

HPV-positive women living in isolated areas in Amazonas, Brazil: Clinical-epidemiological profile and cytological findings

Mulheres HPV-positivas vivendo em área isoladas da Amazônia brasileira: perfil clínico-epidemiológico e achados citológicos

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Abstract

Background: Cervical cancer (CC) is associated with persistent high-risk human papillomavirus (HPV) infection and it causes about 265,000 deaths per year worldwide, thus becoming an important public health problem. **Objective:** This study aimed to describe the epidemiological profile, clinical history, and cytological findings of 55 women infected with HPV living in remote areas in Amazonas, Brazil. **Method:** The samples were obtained by self-collection using the Rovers® Evalyn® Brush for HPV detection through PCR. The cytological examination was performed by liquid-based cytology. **Results:** The mean age of participants was 35 years (SD=14). Most of the women presented low or no schooling (52.7%), lived in stable relationship with a partner (74.5%), and had three to five children (32.7%). Inconsistent use of condoms by most of the participants (“never” — 34.5% and “sometimes” — 41.8%) was observed. Cytopathological examinations showed that 14 (25.4%) women had cytological alterations: 7 atypical squamous cells of undetermined significance (50.0%), 5 low-grade squamous intraepithelial lesions (35.7%), and 2 high-grade squamous intraepithelial lesions (14.3%). **Conclusion:** As cervical lesions have been caused by persistent HPV infection over the years, CC Prevention Programs use effective strategies to follow up on HPV-positive women living in remote areas.

Keywords: Human papillomavirus viruses; uterine cervical neoplasms.

Resumo

Introdução: O câncer cervical está associado com a infecção persistente pelo HPV (*human papilloma virus*) de alto risco e causa cerca de 265 mil mortes todos os anos no mundo, tornando-se um importante problema de saúde pública. **Objetivo:** Descrever o perfil epidemiológico, história clínica e achados citológicos de 55 mulheres infectadas com HPV vivendo em áreas remotas no Amazonas, Brasil. **Método:** Obtiveram-se as amostras por autocoleta, utilizando-se a escova Rovers® Evalyn® para detecção do HPV por meio da técnica de PCR (*polymerase chain reaction*). Realizou-se o exame citológico por citologia em meio líquido. **Resultados:** A média de idade das participantes foi de 35 anos de idade (DP=14). A maioria das mulheres apresentou baixa ou nenhuma escolaridade (52,7%), vivia em uma relação estável com um parceiro (74,5) e tinha de três a cinco filhos (32,5%). Observou-se o



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uso inconsistente de preservativo pela maioria das participantes (“nunca 34,5% e “às vezes” 41,8%). O exame citopatológico mostrou que 14 mulheres (25,4%) tinham alterações citológicas: 7 Ascus (50,0%), 5 L-SIL (35,7%) e 2 H-SIL (14,3%). **Conclusões:** Uma vez que as lesões cervicais são causadas pela infecção persistente pelo HPV, é importante que programas de prevenção do CC usem alternativas eficazes para acompanhar mulheres HPV positivas vivendo em áreas remotas.

Palavras-chave: papillomavirus humano; neoplasias do colo do útero.

INTRODUCTION

Cervical cancer (CC) is an important public health problem and is the fourth most frequent type of cancer worldwide, with an estimated 570,000 new cases per year¹. In Brazil, the National Cancer Institute (INCA) estimated the occurrence of approximately 16,590 new cases of CC in 2022, with an estimated risk of 15.43 cases per 100,000 women. In the Northern region of Brazil, this problem is even greater, as CC is the second most common malignant neoplasia among women, which is the only region in Brazil where breast cancer and CC rates are equivalent¹.

Indigenous women from the Amazon region have the highest rates of CC in the world with less knowledge on the human papillomavirus (HPV)². Persistent infection with high-risk HPV has been described as a necessary cause for the development of precursor lesions and CC and is also associated with other factors such as low schooling, poverty, smoking, other sexually transmitted infections (STIs), immunosuppression, early sexual initiation, and multiplicity of sexual partners³⁻⁵.

In Brazil, the prevention of CC has been performed through conventional Pap smear, as it is able to detect early cellular changes and precancerous lesions, although it does not show high sensitivity. Efficient coverage in CC screening is an essential health service for the success of the prevention strategy but is often still unavailable to women in some developing countries. For women living in isolated, hard-to-reach areas — like much of the Brazilian Amazon — this challenge is even greater^{2,6}. In the Amazon, riverine women — the “*ribeirinhas*” — are predominantly descendants of indigenous people living in the forest and along the banks of rivers and lakes. Most of these villages are far from small towns and are composed of “family nuclei.” Transportation and logistics are limiting factors, and local educational and health services are inadequate^{7,8}. According to the INCA, in regions such as the Brazilian Amazon, women are even affected by the CC still very young¹. This study aimed to characterize the epidemiological, clinical, and behavioral profile of a sample of “*ribeirinhas*” women infected with HPV and to describe the cytological findings in this HPV-positive sample.

