Cervical human papillomavirus infection in older women

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Abstract

Objective: To investigate the presence of cervical infection by human papillomavirus (HPV) and associated factors in older women. *Method:* A cross-sectional, retrospective descriptive study with a quantitative approach was conducted. The sample comprised 106 women aged 60 years or over, seen at public health services of a city in southern Brazil, who underwent cervical cell collection for cytological analysis and molecular detection of HPV DNA. Clinical and sociodemographic data were collected using a standardized questionnaire and from Pap test results. *Results:* Patient age was 60-82 years, with a mean of 64.9 ± 5.1 years. HPV was detected in 14 (13.2%) of the study participants and 8 viral types were identified, the majority (n=7; 87.5%) of high oncogenic risk. Chi-square analysis revealed that positive HPV cases were associated with a higher number of sexual partners (p= 0.018). On cytology, most of the women (n=102; 96.2%) had a negative result for intraepithelial lesion or malignancy, and two (1.8%) had abnormal cytology, but neither were positive for HPV infection on molecular testing. Of the 10 women evaluated at two visits, seven (70%) tested negative for HPV infection on both evaluations, two (20%) eliminated the HPV infection, and one (10%) showed conversion to positive infection status. None of the cases had persistent infection. *Conclusion:* Older women are susceptible to HPV infection and to the lesions caused by the virus. This group should therefore continue regular cytological screening.

Keywords: Screening. Papanicolaou test. Women's Health. Cervical Neoplasms.

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INTRODUCTION

The human papilloma virus (HPV) can infect the epithelial coating of the anogenital tract and other mucosa areas of the body and is responsible for the occurrence of a number of diseases, including cervical cancer[1].

The global incidence of cervical cancer in women aged over 60 years is 35.1 per 100,000 population. In Brazil, this rate is higher at 39.5 per 100,000 population[2]. Persistent infection by oncogenic types of HPV is the main risk factor for developing this neoplasm[3].

The main risk factors for acquisition of HPV infection include number of life-time sexual partners, age at sexual debut, smoking, use of birth control pill, other sexually-transmitted infections (STIs), chronic inflammation, immunosuppression, and parity. Age has also been implicated as a risk factor for the development of cancer, due to cellular changes which take place during the aging process, favoring cellular errors and differentiation[4].

The prevalence of HPV in younger women is high, but declines from age 30 onwards, irrespective of sexual behavior, suggesting that immune response plays a role. However, in some regions of the world, women experience a second peak from 50 years and older[5]. One proposed explanation for this second peak is multiple partners, through which women may be exposed to different types of HPV or to reactivation of latent infection, owing to progressive reduction in specific immunity, exposure to infected partners and hormonal fluctuations. Also, due to the immunosenescence process in older individuals, the virus is not eliminated effectively, particularly for multiple infections that involve different viral types[6,7].

The cytopathological (Pap) test is used in routine screening for cervical cancer[8] as this enables identification of precursor lesions or invasive forms of the cancer[9]. Molecular detection of high-risk HPV can identify women at greater risk of developing neoplasia. Combined use of these two approaches (Pap-HPV co-testing) is recommended by some health organizations and societies and has become part of routine practice in many countries[10].

In Brazil, the Ministry of Health recommends cytopathological testing every 3 years for women aged 25-64 years after 2 consecutive normal annual tests. This testing regimen should continue until the age of 64 and be suspended after at least 2 consecutive positive tests within the past 5 years[8]. However, in women that continue to have an active sexual life after this age, further routine screening may be pertinent, given that the second peak of HPV occurs at older ages[11].

Moreover, it important to emphasize that the older population does not necessarily stop experiencing their sexuality. However, unsafe sex makes this group vulnerable to STIs, including HPV. Additionally, the lack of specific guidelines for management of STIs in older people hampers the individualized care of this group[12].

In this context, although older women are vulnerable to infection by HPV, few studies on this topic involving this population have been conducted[13]. This scenario highlights the need to further investigate and elucidate cervical infection by HPV in older women, allowing the devising of strategies for the prevention and early detection of HPV-induced lesions in this group, including cervical cancer.

Therefore, the objective of this study was to investigate the presence of cervical infection by HPV and associated factors in older women.

