Published in final edited form as:

Gynecol Oncol. 2018 October ; 151(1): 102–110. doi:10.1016/j.ygyno.2018.08.004.

Cervico-vaginal self-collection in HIV-infected and uninfected women from Tapajós region, Amazon, Brazil: High acceptability, hrHPV diversity and risk factors.

Luana L.S. Rodrigues^{a,b}, Mariza G. Morgado^{a,*}, Vikrant V. Sahasrabudhe^c, Vanessa S. De Paula^d, Nathália S. Oliveira^e, Elena Chavez-Juan^f, Diane M. Da Silva^{f,g}, W. Martin Kast^{f,g,h}, Alcina F. Nicol^{i,j}, and José H. Pilotto^a

^aLaboratorio de AIDS e Imunologia Molecular, IOC-FIOCRUZ, Rio de Janeiro, Brazil

^bInstituto de Saúde Coletiva, Universidade Federal do Oeste do Pará, Santarém, Pará, Brazil

^cDivision of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA

^dLaboratorio de Virologia Molecular, IOC-FIOCRUZ, Rio de Janeiro, Brazil

^ePrograma de Pós-Graduação em Patologia, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil

^fNorris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA

^gDepartment of Obstetrics and Gynecology, University of Southern California, Los Angeles, CA, USA

^hDepartment of Molecular Microbiology and Immunology, University of Southern California, Los Angeles, CA, USA

ⁱInstituto Nacional de Infectologia Evandro Chagas, INI-FIOCRUZ, Rio de Janeiro, Brazil

^jLaboratório Interdisciplinar de Pesquisas Medicas, IOC-FIOCRUZ, Rio de Janeiro, Brazil

Abstract

Objective.—We evaluated acceptability of cervico-vaginal self-collection (CVSC) and prevalence of human papillomavirus (HPV) in *Human immunodeficiency virus* (HIV)-infected and HIV-uninfected women living in the Tapajós region, Amazon, Brazil.

* **Corresponding author at:** Laboratório de AIDS e Imunologia Molecular, Avenida Brasil, 4365, Manguinhos, CEP 21040-900, Rio de Janeiro, Brasil; phone +55 (21) 3865-8154., mmorgado@ioc.fiocruz.br (M. G. Morgado); and Instituto de Saúde Coletiva, Universidade Federal do Oeste do Pará, Avenida Mendonça Furtado, 2946, Unidade Amazônia, Fátima, CEP 68040-470, Santarém, Pará, Brasil, luana.rodrigues@ufopa.edu.br (L. L. S. Rodrigues).
Conception and design of the study: L.L.S.R., M.G.M., V.V.S., D.M.D.S., A.F.N., J.H.P. Performed the experiments: L.L.S.R., N.S.O., E.C.J. Analyzed the data: L.L.S.R., M.G.M., V.V.S., V.S. de P., D.M.D.S., W.M.K., A.F.N., J.H.P. Contributed reagents/materials/analysis tools: L.L.S.R., D.M.D.S., W.M.K., A.F.N., J.H.P. Wrote the paper: L.L.S.R., M.G.M., V.V.S., V.S. de P., N.S.O., E.C.J., D.M.D.S., W.M.K., A.F.N., J.H.P.

Potential conflicts of interest. None declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Methods.—Cross-sectional study recruited 153 non-indigenous women (HIV- uninfected, n=112 and HIV-infected, n=41) who voluntarily sought assistance in health services. Peripheral blood for HIV screening and cervical scraping (CS) for HPV detection were collected. Women who accepted to perform CVSC received instructions and individual collection kits. Risk factors for high-risk HPV genotypes (hrHPV) were identified by uni- and multivariate analyses.

Results.—The overall acceptability of CVSC was 87%. Only HIV-infected women had cytological abnormalities (12.2%). Prevalence of any HPV and hrHPV infection was 42.9% and 47.9% for HIV-uninfected and 97.6% and 77.5% for HIV-infected women, respectively. There was significant agreement in the detection of HPV (88%, 0.76, 95% confidence interval [CI], 0.65–0.87) and hrHPV (79.7%, 0.56, 95% CI, 0.41–0.71) between self-collected and clinician-collected samples. The most prevalent hrHPV types were HPV16 and HPV18 in HIV-uninfected and HPV16, HPV51 and HPV59 in HIV-infected women. HIV-infected women with hrHPV infection had multiple hrHPV infections ($p = 0.005$) and lower CD4 count ($p = 0.018$). Risk factors for hrHPV infection included being HIV-infected and having five or more sexual partners.

