the percentage of smokers from 23% to 11%. The average age of sexual debut through the period studied was of 18 years old. HR HPV prevalence was correlated with younger ages, with fewer patients infected by HR HPV when they were older at first sexual activity. Almost 70% prevalence of infection was observed in women who had 4 or more sexual partners. Altered cytology and colposcopy results had significantly higher rates of HR HPV infection. 334 women were referred for biopsy. Of those, 321 had altered colposcopy results and cytopathology of ASC-US/AGC-US, LSILs and HSILs, with 231 biopsies performed by the study. None of the results indicated cervical cancer. HC2 showed higher specificity than cytology, with high positive and negative prediction values (49.8% and 78.6%, respectively).**Conclusion:** The inclusion of HR HPV testing in screening programs in Brazil, according to international policies, will lead to fewer biopsies in women without infection and increased interval between screenings.

**Keywords:**high risk HPV, cervical cancer, hybrid capture, Brazil.

**RESUMO**

**Introdução:** Sabe-se que infecções por HPV de alto risco (HPV AR) estão ligadas ao desenvolvimento de câncer cervical. **Objetivo:** Esse estudo teve como objetivo quantificar a prevalência de infecções por HPV em mulheres da metade sul do Estado do Rio Grande do Sul, Brasil, correlacionando fatores associados ao desenvolvimento de lesões precursoras e câncer cervical. **Métodos:** Para tanto, 643 mulheres foram incluídas no estudo, preenchendo um questionário padronizado e submetidas aos exames de citologia, colposcopia e HPV AR (Captura Híbrida 2). **Resultados:** A maioria das pacientes tinha idade entre 20 e 39 anos (70,6%), houve decréscimo na porcentagem de fumantes de 23% para 11% e a média de idade ao início da vida sexual era de 18 anos. A prevalência de HPV AR é correlacionada com idades mais jovens, com menos pacientes infectadas por HPV AR quando estas eram mais velhas no momento do início da atividade sexual. Prevalência próxima a 70% foi observada em mulheres que tiveram 4 ou mais parceiros sexuais. Resultados citológicos e colposcológicos alterados tiveram taxas significativamente mais altas de infecção por HPV AR. 334 mulheres foram encaminhadas à biópsia. Destas, 321 tiveram resultados de colposcopia alterados e citopatologia ASC-US/AGC-US, LSILs e HSILs, com 231 biópsias realizadas neste estudo. Nenhum dos resultados indicou câncer. O teste de CH2 mostrou mais especificade do que a citologia, com altos valores preditivos positivos e negativos (49,8% e 78,6%, respectivamente). **Conclusão:** A inclusão de testes para HPV AR nos programas de triagem no Brasil, de acordo com as políticas internacionais, levará à redução de biópsias em mulheres não infectadas e aumentará o intervalo entre exames.

**Palavras-chave:** HPV de alto risco, câncer cervical, captura híbrida, Brasil.

## INTRODUCTION

Cervical cancer is the third most common malignancy in women, and the seventh in general, with approximately 270,000 deaths annually[1]. Epidemiologic studies have shown that the main aetiological factor of cervical cancer is the infection with high-risk (HR) types of Human Papillomaviruses (HPVs)[2]; in fact, nearly all of cervical cancer cases test positive for HPV[3].Persistent HPV infection have been unequivocally linked to the development of cervical cancer, and at least 15 HPV sub-types are classified as high risk, including 16, 18, 31, 33 and 51[4]. HPV types 16 and 18 are the most carcinogenic, responsible for approximately 70% of all cervical cancers[5] .

The knowledge of the association between HPV and cervical cancer has led to the development of new screening techniques based on molecular biology testing. Some of these strategies include PCR-based diagnosis or Hybrid Capture 2 (HC2) assays, and between them, HC2 testing has better sensitivity for the detection of High-grade Squamous Intraepithelial Lesions (HSIL)[6–9].

Among the pre-malignancy possible results of Pap smear, HSIL shows more severe cell abnormalities and are more likely to progress into cancer if left untreated[10]. Hence the importance of identifying HR HPV in patients with a HSIL diagnosis.

Reviews on HPV infection, screening processes and treatment options indicate a lack of studies in developing countries[7]. The current study aims at quantifying HPV infection in women

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from the Southern part of the State of Rio Grande do Sul, and describes factors associated with the development of precursor lesions and cervical cancer.