METHODOLOGY

This is a descriptive, cross-sectional study conducted between August 2014 and February 2015. Fifty-five riverine women infected with HPV who were screened in earlier research⁶ participated in this study, whose inclusion criteria were to be sexually active and to be 18 years old or older. Pregnant, hysterectomized, virgin, and menstruating women as well as those who were using vaginal cream were excluded from the study. HPV detection was performed by PCR using the generic primers PGMY09/11⁹ from auto-collected vaginal samples obtained using the Rovers[®] Evalyn[®] Brush (Rovers[®] Medical Devices). These women were summoned through active search in their residences for the cytological examination — liquid-based cytology (LBC) (BD SurePathTM). They also answered a standard questionnaire with questions covering socioeconomic aspects, clinical history, and behavioral facts.

The results of the Pap smear were categorized according to the Brazilian Nomenclature for Cervical Reports: negative for neoplasia; atypical squamous cells of undetermined significance, possibly non-neoplastic (ASCUS); atypical squamous cells of undetermined significance, high-grade lesions cannot be excluded (ASC-H); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); atypical glandular cells (AGC); adenocarcinoma *in situ* (Adeno *in situ*); and invasive carcinomas. Women who had cytological changes were sent for colposcopy examination and biopsy (if necessary).

Statistical analysis was performed using the SPSS program (Statistical Package for the Social Sciences) 16.0 for Windows, and the mean and standard deviation or median, minimum, and maximum were calculated. This study was approved by the Research Ethics Committee of the Center for Oncology Control Foundation of the State of Amazonas (FCECON).

RESULTS

The 55 HPV-positive women studied had a mean age of 35 years (SD=14), with a higher frequency of young women in the 18–29 years age group (38.2%). Most of the women had low or no schooling, as 40% of them had only up to 5 years of schooling and 12.7% had never studied. Most women (67.3%) had low economic status, with monthly family income below minimum wage — \$258, at the time. Data on the clinical and behavioral profile of these HPV-positive women showed that most of them (74.5%) were living together with a fixed partner, being “married” or “in stable union,” while 25.4% said that they were not living together with a partner (being single, widowed, separated, or divorced). Most women reported having had their first sexual intercourse before 15 years of age (54.5%) and having had two or more sexual partners throughout their lives (65.5%). Regarding the use of condoms, inconsistent condom use predominated (41.8% reported using only “sometimes”), followed by not using (36.6% reported using “never”). When asked why they do not use it, the main reason cited by them was “trust in the partner,” followed by “the partner does not like.” Most women (50.9%) had two to five children at the time of collection, and a significant number of women had more than five children (27.3%). Regarding the frequency with which the women performed the preventive exam, the majority (61.8%) reported to perform the test once a year. These data are detailed in Table 1.

The suitability of the cellular material was classified as satisfactory with a percentage of 100% for cytopathological analysis. A variation in the representativeness of epithelium in the collected cervical samples was observed: the majority of the slides (65.4%) obtained the standard representativeness of the squamous and glandular epithelium, 18.2% had only squamous epithelium, and 16.4% had squamous, glandular, and metaplastic epithelia. Cytological alterations were found in 14 women (25.5%), of whom 5 cases were LSIL (35.7%), 2 cases of HSIL (14.3%), and 7 cases of ASCUS (50%).

DISCUSSION

CC screening through the Pap smear aims at the early diagnosis and consequent reduction in mortality due to this pathology. In developed countries, screening programs for the CC are organized, which enables adequate coverage of women eligible for the examination. This is not the case in most developing countries, like Brazil¹⁰, although there has been an improvement in exam coverage in recent years¹¹. In countries such as Brazil, Mexico, China, and some African countries, there is a large difference in coverage when stratified by area of residence, which is much smaller in rural areas than in urban areas, limiting access to timely diagnosis and treatment for CC^{12–14}. Brazil has a very large territory and presents many isolated and hard-to-reach communities, mainly in the Northern region of the country, a region predominantly covered with forests and rivers, showing high demographic dispersion^{7,8}. These geographical features make it difficult for the population to access many health services, such as the Pap smear, so the rates of HPV infection and CC in these regions are the highest in the country, including indigenous women^{1,2,15}.