METHOD

Study characteristics and ethics aspects

A retrospective, descriptive, cross-sectional study with a quantitative approach analyzing data from studies performing cytological assessment and detection of DNA-HPV in cervical samples was conducted. These studies are in compliance with Resolution nos. 466/2012 and 510/2016, and were approved by the Research Ethics Committee under permits 078.0.417-09, 1.506.860, 2.790.225 and 4.984.176.

Sample characteristics

A total of 106 older women were included in the sample, drawn from the database associated with the projects outlined above, according to the inclusion criteria of the present study, namely: age ≥60 years, and results of Pap and HPV molecular tests available on the database.

The sample of women studied was seen under the Cervical Cancer Screening Program, run as part of the Family Health Strategies, at the city of Cruz Alta (RS) to perform routine cytopathology tests during the periods January-June, 2010 (n=337), January-November, 2012 (n=285), March-November, 2013 (n=374), August-November, 2018 (n=89) and April-June, 2019 (n=78). The women were seen under the screening program both opportunistically, i.e. underwent testing when seeking health services for other reasons, or by prior appointment for the test.

Intentional sampling was used and participants included in the study were grouped as follows: (i) women who made only one visit to the services included over the study period; and (ii) women who made 2 visits to the services included in the study, with a minimum interval of 1 year between them, over the study period (Figure 1).

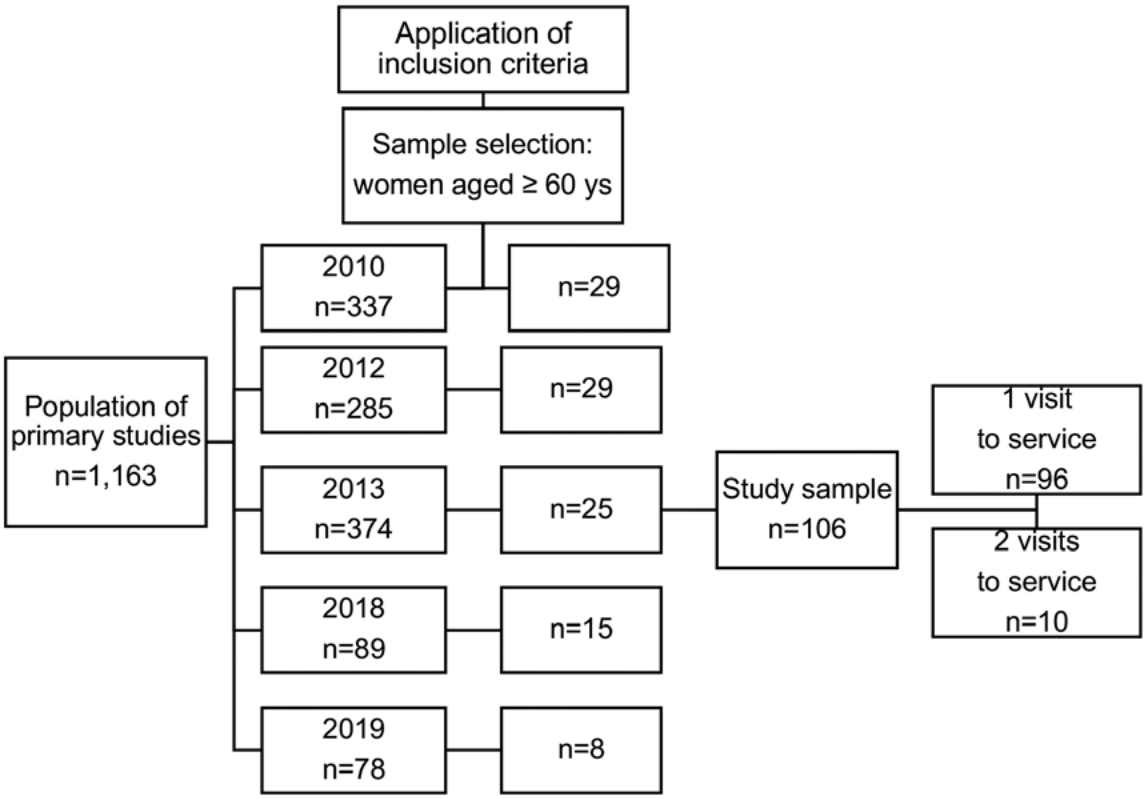

Figure 1. Flow diagram of sample selection process. Cruz Alta, Rio Grande do Sul state, 2023.