## METHODS

A cross-sectional study was performed at the Gynecological Cytology Specialized Clinic (SECIG, Pelotas, RS-Brazil). All women included in the study were the patients who were referred for test of HPV, by using uterine cervix samples and Hybrid Capture 2 (HC2) method, between January of 1998 and July 2012. This population comprises patients of gynecological practices from the entire Southern region of the State of Rio Grande do Sul (Brazil), private and insured. SECIG is the only service in the region, performing all the three screening tests such as cytopathology, videocolposcopy and HC2, being a reference also for the treatment of uterine cervix pathologies.

The study population comprised of 643 women aged between 15 to 81 years. The hybrid capture test was requested by the patient's physician mostly based on previous history of HPV infection or the presence of clinical findings. Pregnant women, patients with diagnosis or suspicion of immunosuppression and those who underwent total hysterectomy were excluded. Patients answered a standardized questionnaire which included the following information: age, number of pregnancies, age of first sexual intercourse, number of sexual partners, smoking, previous history of HPV, and birth control method of choice. Gynecological exam was then performed and screening test samples were obtained according to the following protocol:

For cervical cytology, an Ayre's spatula was used to collect cells from the transformation zone and a cytobrush for endocervical cells. Slides were fixed in 95% ethanol and Papanicolaou stained was used. A single pathologist analyzed all cytological tests and results were described using Bethesda International Classification[11].

Colposcopy was performed using a *DFVasconcellos*® videocolposcope model CP-M1250, according to the literature[12]. Colposcopy findings were classified according to the International Federation of Cervical Pathology and Colposcopy (IFCCP) classification[13] and the images were digitalized. Results were catalogued as normal and abnormal according to the presence of: aceto-whitening at different levels, changed patterns of the blood vessels, and the stain pattern with iodine.Testing was unsatisfactory when the totality of the squamocolumnar junction was not visualized or intense atrophy and inflammatory process that prevented evaluation was observed.

Patients with altered colposcopies were submitted to biopsy, using *MedGyn*® BabyTischlerCervicalBiopsy forceps, and subsequent histologic analysis with Hematoxylin/Eosin coloration. At colposcopy, two or more biopsy specimens should be taken. The used nomenclature was: low-grade Cervical Intraepithelial Neoplasia (CIN1), high-grade Cervical Intraepithelial Neoplasia (CIN2, CIN3) and invasive cervical carcinoma[14].

For the detection of HPV DNA by HC2, material was collected from the cervix using the swab and collection kit provided by *Digene*® HC2 HPV DNA Test (Qiagen N.V., Netherlands). The material was processed according to manufacturer's instructions. Samples were analyzed for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The usual limit of 1 pg/mL of HPV DNA 16 was used as positive control, determining both quantitative and qualitative results (1 pg/mL equals 0.1 viral copy by cell). Positive results were then categorized in low (1 pg/mL to 10 pg/mL), moderate (10 pg/ml to 100 pg/mL) and high viral load (>100 pg/mL).Bivariate analyses were performed using chi-square and chi-square for linear trend. Statistical tests were considered to be statistically significant at a $p < 0.05$.

The study protocol was approved by the Ethics Committee of the Universidade Federal de Pelotas (Approval report number 425.607).

## RESULTS

The majority of the patients were aged between 20 to 39 years (70.6%) (**Table 1**), and the age distribution did not changed in different periods throughout the of the study. The prevalence of smoking was 23% at the first period of years and showed a significant reduction through the years, reaching 11% at the last period. Regarding the age of sexual initiation, there was an increase in the number of women having their sexual debut before the age of 15 years, and a decrease in those who started at the age of 20 or more.

The number of sexual partners was evenly distributed between 1, 2 or 3, and 4 or more for the first third of the study. From 2003 onwards we can see a trend of increase in the number of sexual partners in this population. The number of pregnancies did not vary throughout the studied period. There was a significant trend of decrease in the number of women infected by HR types of HPV on recent years (**Table 1**).

**Table 2** shows the positivity for HR HPV according to the sample characteristics. Regarding HR HPV prevalence, there was a linear correlation between a positive result for HPV and age, with a clear decrease in HPV infection with the increase in age ($p < 0.001$). This study did not find a significant correlation between HPV infection and smoking ($p = 0.4$). There was, however, a linear trend for reduction in HR HPV infection with the increase in the age of sexual initiation ($p < 0.001$).

We found that an increase in the number of sexual partners was associated with an increase in HR HPV infection ($p < 0.001$), reaching almost 70% of prevalence in those who had 4 or more partners. Moreover, no correlation was found between the number of pregnancies and infection by HR HPV (**Table 2**). Although infection decreased in a linear pattern between those with no children and those with 1 or 2 pregnancies, the trend was not maintained. Those women with 3 or more pregnancies showed similar values of HR HPV infection than women with only one pregnancy (**Table 2**).