The women participating in our research were recruited from the study conducted previously by Torres et al.⁶, a screening survey performed in 32 riverside communities belonging to the city of Coari, in the State of Amazonas, which found 79 (19.1%) HPV positivity among the participants (n=412). There are few Brazilian studies with a population similar to ours, that is, women living in rural communities and isolated areas in the Brazilian Amazon region. Pinto et al.¹⁶ conducted a study with riverine women in the city of Tucuruí, in the state of Pará, and found a prevalence of 14.2% of infection. Tamegão-Lopes et al.¹⁷, studying women in the city of Juruti, state of Pará, found a positivity of 29.4% for HPV. An important study by

Table 1. Socio-demographic, clinical, and behavioral profile of HPV-positive riverine women from Coari City, Amazonas (2015).

Socio-demographic, clinical, and behavioral variables	(n=55)	%
Age (years)		
18–29	21	38.2
30–39	17	30.8
40–59	14	25.5
60 or more	3	5.5
Marital status		
Single	8	14.5
Married	41	74.5
Widowed/divorced/separated	6	11.0
Schooling		
Illiterate	7	12.7
1–5 years of schooling	22	40.0
6–10 years of schooling	15	27.3
>10 years of schooling	11	20.0
Monthly income *		
<1 MW	37	67.3
1 MW	12	21.8
2–3 MW	6	10.9
Age of first sexual intercourse		
<15 years	30	54.5
15–20 years	24	43.6
>20 years	1	1.8
Condom use		
Never	19	34.5
Sometimes	23	41.8
Always	13	23.6
Number of sexual partners throughout life		
1	19	34.5
2–5	32	58.2
6–10	2	3.6
>10	2	3.6
Number of sexual partners in the last 12 months		
0	5	9.1
1	42	76.4
2	6	10.9
>2	2	3.6
Number of children		
0	6	10.9
1	6	10.9
2–5	28	50.9
>5	15	27.3
Regularity in the accomplishment of Pap smear**		
Non-regular	21	38.2
Regular	34	61.8

*Minimum wage — \$ 258.00 at the time (from August 2014 to February 2015); **regularity in the accomplishment of Pap smear — women taking the preventive examination once a year.

Fonseca et al.² found a very high prevalence of 45.9% of infection in indigenous women living in almost complete isolation (Yanomami tribe, in the state of Roraima, with access only by air) and 34.5% in indigenous women with greater contact with non-indigenous people, also in the state of Roraima. In another study by Rocha et al.¹⁵ also performed in Coari City, a prevalence of 29.1% among women residing in the urban area of the city was observed (in this case, we emphasize that in the Rocha et al.¹⁵ study, nested-PCR was used to detect HPV, which has a higher sensitivity).

In this study, the women who participated were diagnosed with HPV from vaginal samples obtained by self-collection (Rovers[®] Evalyn[®] Brush), as the self-sampling methods are part of a set of strategies that have been tested to increase the coverage of the screening in remote and low-resource places^{13,14,18}. The women were summoned by active search, but of the 79 HPV-positive women, 22 did not attend the follow-up (LBC and colposcopy), most likely because it was performed in the urban area of the city. Of the 32 participating rural communities, 13 were located between 20 and 40 km from the city's headquarters, and 6 communities were located for more than 40 km (data not shown). Depending on the type of transport used (boat engine power) and the period of the year (dry or full), these distances can last from a few hours to 3 days, limiting the access of the riverine women to the urban area of the city⁷. Of the 55 participating women, 21 (38.25%) reported that they did not periodically undergo Pap smears, which may be due to economic, educational, cultural, and personal difficulties, in addition to geographic barriers and access to health services.

Of the 55 HPV-positive women who participated in the study follow-up, the mean age was 35 years (SD=14 years), ranging from 18 to 69 years. Similar data were found in a study by Rocha et al.¹⁵ with 105 HPV-positive women living in the urban area of Coari City who attended the Basic Health Units for routine gynecological examination, in which the mean age of these infected women was 36.4 years (SD=13.2). Something that should be taken into account is that both in this study and in Rocha et al.¹⁵, adolescents (under 18 years of age) did not participate, which could certainly decrease the average age of infected women. Detection of HPV DNA is greater in young women under 25 years of age, with a decline in age groups above this one¹⁹. A study by Rama et al.¹⁹ on high-risk HPV infection in 2,300 women between 15 and 65 years old in the cities of São Paulo and Campinas (São Paulo, Brazil) also observed that the highest prevalence of infection was found in the age group under 25 years. In Brazil, we believe that we are going through the transition phase of these numbers, once the implementation of the anti-HPV vaccine began in 2014 in Brazilian teenage girls.