Conclusions.—CVSC had high acceptability and high prevalence of hrHPV types in women living in the Tapajos region, Amazon, Brazil.

Keywords

HPV; HIV; self-collection; cervical cancer; epidemiology

INTRODUCTION

The World Health Organization (WHO) estimates that more than 290 million women worldwide have a HPV infection [1]. Sexually transmitted infections (STIs) continue to be a health problem worldwide, as they can cause a wide range of acute clinical conditions in addition to severe complications and long-term consequences such as cervical cancer and AIDS [2]. Despite numerous prevention strategies, cervical cancer incidence and mortality remains high, especially in resource-limited settings [3].

In Brazil, the National Cancer Institute estimates an annual cervical cancer incidence rate of 24.91 per 100,000 inhabitants for the Northern region by 2018, which is the most common incident cancer in the Amazon region [4]. The Tapajos region, which is the focus of our study, is located in Northern Brazil and is comprised of two mesoregions of the state of Para, the Lower Amazon and the Southwest (Fig. 1). It encompasses part of the Amazon rainforest in a territory of 281,082 mi² [5]. Traveling to access health services at Santarem, the city that has the best infrastructure in the region, tends to be arduous and challenging, located approximately 486,22296 mi by highway from one of the most remote municipalities of the region or 93,2057 up to 178,334 mi by river from one municipality not so far. [6]. Santarem receives people from 22 municipalities who are looking for medical care throughout the Tapajos region [5], as depicted in Fig. 1.

In this context, the implementation of a CVSC method, to facilitate widespread hrHPV-DNA testing, and which would yield comparable results as obtained with the clinician-collected cervical samples [7,8] is an important strategy to encourage women to participate in the

cervical cancer screening program and increase coverage and uptake of HPV testing in the Tapajos region. The aim of this study was to evaluate the acceptability of CVSC and prevalence of HPV in HIV-infected and HIV-uninfected women living in the Tapajos region, Amazon, Brazil.

METHODS

Ethical issues

Women were invited to participate in the study after signing a written informed consent form, followed by an epidemiological interview. Written informed consent was obtained from all participants in strict compliance with the Brazilian ethical guidelines involving human subjects (protocol numbers 1.099.852 and 1.059.253).

Study design and population

A cross-sectional study was conducted from August 2015 to August 2016 that included a total 186 women were approached and invited to participate and 153 of those accepted to enter the study. The 153 subjects were consisting of non-indigenous HIV-infected and HIV-uninfected women living in the Tapajos region. The women were recruited in nine public health units strategically spread out between the center, semiperiphery and periphery of Santarem, making the study group representative of the population of the Tapajós region. The HIV-infected women being followed up at the Santarem counseling and testing center were also invited. This is the only referral center for the care and follow up of people living with HIV in this region. All women were first screened for HIV infection by HIV rapid diagnostic testing (Alere Determine™ HIV-1/2 Kit, Chiba, Japan). For those with reactive results, we further collected intravenous blood samples to prepare aliquots of serum and plasma, for conventional HIV serology confirmatory testing (4th-generation ARCHITECT assay, Abbott, Germany) and HIV viral load determination (Abbott Real Time HIV-1® kit, Abbott, Germany; limit of detection 40 copies/mL) and of whole blood samples for CD4+ T-lymphocytes (Alere™ PIMA CD4 Test, Jena, Germany), according to the manufacturer's instructions.