**Table 3** shows the relationship between positive results for HR HPV tests, colposcopy and cytopathology exams. Patients with altered colposcopy had significantly higher rates of HR HPV infection when compared with those patients whose colposcopy showed no alteration ($p < 0.001$). There was also a significant difference according to the results of the cervical cytopathology tests. More severe results showed higher prevalence of HR HPV infection. Results varied from a prevalence of 37.2% of HR HPV infection among patients with normal cytopathology to a 98.4% prevalence among patients with HSILs.

**Table 1 –** Characterization of the cohort, on the three periods of the study, according to the variables from the standardized questionnaire

| Variables | 1998 – 2002 | 2003 – 2008 | 2009 – 2012 | Total | p-value |
|---|---|---|---|---|---|
| | n = 199 | n = 227 | n = 217 | n = 643 | |
| **Age** | | | | | **0.34*** |
| Up to 19 years old | 12.6 | 8.8 | 6.9 | 9.3 | |
| 20 to 29 years old | 45.2 | 56.4 | 45.6 | 49.3 | |
| 30 to 39 years old | 18.1 | 21.2 | 24.4 | 21.3 | |
| 40 to 49 years old | 14.6 | 11.5 | 14.8 | 13.5 | |
| 50 years old or more | 9.6 | 2.2 | 8.3 | 6.5 | |
| **Smoking** | | | | | **0.001*** |
| No | 76.9 | 85.5 | 88.9 | 83.9 | |
| Yes | 23.1 | 14.5 | 11.1 | 16.0 | |
| **First intercourse** | | | | | **0.005*** |
| Before 15 years old | 19.1 | 19.8 | 24.0 | 21.0 | |
| 16 to 19 years old | 54.3 | 56.8 | 62.2 | 57.9 | |
| 20 years old or more | 26.6 | 23.4 | 13.8 | 21.2 | |
| **Number of partners** | | | | | **< 0.001*** |
| 1 | 36.2 | 25.6 | 17.1 | 26.0 | |
| 2 to 3 | 31.7 | 34.4 | 33.2 | 33.1 | |
| 4 or more | 32.2 | 40.1 | 49.8 | 40.9 | |
| **Pregnancies** | | | | | **0.40*** |
| 0 | 64.8 | 72.3 | 64.5 | 67.4 | |
| 1 | 10.6 | 13.7 | 17.1 | 13.8 | |
| 2 | 17.1 | 7.9 | 13.4 | 12.6 | |
| 3 or more | 7.5 | 6.2 | 5.1 | 6.2 | |
| **HR HPV** | | | | | **0.05*** |
| Negative | 38.2 | 28.2 | 47.0 | 37.6 | |
| Positive | 61.8 | 71.8 | 53.0 | 62.4 | |

*Chi-square for linear trend.

**Table 4** presents results for colposcopy and cytopathology exams, since the criteria for referral to histological analysis by biopsy was the correlation between altered colposcopy and cytology results. The results on this table indicate that the patients that should be referred to biopsy are those with altered colposcopy results (atypical transformation zone) and cytopathologic results of ASC-US/AGC-US, LSILsand HSILs. This group corresponds to 321 women. Nevertheless, a further 6% of patients (13 women) were referred to biopsy despite having normal cytopathologic results. Their colposcopy findings included intense reaction to acetic acid, mosaic and punctation.

Therefore, 334 women were referred for biopsy, but only 231 biopsy exams were performed, since some patients returned to their physicians of choice to undergo the procedure. In the group of 231 women that had biopsy exams done by our team, results were as follows: 10% presented chronic cervicitis, 43% CIN I, 46% CIN II or III. None of the performed biopsies suggested cervical cancer.

From the total of patients with CIN II or III (107 women), 75% were referred to Loop Electro-surgical Excision Procedure (LEEP), 10% to cold knife conization, and 3% to electrocauterization of the cervix. The remaining patients were treated by their physician of choice. Histological surgical results, for both LEEP and cone, were 3% negative, 16% CIN I, 77% CIN II or III, and 4% invasive carcinoma. Regarding the margins of LEEP/cone samples, 85% had noninvolved (negative) margins.

**Figure 1** presents sensibility and specificity values of the methods when compared to the histological findings, while **table 5** presents positive and negative prediction values according to the tests.