Current guidance for screening in Brazil recommends that collection of this test should commence at 25 years of age for women who have debuted or are sexually active and cease at 64 years, for individual with no prior history of pre-neoplastic disease and at least 2 consecutive negative tests in the past 5 years. Women aged older than 64 years who have never performed the test should undergo 2 tests with a 3-year interval between them. In the event that both tests prove negative, these individuals need undergo no further testing[7].

Data Collection

The following information were obtained from the database of the primary studies: results of cytological (Pap) test, results of HPV detection test, and both clinical and sociodemographic data.

The result of the cytological test was based on the Papanicolaou technique, with samples examined by 2 cytopathologists and results classified according to the Bethesda system[9]. Data for the HPV test were obtained using molecular biology techniques, with DNA extraction by the silico method, DNA amplification using Nested-PCR assays[14] and genotyping by direct sequencing or restriction fragment length polymorphism (RFLP) from PCR-amplified DNA fragments, with classification of viral types according to de Villiers et al.[15] and de Villiers[16].

Women who had made two visits to the services included in the study, at least 1 year apart, were assessed on both occasions by applying the Pap test and molecular HP detection test to check for the persistence, conversion to positive status, or elimination of the infection. This group was categorized according to HPV infection status, as follows: (1) persistent infection, when DNA-HPV was detected at both assessments; (2) conversion, when DNA-HPV was negative at the first consultation, but subsequently detected during follow-up; (3) elimination, when the presence of DNA-HPV was detected only at the first assessment; and (4) no HPV infection, when DNA-HPV was negative at both visits.

Clinical data (sexual debut, date of last Pap test, use of condom during sexual intercourse, number of sexual partners) and sociodemographics (age, education, number of children) were obtained by applying a standardized questionnaire and from the findings of the Pap test applied to participants.

Statistical Analysis

Statistical differences among qualitative variables were determined using Pearson´s chi-square test or Fisher´s Exact Test, as applicable. All statistical analyses were two-tailed with a pre-defined significance level for alpha error of 5% ($p < 0.05$).

RESULTS

HPV was detected in 14 (13.2%) of the 106 women assessed in the study. A total of 8 viral types were identified, including 7 (87.5%) of high-oncogenic risk (16, 31, 45, 53, 58, 64 and 70) and 1 (12.5%) of low oncogenic risk (cp8304). For 5 of the positive samples, the type could not be determined due to insufficient sample to perform the technique available to the research group. The most prevalent type was HPV 53 (2 cases).

Patient age ranged from 60-82 years, with a mean of 64.9 (SD±5.1) years. The other characteristics of the study population, according to HPV infection status, are presented in Table 1. The chi-square analysis revealed that positive HPV cases were associated with higher number of sexual partners (p= 0.018).

For cytological characteristics, most participants (n=102; 96.2%) tested negative for intraepithelial or malignancy, while only 2 (1.8%) individuals exhibited changes in cell cytology. Of the cases with abnormal cytology, none were positive for HPV infection on molecular testing (Table 1).

Table 1. Characteristics of population assessed, according to Human Papillomavirus (HPV) infection status (N= 106). Cruz Alta, Rio Grande do Sul state, 2023.

Variables	All participants (N= 106) n (%)	HPV absent (n= 92) n (%)	HPV present (n= 14) n (%)	*p-value*[c]
Education[a]				
≤ Primary	64 (82.0)	57 (83.8)	7 (70.0)	0.373
≥ Secondary	14 (18.0)	11 (16.2)	3 (30.0)	
No. of children[a]				
≤ 2	22 (41.5)	20 (43.5)	2 (28.6)	0.686
≥ 3	31 (58.5)	26 (56.5)	5 (71.4)	
Sexual debut[a]				
< 20 years	21 (38.8)	18 (38.3)	3 (42.9)	0.999
≥ 20 years	33 (61.2)	29 (61.7)	4 (57.1)	
No. of partners[a]				
< 2	43 (81.1)	40 (87.0)	3 (42.9)	**0.018**
≥ 3	10 (18.9)	6 (13.0)	4 (57.1)	
Use of condom[a]				
Yes	05 (9.4)	03 (6.5)	02 (28.6)	0.124
No	48 (90.6)	43 (93.5)	5 (71.4)	
Last Pap test				
Does not remember	2 (1.9)	1 (1.1)	1 (7.1)	0.610
≤ 3 years	94 (88.7)	83 (90.2)	11 (78.6)	
≥ 4 years	10 (9.4)	8 (8.7)	2 (14.3)	
Papanicolaou test				
Normal	102 (96.2)	88 (95.7%)	14 (100.0)	0.729
Abnormal[b]	2 (1.8)	2 (2.2)	0 (0.0)	
Unsatisfactory	2 (1.8)	2 (2.2)	0 (0.0)	