Cervical samples collection

All women underwent a gynecological evaluation for cytology (Pap smear). The sample was collected with an endocervical brush by the health unit personnel and preserved in ThinPrep Pap Test (Hologic, Inc., San Diego, California, USA) preservative vials, constituting the CS. Soon after the women were invited to perform the CVSC, and those who agreed received an individual collection kit and specimen collection instructions with the aid of an illustrated, self-explanatory pamphlet, with step-by-step procedures [7,9]. CVSC was done immediately after the PAP smear. The procedure was done in a separate office within the health care providers office, where the patient was alone to perform it. After the procedure those women handed the CVSC sample immediately to the health care provider for identification and storage. All collected samples were locally labelled and stored at 5oC up to five hours, transported to the Federal University of Western Pará, in Santarém, PA, for storage until sending to the Aids and Molecular Immunology Laboratory at the Oswaldo Cruz

Foundation, Rio de Janeiro, Brazil and, later on, to the University of Southern California, Los Angeles, CA, USA.

DNA extraction and HPV DNA detection

DNA extraction from the cervical samples was performed with the QIAamp DNA Mini Kit (Qiagen, Valencia, California, USA), according to the manufacturer's protocol, and quantified by spectrophotometry on Nanodrop (ND 1000, Fisher Scientific, Wilmington, USA). Nested PCR was performed to detect HPV DNA targeting the L1 gene. In the first round PGMY09 and PGMY11 primers were used to amplify a fragment of 450 base pairs (bp) and the second round GP5+ and GP6+ primers were used for amplification of 110 bp, as previously described [10,11], in an automatic thermocycler (GeneAmp PCR System 9700 - Applied Biosystems). PCR for the β - globin gene was performed on HPV-negative samples as a control of DNA extraction and viability.

HPV genotyping and multiple HPV types

Nested PCR products were purified with Wizard® SV Gel and PCR Clean-up System (Promega Madison, WI, USA), and sequenced using the ABI Prism™ BigDye Terminator Cycle Sequencing kit on an ABI 3730x/automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences analysis was done using Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0.18. Sequence characterization was performed in the Basic Local Alignment Search Tool using nucleotide (BLASTn) (<https://blast.ncbi.nlm.nih.gov>). Samples presenting DNA sequencing electropherograms with double or multiple peaks were subject to detection of multiple HPV infections using the Novaplex™ II HPV28 Detection Assay (Seegene, Seoul, South Korea) in the CFX96™ Real-time PCR System (Bio-Rad, Hercules, California, USA) [12]. HPV samples were grouped into three groups considering the oncogenic risk classification: high-risk, “probable” or “possible” carcinogenic and low- risk [13].

Statistical analysis

Descriptive statistics of the qualitative variables was determined by frequency distribution and quantitative variables by medians and interquartile ranges (IQR) or mean and standard deviation (SD). Chi-square test was used for categorical variables and Mann-Whitney U test for continuous variables, both at 95% CI and p value ≤ 0.05 . For the identification of risk factors, we initially used univariate analysis and Odds Ratios (OR) were calculated. Variables with p value ≤ 0.20 were used in the multivariate analysis. In the last model of the multivariate analysis only the variables with p value ≤ 0.05 remained in the model and the adjusted Odds Ratios (aOR) were calculated. Spearman's ρ test was performed to evaluate the linear relationship between CD4+ T-cell count (as a proxy of immunosuppression levels) and number of hrHPV. We considered 39 copies/mL for the viral load below limit of detection (40 copies/mL) for statistical purposes. The agreement rate between CVSC and CS was performed using the Kappa test with 95% CI and p value ≤ 0.05 . All statistical analyzes were performed using the Statistical Package for Social Sciences (SPSS) software version 19.0 (IBM Corp., Armonk, USA).

RESULTS

Sociodemographic characteristics of the study groups

A total of 287 cervical and cervico-vaginal specimens were obtained from 153 women who agreed to participate in the study. Participants were divided into two groups: HIV-uninfected women (N = 112) and HIV-infected women (N = 41). The mean age of participants was 36.9 ($\pm$ 12.9) years, ranging from 17 to 75 years. Compared to HIV-uninfected women, HIV-infected women were more likely to be single ($p = 0.006$), to live outside the main city of Tapajós region (Santarém) ($p = 0.005$), have higher number of pregnancies ($p = 0.050$), more likely to report regular use of condom ($p = 0.010$) and less likely to report use of hormonal contraception ($p = 0.004$) (Table 1).