**Table 2 –** High Risk HPV prevalence, according to characteristics of the cohort

| Variables | High Risk HPV | | p-value |
|---|---|---|---|
| | Negative | Positive | |
| | n = 242 | n = 401 | |
| **Age** | | | **< 0.001*** |
| Up to 19 years old | 25.0 | 75.0 | |
| 20 to 29 years old | 30.3 | 69.7 | |
| 30 to 39 years old | 36.5 | 63.5 | |
| 40 to 49 years old | 57.5 | 42.5 | |
| 50 years old or more | 73.8 | 26.2 | |
| **Smoking** | | | **0.40** |
| No | 38.3 | 61.7 | |
| Yes | 34.0 | 66.0 | |
| **First intercourse** | | | **< 0.001*** |
| Before 15 years old | 26.7 | 73.3 | |
| 16 to 19 years old | 37.1 | 62.9 | |
| 20 years old or more | 50.0 | 50.0 | |
| **Number of partners** | | | **< 0.001*** |
| 1 | 50.9 | 49.1 | |
| 2 to 3 | 33.8 | 66.2 | |
| 4 or more | 32.3 | 67.7 | |
| **Pregnancies** | | | **< 0.001*** |
| 0 | 31.4 | 68.6 | |
| 1 | 46.1 | 53.9 | |
| 2 | 55.6 | 44.4 | |
| 3 or more | 50.0 | 50.0 | |

*Chi-square for linear trend.

**Table 3 –** High risk HPV prevalence, according to colposcopy and cytology results

| Variables | HR HPV viral load | | | p-value |
|---|---|---|---|---|
| | Negative | Positive | Total | |
| | n = 242 | n = 401 | n = 643 | |
| **Colposcopy** | | | | < 0.001 |
| Normal | 64.1 | 35.9 | 44.2 | |
| Altered | 16.7 | 83.3 | 55.8 | |
| **Cytopathologic** | | | | < 0.001 |
| Normal | 62.8 | 37.2 | 41.8 | |
| ASC-US/AGC-US | 38.5 | 61.5 | 14.2 | |
| CIN I | 16.8 | 83.2 | 34.2 | |
| CIN II e III | 1.6 | 98.4 | 9.8 | |

Sensibility for altered cytopathology test was 98.1% and positive prediction of 48.4%. Specificity was 9.7% and negative prediction of 53.7%. Regarding HR HPV, sensitivity was 94.4%and positive prediction of 49.8%, while specificity was 17.7% and negative prediction was of 78.6%.

## DISCUSSION

There was an increase in the number of women with age above 30 who were seeking medical care with physicians for HPV-related exams throughout the studied period. From 2009 onwards it was observed a decrease in the proportion of patients aged less than 29 years and an increase in those aged over 30, relative to the two previous study periods. This agrees with current policy guidelines that advise the cotesting for HPV and cytopathology for women aged 30 or older[10]. It was also observed a decline in smoking habits throughout the study, reaching less than half of the

**Table 4 –** Relative results between colposcopy and cytology tests

| Cytopathologic | Colposcopy | | | | p-value |
|---|---|---|---|---|---|
| | Normal | | Altered | | |
| | n | % | n | % | < 0.001 |
| Normal | 231 | 85.9 | 38 | 14.1 | |
| ASC-US/AGC-US | 33 | 36.3 | 58 | 63.7 | |
| CIN I | 20 | 9.1 | 200 | 90.9 | |
| CIN II e III | 0 | 0 | 63 | 100.0 | |

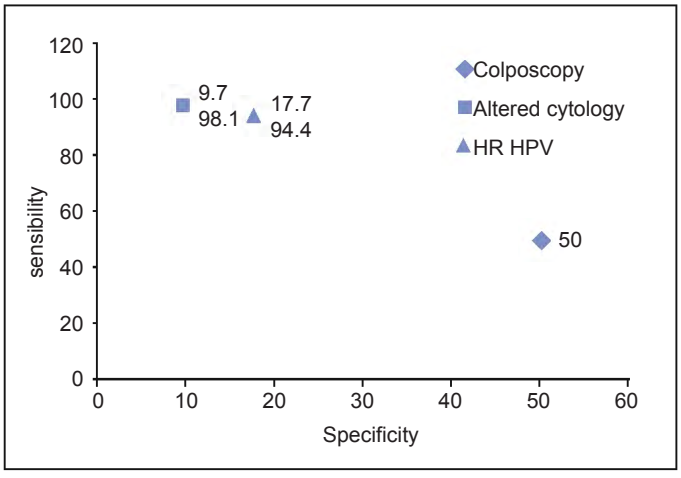


**Figure 1 –** Sensibility and specificity results for colposcopy, cytopathology and HR HPV tests.

**Table 5 –** Positive and negative predictive values (PPV and NPV, respectively) for precursor lesion prediction on colposcopy, cytopathology and HR HPV tests

| | PPV | NPV |
|---|---|---|
| Colposcopy | 46.3 | - |
| Altered cytology | 48.4 | 53.7 |
| HR HPV | 49.8 | 78.6 |

initial proportion of smokers. This trend has also been observed in other countries[15,16]. In the United Kingdom there was a significant reduction in the percentage of female smokers, from 40% in the 1970's to 20% in 2007, an estimate that remains constant[15]. The proportion of 16% for smoking found in our study is the same described in the United States[16].