[a] Total tally does not match due to missing data for this variable.

[b] Includes 1 case of atypical squamous cells of undetermined significance (ASC-US) and 1 case of atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H).

[c] Pearson´s or Fisher´s Exact tests, as applicable

Of the 10 participants assessed at 2 visits, with at least a 1-year gap between consultations, most (n=7; 70%) tested negative for HPV infection on both assessments. Two individuals (20%) eliminated the HPV infection, where 1 case initially tested positive for HPV 16 and the other for HPV 53, both of whom had normal cytology findings on the two Pap tests performed. Only 1 (10%) participant showed conversion to positive status, exhibiting infection by HPV 31 at the second visit, but no cytological abnormalities. There were no cases of persistent infection.

DISCUSSION

The present cross-sectional study involved older women seen by the public health services of the city of Cruz Alta, situated in the interior of Rio Grande do Sul state. The 13.2% prevalence of HPV infection found is higher than the 4.3%[11] and 4.1%[13] rates reported by previous studies investigating older women[13]. With regard to viral types, the most commonly identified type was HPV 53, differing to the types found by other studies cited[11,13].

In Brazil, studies investigating the prevalence of cervical infection by HPV reported type 16 as the most common type detected, both in younger and older women[17-20]. Similarly, worldwide, HPV 16 is the most prevalent type in women with cervical cancer and also in individuals presenting cytological changes[1]. Nevertheless, it is important to note that the HPV 53 type is also frequently detected in women. This type is classified is as high oncogenic risk and is associated with malignant lesions, but can also be found in benign lesions[14].

In the present study, HPV infection was found to be associated with a higher number of sexual partners (p= 0.018). Increased life expectancy[21,22] and changes in sexual behavior, including higher divorce rates, contribute to multiple and new sexual partners, factors associated with greater risk of HPV acquisition[4,23].

The prevalence of HPV peaks in younger women (age 20-24 years) and steadily declines with age. However, curves of HPV prevalence versus age are parabolic, i.e. show an increasing prevalence in older women. This pattern might occur due to reactivation of HPV infection and by changes in sexual behavior of middle-aged men and women[24]. Hormonal changes which alter immunological function may also contribute to reactivation of latent HPV infection in older women, particularly among those infected by types of high oncogenic risk[25].

The study by Strander, Hällgren & Sparén[26] showed that women previously diagnosed with Cervical Intraepithelial Neoplasia – Grade 3 (CIN 3), which corresponds to High Grade Squamous Intraepithelial Lesion (HSIL), have high risk of progressing to invasive cervical cancer, a risk which increases after age 60, in the same way as risk of death rises after age 70.

On the cytological analysis performed in the present study participants, only 1.8% exhibited abnormal changes. However, unexpectedly, HPV infection was not detected on molecular testing in these cases. Analysis of Pap tests of post-menopausal women should be performed with caution, especially in the presence of atrophic changes, which may exhibit different cytomorphological patterns owing to low hormone concentration in epithelial tissues[8]. Atrophic changes associated with vaginitis pose a diagnostic challenge because degenerated cells may resemble tumor cells[27]. Moreover, due to hormonal changes in post-menopausal women, the squamocolumnar junction – a preferential region for the development of precursor lesions of cervical cancer – is situated within the cervical canal, hampering access for biopsy and collection of adequate sample size for cytological study, reducing its sensitivity[28].