Cytological results

All 153 CS were submitted to cytology. Self-collection samples were not submitted to this analysis. None of the women included in the study had been HPV tested or had received the HPV quadrivalent vaccine. All HIV-uninfected women had negative cytology. Among HIV-infected women, 12.2% (5/41) had cytological abnormalities, of which three cases (7.3%) of low grade squamous intraepithelial lesion (LSIL) and two cases (4.8%) of high grade squamous intraepithelial lesion (HSIL). Women diagnosed with HSIL were referred for colposcopy and biopsy for confirmation and medical care follow up.

Acceptability of CVSC

The overall acceptability of CVSC was 87% (133/153). The remaining women (20/153) did not accept performing an additional assay. There was no statistically significant difference in the education level between the women who accepted and those who did not take part in the self-collection option ($p = 0.760$). Roughly 84% (94/112) of HIV-uninfected women and 95% (39/41) of HIV-infected women agreed to perform the CVSC, demonstrating a high acceptability in both groups.

HPV infection in CVSC and CS specimen

The overall prevalence of HPV infection was 42.9% (48/112) and 97.6% (40/41) for HIV-uninfected and infected women, respectively, when considering positivity in at least one of the two clinical sample type specimens (Table S1).

There was significant agreement in the detection of HPV-DNA and hrHPV-DNA between the two sample collection methods. Comparing both collection methods, the agreement rate between CVSC and CS among in study participants was 88.0%, kappa = 0.76 (95% CI, 0.65–0.87, $p < 0.0001$) for HPV detection and 79.7%, kappa = 0.56 (95% CI, 0.41–0.71, $p < 0.0001$) for hrHPV detection. The agreement rate for detection HPV-DNA in HIV-uninfected women between the two methods was 85.1%, kappa = 0.68 (95% CI, 0.52–0.83, $p < 0.0001$). Such calculations were not possible among HIV-infected women as there was no concordant HPV-DNA negative samples in the two collection methods. The agreement rate for detection of hrHPV-DNA between the two methods was 81.9%, kappa = 0.40 (95% CI, 0.17–0.64, $p = 0.0001$) in HIV-uninfected women and 74.4%, kappa = 0.28 (95% CI, –0.06–0.63, $p = 0.083$) in HIV-infected women.

HPV types in HIV-uninfected and HIV-infected women

HPV genotyping revealed a high frequency and diversity of the HPV types found in this study (Table 2; Fig. 2). Among the HIV-uninfected women 89.2% (33/37) had single infections and 10.8% (4/37) multiple infections in the CVSC, whereas 97.3% (36/37) had single infections and 2.7% (1/37) had multiple infections detected in their CS (Table 2). In HIV-infected women, however, multiple infections were much more prevalent at 84.2% (32/38), and single infections accounted for 15.8% (6/38) in the CVSC, similar to the 15.4% (6/39) of single infection and 84.6% (33/39) of multiple infections in CS (Table 2). The overall prevalence of hrHPV was 47.9% (23/48) in HIV-uninfected women and 77.5% (31/40) in HIV-infected women considering positivity in at least one of the two clinical sample type specimens. In HIV-uninfected women high- risk HPV16 and HPV18 represented 24.3% of the mono-infections in the CVSC and 30.5% in the CS. In HIV-infected women, a greater diversity of HPV was identified, with high-risk HPV16, HPV51 and HPV59 being the most frequent in CVSC (18.9%) and CS (16.7%) (Table 2).

All five cases of HIV-infected women with pre-neoplastic lesion were hrHPV positive. In the two HSIL cases, high-risk HPV31 and HPV31-HPV35 co-infection were identified. In the three cases of LSIL the following high-risk HPV types were identified: HPV56, hrHPV51–58 co-infection and multiple infection with HPV16-HPV18-HPV51-HPV56-HPV59 (Fig. 2).

No statistically significant difference was found between age range and the two groups ($p = 0.455$). The hrHPV infection seems to be more concentrated in the age groups encompassing 21–40yo (35.3 ± 12.3) for HIV-uninfected and 31–50yo (37.6 ± 12.5) for the HIV-infected women.