A change in the age of sexual initiation is evident in this population, with a trend for earlier initiation. Other studies have shown an average decrease of four years in the sexual debut [17]. Bajos *et al.*[17] also observed an increase in the number of sexual partners between the years of 1970 (1.8) to 2006 (4.4). The same was observed in the present study. As most middle-income countries, Brazil has made the transition to a low-fertility country, with women having less children, having near zero population growth rate[18]. The data seen is this population reflects this tendency for smaller families. Nonetheless, we could not define a trend in HR HPV infections, with the prevalence fluctuating between different time periods.

Our study has shown that younger women were at greater risk of contracting HR HPV. This finding agrees with previous findings[19]. Also, the early beginning of sexual activity may lead to an increased number of partners in life, and both variables increase the likelihood of HR HPV infection. In this study 21% of women had started sexual life before the age of 15, and over 73% of them tested positive for HR HPV infection. Although there is limited information of sexual behavior data for Brazil[1], this report agrees with previous findings of another Brazilian group, where 20% of women reported having had their sexual debut at the age of 15 or younger[20].

With the average age of sexual initiation being 18 years, women who started sexual activity after the average age had lower risk of HR HPV infection than those whose onset was below average age. Regarding the number of partners, there was a significant increase (38%) in HR HPV infection with higher number of sexual partners.

Although there is an indication that smoking could be an independent factor associated with squamous cell carcinoma and adenocarcinomas[20,21], this study did not find correlation between smoking and infection by HR HPV. However, the actual role of independent factors still bears a level of uncertainty.

According to the literature, there is a relative increase of risk to develop cervical cancer with, not only an increasing number of sexual partners, but also with younger age at first intercourse, younger age at first full-term pregnancy, increasing duration of oral contraceptive use, and also with increasing parity[22]. The findings of this study don't follow such trend. However, this may be explained by the distribution of the cohort. The number of women who had 3 or more pregnancies and 4 or more partners is only 5% of the number of women without children with 4 or more partners (data not shown). The absence of linearity for this variable may be due to the fact that, for this study, an increase in the number of pregnancies was proportional to a decrease in the number of sexual partners (data not shown).

The high sensitivity of HR HPV test is very important when colposcopy and cytology tests give false positive results or to reassure patients that had inconclusive results from the aforementioned tests. Over 38% of patients diagnosed with ASC-US/AGC-US through cytopathology were HR HPV negative. Since the patient won't need to treat an abnormality that will probably disappear without treatment, the psychological tension associated with a diagnosis that could lead to cancer is considerably lowered[10]. Also, increasing the interval on follow-up visits benefits the patient emotionally, since the negative result on the HR HPV test would restrict the physician from referring the patient to colposcopy. In the Brazilian case, this wouldn't account for a significant financial difference, as colposcopy tests cost R$ 3.38 on the Public Health System (SUS)[23]. However, in countries where the test is only available through private medical care, the increase in costs might make a significant difference on both access to the test and its combination with other tests.

Although most research groups find that HPV testing is more sensitive, but less specific than cytology[7,19,24], we report different findings. As seen in **Table 5**, HR HPV testing showed opposite results, with lower sensitivity (although high percentage value) and higher specificity than cytological test. Since, results showed higher positive and negative predictive values (PPV and NPV, respectively), the tendency is for higher costs of screening. As cytology is cheaper than HR HPV tests, when it has higher PPV values, this can reduce the referral of patients to colposcopy. A thorough screening strategy, as the one presented here, prevents the development of cervical cancer in apparently adequately screened women. Also, with such high NPV, the use of HR HPV test can lead to an extension on screening interval.

## CONCLUSION

Therefore, from 14 years of analysis included in this study, we see that women in the Southern part of Rio Grande do Sul are now smoking less, have first intercourse at younger age, and increasing the number of partners throughout their life and hence exposing themselves to higher risks of HR HPV infection. However, there has been a decrease in the number of children they have. Women and physicians are complying with international policies for HR HPV testing, since the percentage of women aged 30+ referred to testing increased. And with HR HPV tests showing high levels of specificity, screening intervals may be increased without compromise to the patient's health.

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### Conflict of interests

The author declare no conflict of interests.

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