Early onset of sexual activity and a high number of sexual partners are causes for concern, as these factors may predispose to HPV infection and consequently to a greater prevalence of precursor lesions of the cervix. Our results showed that the age of the first intercourse ranged from 11 and 21 years, with a mean of 15 years (SD=2.1); most of the participants (54.5%) reported that the first sexual relationship occurred before the age of 15 years and having 2–5 lifetime sexual partners (58.2%). Brazilian studies are divergent regarding these factors related to sexual behavior. The study by Rama et al.¹⁹ found a statistically significant relationship between HPV infection and the high number of sexual partners and no relation to the early age of the first sexual intercourse. Rocha et al.¹⁵ found no relation between any of these variables and the presence of HPV. We know, however, that these questions about sexual behavior may not always be answered with total sincerity by the participants, for even if the research team clarifies that the data will be completely confidential, fear of exposure and judgment may prevent them speak the truth completely, making it difficult for correlations to be established with confidence.

Our results showed that most women were married or lived in stable union with a partner, allowing the deduction of greater fixed and monogamous sexual activity (most women (76.4%) reported having had only one sexual partner in the past 12 months). However, this does not mean that these women are less at risk for HPV infection and other STIs, as we know that this depends directly on the partner's sexual behaviors and condom use by the couple. This disconnection is evident in this study because our sample consists of only HPV-positive women.

The fact that most of the women in this study are married or living in a stable union with a partner is directly related to poor adherence to condom use, as 76.3% of the women reported using only “sometimes” or “never.” According to Jiménez et al.²⁰, even in the so-called “modern” societies, heterosexual couples with relationships considered them as “fixed” and the sexual relationship is also “affective” and “enduring,” which implies “knowing” and “trusting” the partner, it is difficult for the use of condoms as a means of preventing STI to be considered. At most, the condom is considered a contraceptive method. Gender relations, that is, the power relations existing between men and women, are certainly a great aggravating factor of women’s vulnerability, which are now considered a “permanent state of vulnerability”^{20,21}. Additionally, we emphasize that the socioeconomic situation of these women is very precarious, and condoms are not available for sale in riverside communities. There are no drugstores in these villages for women to buy condoms; let alone distribution of condoms by the government in the few health services offered that reach the communities. Many women spend many months without going to the nearest urban center, usually leaving their partners to do the month’s purchases of groceries, medicine, and other things with the less money they have. Buying condoms is certainly not a priority.

Although it was not the purpose of this research to focus on the relationships of trust or marital agreements between women and their partners, this low rate of adherence to the use of condoms in HPV-positive women makes clear this state of female vulnerability, as the use of the condoms is a means of prevention against HPV infections and other STI. However, among young women, the study by Pinto et al.¹⁶ observed that among riverine women between 13 and 25 years old, the single/divorced/widowed marital status was a risk factor statistically associated with HPV infection, which could be explained by being this age group in which the sexual life begins, being characteristic the greater frequency of sexual activity and search for new partners, irregular use of barrier contraceptive methods, and fragility of the uterine cervix at this stage of life. In women over 25 years of age, Pinto et al.¹⁶ found no relationship between conjugality and HPV infection. In contrast, the study by Rama et al.¹⁹ showed that monogamy was a protective factor against HPV infection in their sample.

Most of the women participating in our study (52.7%) had low educational levels (at most 5 years of study), with a very high illiteracy rate (12.7%). The study by Gama et al.⁷ carried out with the aim of characterizing various socioeconomic, demographic, and health aspects of the Coari riverine people by means of a probabilistic sample by conglomerates composed of 492 individuals found an illiteracy rate of 9.7%. These authors also showed through secondary data extracted from the Atlas of Human Development in Brazil that illiteracy rates are historically much higher in Coari than those found in Amazonas and Brazil. Education is a collective good, essential for social insertion, presenting a visible impact on the general conditions of the population’s life. It is a right of every human being, as from it, individuals can enjoy other rights proper to an organized society. Health and Education cannot be dissociated, as they go together and are articulated as social practices²². Remote areas, such as the Brazilian Amazon region, are characterized by the poor quality of health and education services, and public investments to improve the quality of life of these populations are lacking. These populations live without good education and health basic services, living in a state of “self-care,” which increases the risks of these women in acquiring many diseases⁷. Hernández-Márquez et al.¹² conducted a study with Mexican women and observed that the level of knowledge about the relationship between HPV and CC was higher among women with higher schooling and younger. Almeida et al.²³ found a higher prevalence of HPV infection among women with lower educational levels among women in the city of Campo Grande, in the state of Mato Grosso do Sul, Brazil. In our study, the fact that most of these women are farmers (80%), living in rural areas with low family incomes, with the majority (67.3%) earning less than the minimum wage (\$ 258.00 at the time), adds to their difficulty in obtaining adequate access to good quality education and health services.