All HIV-infected women were on combination antiretroviral therapy (cART). No significant association was found between median HIV viral load and hrHPV infection ($p = 0.893$). However, hrHPV infection was significantly related to a lower median of CD4+ T-cell count ($p = 0.018$) (Table S2).

In addition, Spearman's correlation test showed that HIV-infected women with lower CD4+ T-cell counts carried a significantly higher number of distinct hrHPV types ($r = -0.432$; $p = 0.005$) (Fig. 3).

Risk factors for hrHPV infection in women living in the Tapajos region

Univariate analysis showed the following variables as risk factors for high risk oncogenic HPV infection ($p < 0.20$): more likely to be HIV-infected (OR = 18.0, 95% CI = 6.48–49.98), more likely to be single (OR = 1.69, CI 95% = 0.87–3.28), more likely to live outside the main city of Tapajos region (Santarem) (OR = 6.77, CI 95% = 0.77–59.44), have no regular Pap smear screening (OR = 1.95, 95% CI = 0.74–5.13), more likely no previous history of HPV such as warts, condylomas and cervical dysplasias (OR = 1.68, CI 95% = 0.74–5.13), more likely alcohol use (OR = 2.34, CI 95% = 1.16–4.75), and have five or more sexual partners (OR = 2.07, 95% CI = 1.07–4.00) (Table 3). In the multivariate analysis, all variables were adjusted and in the last model the following variables remained in the model as risk factors ($p < 0.05$): being HIV-infected (aOR = 17.06; 95% CI = 5.96–48.83); use of

alcohol (aOR = 2.49; CI 95% = 1.09–5.71) and to have five or more sexual partners (aOR = 1.32, 95% CI = 0.60–2.90) (Table 3).

DISCUSSION

In the present study we found a high acceptability rate (87%) of women who agreed to perform CVSC, similar to that observed in studies conducted in developed countries, where people are expected to have higher educational levels [14,15]. This study was conducted in an economically poor region of Brazil and with women who had a medium to low level of education. Regardless, it was felt critically important to have adequate explanations about the instructions on self-collection to give her support to decide whether or not to perform it, as has been seen in a study with women with low levels of education and socioeconomic status in Mexico [16]. We also highlighted the advantage of educating women about the role of HPV in the etiology of cervical cancer during the process of providing instructions for self-collection.

Some women reported having difficulties undergoing routine cervical cancer screening due to many issues, like the cost and time spent traveling to the health service centers, the lack of collection kits at the health unit, the long intervals between clinical consultation and collection, household duties such as having to take care of their children, the partner not allowing the process of vaginal collection, or just because they would feel embarrassed during collection. Others also confessed that the only reason they perform the examination is to update their registration in the Brazilian social program, to keep receiving financial assistance by the government. Some women also felt unmotivated to return to the clinic to receive their results, because if the result showed abnormal cytology, they were not confident they would receive follow-up medical care. Corroborating with the above mentioned issues, the same barriers for acceptance were observed for the Pap smear screening in a qualitative study with low-income indigenous women living in rural areas of Mexico [17].

The CVSC showed a significant agreement rate in the detection of HPV and hrHPV compared with CS on the HPV detection among all study participants and also when restricted to HIV-uninfected women. In addition, a gross similarity was observed in the rates of single- and multiple HPV infections in both methods between the two women groups. The slight difference between the HPV types detected in the CVSC and CS can be explained by the possibility of collecting cells from the epithelial lining of the vagina in addition to cervical cells in the CVSC. New technologies in cervical cancer screening have been proposed to directly benefit high-risk populations [18]. Several studies showed the safety of self-collection compared to the clinician-collected cervical samples [14–16,19–21] and the improved performance of a primary HPV test in early detection of HSIL compared with negative cytology [22–26]. HPV-DNA molecular testing coupled with samples obtained by CVSC is a valid alternative to be considered in the practice of cervical cancer screening in the Tapajós region, because of the ease and feasibility both for women who routinely attend clinics, such as HIV-infected women, and those who undergo it for the first time or less regularly, such as those from our study.