In this study, one of the patients with abnormal cytology findings had a result consistent with Atypical Squamous Cells of Undetermined Significance (ASC-US). The prevalence of ASC-US and positivity for HPV DNA of high oncogenic risk tends to decline with age[29]. Hence, the cytological analysis of samples obtained from women peri and post-menopause should be rigorous, since slight nuclear increase may suggest an ASC result. However, this change without significant hyperchromasia or irregular nuclei is generally not associated with the cytopathic effect of HPV, but rather with inflammatory modifications[9].

One case of atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) was detected in a woman with an atrophic smear. In patients deemed high risk, the presence of atypia on atrophic smear can explain this result. Nonetheless, the interpretation of atypia can be difficult in an atrophic context due to the lack of maturity and high nucleus-cytoplasm ratio of the cell, which resemble small atrophic cells and dysplastic cells[9]. In these case with diagnostic difficulty because of atrophy, estrogenization can be an alternative, improving the quality of the smear and reducing degenerative cell changes[8].

When the organism develops an efficient cell immune response, regression of HPV infection and control of viral replication occur[22], thus, immune response is a determinant of the progression of carcinogenesis. None of the study participants assessed at 2 visits to the services had persistent infection. However, the literature shows persistency

occurs in older women[11,13]. It is therefore critical to perform cytological follow-up in these women in order to allow monitoring and early detection of precursor lesions of cervical cancer[27], with implementation of interventions where necessary.

In immunocompetent women, most HPV infections are detected transiently, with subsequent loss of viral detection. However, the infection can persist in a non-productive stage, which is not eliminated and becomes latent in undifferentiated basal calls of the cervical epithelium. In this case, among older women, reactivation of the infection can occur when they undergo age-related hormonal and immunologic changes[30]. Nevertheless, a proportion of HPV infections may also be attributed to new sexual partners, including in older people[26], perhaps explaining the current study finding of conversion of the infection status in 10% of participants between the two assessments performed during the course of the study.

Prevention of cervical cancer in older women remains a challenge, because of the lack of specific guidelines and of consensus on the age at which routine screening should cease. The Brazilian guidelines for cervical cancer screening emphasize there is scant objective evidence on when women should cease screening for the disease[8].

The literature suggests that, upon leaving the screening program, women should be tested for HPV, with continued monitoring of those who test positive for HPV. Furthermore, the impact of changes in hormonal and immunologic factors with advancing age on reactivation of latent HPV infection or the reduced likelihood of eliminating a new infection, and the course of new HPV infections in older women, all warrant future investigation.

Thus, although older women are vulnerable to infection by HPV, scant evidence exists on the course of HPV infection in this population group. The ideal age at which screening should be discontinued is also unclear and a topic requiring further debate. Hence, future studies focusing on this population are needed to devise strategies for prevention and early detection of HPV infections, effectively reducing cervical cancer rates in this age group.

Limitations of the study include the small sample size, perhaps due to the low number of older women seeking testing, and also the fact that almost half of the HPV-positive samples were not genotyped.

CONCLUSION

The results of this study showed that, although no intraepithelial lesions or neoplasia were detected on the Pap tests performed, older women are infected by HPV and should therefore continue to undergo routine cytological screening. This recommendation is supported by the finding of conversion of infection status (when DNA-HPV was negative at first consultation, but subsequently detected during follow-up) between health service visits in 10% of the women assessed. The study found that older women with multiple sexual partners were more susceptible to HPV infection and so this group should be prioritized in screening programs for cervical cancer.

In addition, this investigation also prompts reflection on the guidelines of the current cervical cancer screening program proposed in Brazil, which recommend routine Pap smears for women aged between 25-64 years. Beyond this age, screening is automatically halted when the woman has at least 2 negative consecutive tests in the past 5 years. The present study underscores the importance of continued cytological screening in older women for prevention and control of HPC-induced lesions, particularly cervical cancer, given that under the current program, many may go unmonitored having discontinued Pap smears in later life, despite still engaging in an active sexual life.

AUTHOR CONTRIBUTIONS

- Jaqueline A. Bessa – Involved in all aspects of the study.
- Tatiana Mugnol – Data analysis and interpretation, writing of article and critical review.

- Jonas Wolf – Data analysis and interpretation, analysis and approval of draft to be published.
- Thais R. Boeira – Data analysis and interpretation, analysis and approval of draft to be published.
- Vagner R. Lunge – Data analysis and interpretation, analysis and approval of draft to be published.
- Janaina Coser – Involved in all aspects of the study.

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