Regarding the cytological alterations in these HPV-positive women, 41 (74.5%) samples presented negative results for neoplasia, and 14 (25.5%) samples presented altered results. Among the altered results, we found 50% of ASCUS, 35.7% of LSIL, and 14.3% of HSIL. Although

it is not ruled out that these infected women may suffer viral clearance, these HPV-positive women without cytological alterations should be monitored with more attention, as the CC takes about 10 years to establish itself, and these women are at a higher risk than uninfected women. The suitability of the cellular material was classified as satisfactory with 100% for cytopathological analysis. Regarding the epithelial representation in the slides, the presence of squamous and glandular epithelium (65.4%) was observed in most of the exams. According to the Ministry of Health, for a good-quality cytopathological examination, it is expected that the representation of the glandular and/or metaplastic epithelium is at least equal to the squamous one. One of the quality indicators of the collection is the presence of metaplastic cells or endocervical cells, representative of the squamocolumnar junction (SCJ), where most CC begins²⁴. A good technique used in performing the preventive examination is also essential for obtaining satisfactory samples. We used LBC, which consists of a method in which the collected cervical cells are immersed in a preservative liquid prior to the preparation of the slide. The collection kit used in this methodology allows the use of almost all the cells collected from the cervical region. The improved quality of the slides results in a greater sensitivity than that of the conventional Pap smear, allowing the residual material to be used for genetic analysis and molecular diagnosis of various microorganisms^{25,26}. Ensuring adequate collection for the cytopathological diagnosis of riverine women living in remote areas is very important, due to the logistic difficulties mentioned above for the accomplishment. Without the need to repeat collections unnecessarily, we also increased women's adherence to the examination.

Integrating HPV testing in CC screening programs can identify women at risk of developing CC and, when coupled with appropriate treatment, can reduce CC incidence and mortality. There are several options: immediate treatment, triage with another test — cytology or visual inspection with acetic acid — or referral for colposcopy. The choice will depend on the resources available in the health system and the country's guidelines²⁷. In Brazil, the incorporation of these tests into the screening arsenal should also consider the geographical peculiarities of the Amazon that make it difficult for women to access health services. In this context, screening using samples obtained by self-collection allied to molecular diagnostic tests may be promising. What is important is that all efforts are made to ensure that women with HPV-positive test results receive follow-up care.

CONCLUSION

Our results showed that in our sample of positive HPV women living in remote areas of the Brazilian Amazon, most of them were young and married and had low schooling and low income. Most of these women had their sex life started before the age of 15 years, did not consistently use condoms, and had between 2 and 5 sexual partners throughout their lives but only 1 partner in the last 12 months. We found a prevalence of cytological changes of 25.5%, with 50% ASCUS, 35.7% LSIL, and 14.3% HSIL among the altered women. The LBC proved to be an excellent methodology for sample collection and preparation of the cytopathological slide, with 100% suitability in our research. This is very important because ensuring the optimum suitability of the material so that it is not necessary to repeat the exam is a way to reduce existing logistical obstacles due to geographic isolation and increase women's adherence to screening. As HPV plays an important role in the development of premalignant and malignant lesions and the progression of lesions to CC occurs over the years, HPV-positive women screened for molecular examinations should be closely monitored.

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AUTHORS' CONTRIBUTIONS

JMM: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft.

DAPR: Data Curation, Formal Analysis, Writing – Original Draft.

RSR: Data Curation, Formal Analysis, Writing – Original Draft.

MGS: Data Curation, Formal Analysis, Writing – Original Draft.

SCCS: Data Curation, Formal Analysis, Writing – Original Draft.

JCAP: Data Curation, Formal Analysis, Writing – Original Draft.

MHR: Data Curation, Formal Analysis, Writing – Original Draft.

JEL: Conceptualization, Formal Analysis, Project Administration; Resources, Writing – Review & Editing.

KLTS: Conceptualization, Formal Analysis, Project Administration, Resources, Writing – Review & Editing.

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