To the best of our knowledge, this is the first study that has evaluated CVSC in women from the Tapajos region, Amazon. In fact, there are few data on HPV infection in Northern Brazil, using conventional cervical scraping. Most of the prevalence surveys in Pará were conducted with women recruited in Eastern and Northeast Pará, with reported ranges in non-indigenous women between 10.5% to 22.8% for HPV [27–29]. A study conducted in Manaus, Amazonas reported a prevalence rate of 61.6% of hrHPV infection in non-indigenous women living with HIV/AIDS [30]. In contrast, much higher prevalence of HPV and hrHPV infections were found in our study conducted in the Tapajós region, including both in HIV-uninfected with normal cytology and HIV-infected women who had abnormal cytology or not.

The most prevalent hrHPV types were HPV16 and HPV18 in HIV-uninfected women, whereas, in HIV-infected women there was greater diversity and multiplicity of HPV types, including those not covered by the quadrivalent or bivalent HPV vaccines, HPV16, HPV51 and HPV59 being the most frequent. In a study of socially isolated and non-isolated indigenous women living in the Amazon forest, 39.7% prevalence of HPV infection and 26.5% of hrHPV were identified, where HPV16 (9.5%), HPV18 (7.2%), HPV31 (8.8%), HPV53 (3.0%) and HPV 68 (3.8%) were the most frequent [31]. The general population of women living in the Tapajos region has different types of circulating hrHPV.

Our findings and other evidence [28,31] make us speculate that high prevalence and long-term persistence of HPV infection is a hereditary characteristic of the prehistoric peoples of the Amazon and it seems that those living farther away from the urban centers of the Amazon are more likely to be infected by hrHPV. Social factors such as difficult access to communities and lack of access to basic public health services have a higher impact on the consequences of long-term persistence HPV infection for women. Cultural and behavioral factors, such as low frequency of condom usage and limited hygienic conditions, might explain recurrent infections, which are associated with high HPV prevalence and diversity [32]. In addition, the high prevalence of HPV and hrHPV found in this study may be related to the high sensitivity and specificity of the laboratory techniques used here for HPV genotyping.

CD4+ T-cell counts were shown to be a better marker for the detection of multiple hrHPV types than HIV viral load, similar to the ratio reported in a study done with women in Senegal, West Africa [33]. Although it was not possible to perform a statistical analysis, 12.2% of cases of cytological abnormalities in HIV-infected women were identified and all were positive for hrHPV-DNA, including multiple infections. Combined antiretroviral therapy appears not to have fully protected women from the development of pre-neoplastic lesions and multiple hrHPV infections. In agreement with our data, a recent study developed in Manaus found a high prevalence of hrHPV types among HIV infected women, with high prevalence of multiple types [34]. Treatment for HIV does not appear to impact the rate of hrHPV infection, it decreases viral load and consequently delays immunosuppression. But coinfection by HIV and hrHPV significantly impacts immunosuppression, and HIV-infected women with lower CD4+ T-cell counts carried a significantly higher number of distinct hrHPV types. The association of immunosuppression with a higher frequency of hrHPV, and

still a higher risk of cervical dysplasia, was also found in HIV seropositive women from Johannesburg South Africa [35].

In this study, there was no significant difference between the prevalence of HPV and hrHPV infection by age in the two studied groups. Risk factors identified were having HIV infection, being single, having five or more sexual partners and alcohol use. It is well established in the literature that a set of factors are necessary for the development of cervical cancer, and persistent infection with hrHPV is the main one but there are other ones like HIV infection and herpes simplex virus type 2 (HSV-2) [3,36,37]. Further studies should be performed in the Tapajós region for a better understanding of the relationship between age, hrHPV infection and cancer risk.

Our study has some limitations. The cross-sectional epidemiological design does not allow the temporal relationship between exposure and effect to be established, which makes it difficult to establish a causal relationship. The sample size, particularly of HIV-infected women, may have been influenced by the difficulty of access and the infrastructure of the health units in Santarém, as well as the lack of data in the literature on the prevalence of HIV and HPV infection in the population studied. In order to maintain homogeneity between the two groups of women, we established the criterion of including only HIV-infected women who had been diagnosed with HIV infection during the same study period from August 2015 to August 2016 and in the same age groups as HIV-uninfected women (36.9 ± 13.1).

As CVSC is a patient-centered method and is more inclusive and, therefore, the reasons that women can't or did not want to go to health centers to do Pap smear collection (CS) would be avoided. The woman with hrHPV infection detected on selfcollection should be referred for medical follow-up that should follow the flow of care established by the Brazilian cervical cancer screening guidelines, which is to perform cytological screening by Pap smear and, if the result is abnormal, perform the colposcopy, and depending on the degree of cervical lesion observed, perform the biopsy for confirmation. The gap that currently exists in cervical cancer screening is that many women are still not being reached or are not efficiently attained. CVSC is a new technology for screening that can change this scenario.

The data in this study about CVSC and HPV infection in women living in the Tapajós region reveal differences on the prevalence and diversity of HPV and hrHPV and can add to the body of evidence on this topic and inform global meta-analyses and systematic reviews to support evidence-based policies of cervical cancer prevention and control. Self-collection of specimens for use in cervical cancer screening programs will be the appropriate approach for expanding cervical cancer screening services for this population that lives in a limited-resource and geographical remote region in Northern Brazil with a high incidence and mortality of cervical cancer.

CONCLUSIONS

CVSC had a high acceptability in HIV-negative and HIV-positive women living in the Tapajós region. We identified a much higher prevalence rate of HPV and hrHPV than those reported in the literature in our population and with different types of hrHPV that are not

included in the quadrivalent HPV vaccine. We encourage public health care officials to include CVSC along with hrHPV-DNA testing as a primary approach alternative in Northern Brazil, where cervical cancer persists with the highest incidence and highest mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all the patients participating in the study. The Federal University of Western Pará for granting leave to official for doctorate to L.L.S.R. The Secretaria Municipal de Saúde and Centro de Testagem e Aconselhamento of Santarém, Pará for having authorized the recruitment of the women in the health units. Dr Charlotte Gaydos and Justin Hardick from Johns Hopkins University, USA, for logistical support with the samples to the University of Southern California, USA where the HPV typing was done in the Immune Monitoring Core. Dr Miguel Moreira of the National Cancer Institute of Brazil for training in nucleotide sequencing analysis. Dr Katrini Martinelli for performing the statistical analyzes and Jonas Aguiar for the excelent Figure from the Tapajós region.

Financial support

This work was supported by Coordination of Personal Improvement of Higher Education (CAPES) Foundation, Ministry of Education of Brazil [Process number 88881.131796/2016-01 to L.L.S. Rodrigues], Programa Estratégico de Apoio a Pesquisa em Saúde (PAPES VII), Fundação Oswaldo Cruz (FIOCRUZ)/ Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grants numbers 401810/2015-1 to A.F. Nicol] and National Institutes of Health [grants numbers R01 CA074397 and P30 CA014089 to W.M. Kast].

References

- [1]. de Sanjosé S, Diaz M, Castellsague X, Clifford G, Bruni L, Muñoz N, Bosch FX, Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis, *Lancet Infect. Dis* 7 (2007) 453–459. doi:10.1016/S1473-3099(07)70158-5. [PubMed: 17597569]
- [2]. Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, Stevens G, Gottlieb S, Kiarie J, Temmerman M, Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting, *PLoS One*. 10 (2015) 1–17. doi: 10.1371/journal.pone.0143304.
- [3]. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-tieulent J, Jemal A, Global Cancer Statistics, 2012, *CA a Cancer J. Clin* 65 (2015) 87–108. doi:10.3322/caac.21262.
- [4]. Instituto Nacional do Câncer, Estimativa 2018 Incidência de Câncer no Brasil - Neoplasia maligna da mama feminina e colo do útero, <http://Www.Inca.Gov.Br>. (2018) <http://www.inca.gov.br>. <http://www.inca.gov.br/estimativa/2018/mapa-mama-feminina-colo-uterio.asp> (accessed June 2, 2018).
- [5]. Loyola Cissa, Conheça a estrutura do estado do Tapajós, Tapajos Meu Estado. (2018). <http://www.tapajosmeuestado.com.br/2018/02/conheca-estrutura-do-estado-do-tapajos.html> (accessed June 2, 2018).
- [6]. Instituto Brasileiro de Geografia e Estatística, *Uso da terra no Estado do Para : relatório técnico*, 95892nd ed., Rio de Janeiro, 2013. doi:95892.
- [7]. Lazcano-Ponce E, Lorincz AT, Cruz-Valdez A, Salmerón J, Uribe P, Velasco-Mondragón E, Nevarez PH, Acosta RD, Hernández-Avila M, Self-collection of vaginal specimens for human papillomavirus testing in cervical cancer prevention (MARCH): A community-based randomised controlled trial, *Lancet*. 378 (2011) 1868–1873. doi:10.1016/S0140-6736(11)61522-5. [PubMed: 22051739]
- [8]. Smith LW, Khurshed F, van Niekerk DJ, Krajden M, Greene SB, Hobbs S, Coldman AJ, Franco EL, Ogilvie GS, Women's intentions to self-collect samples for human papillomavirus testing in an organized cervical cancer screening program, *BMC Public Health*. 14 (2014) 1060. doi: 10.1186/1471-2458-14-1060. [PubMed: 25303975]

- [9]. Chen KW, Ouyang YQ, Hillemanns P, Jentschke M, Excellent analytical and clinical performance of a dry self-sampling device for human papillomavirus detection in an urban Chinese referral population, *J. Obstet. Gynaecol. Res* 42 (2016) 1839–1845. doi:10.1111/jog.13132. [PubMed: 27647799]
- [10]. Oliveira NS, Andrade CV, Grinsztejn B, Friedman RK, Cunha CB, Veloso VG, Coutinho JR, Chicarino-Oliveira JM, Ferreira DDC, Pereira LOR, Amaro-Filho SM, Moreira MA, Nuovo GJ, Nicol AF, In situ FoxP3+ and IL-10 over-expression is associated with high grade anal lesions in HIV infected patients, *Int. J. Clin. Exp. Pathol* 9 (2016) 1520–1532.
- [11]. Nicol AF, Brunette LL, Nuovo GJ, Grinsztejn B, Friedman RK, Veloso VG, Cunha CB, Coutinho JR, Vianna-Andrade C, Oliveira NS, Woodham AW, da Silva DM, Kast WM, Secretory leukocyte protease inhibitor expression and high-risk HPV infection in anal lesions of HIV positive patients., *J. Acquir. Immune Defic. Syndr* 73 (2016) 27–33. doi:10.1097/QAI.0000000000001049. [PubMed: 27149102]
- [12]. Cornall AM, Poljak M, Garland SM, Phillips S, Tan JH, Machalek DA, Quinn MA, Tabrizi SN, Anyplex II HPV28 detection and Anyplex II HPV HR detection assays are highly concordant with other commercial assays for detection of high-risk HPV genotypes in women with high grade cervical abnormalities, *Eur. J. Clin. Microbiol. Infect. Dis* 36 (2017) 545–551. doi:10.1007/s10096-016-2831-5. [PubMed: 27822653]
- [13]. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA, The biology and life-cycle of human papillomaviruses, *Vaccine*. 30 (2012) F55–F70. doi: 10.1016/j.vaccine.2012.06.083. [PubMed: 23199966]
- [14]. Igidbashian S, Boveri S, Spolti N, Radice D, Sandri MT, Sideri M, Self-Collected Human Papillomavirus Testing Acceptability: Comparison of Two Self-Sampling Modalities, *J. Women's Heal* 20 (2011) 397–402. doi:10.1089/jwh.2010.2189.
- [15]. Leinonen MK, Schee K, Jonassen CM, Lie AK, Nystrand CF, Rangberg A, Furre IE, Johansson MJ, Trope A, Sjoborg KD, Castle PE, Nygard M, Safety and acceptability of human papillomavirus testing of self-collected specimens: A methodologic study of the impact of collection devices and HPV assays on sensitivity for cervical cancer and high-grade lesions, *J. Clin. Virol* 99–100 (2018) 22–30. doi:10.1016/j.jcv.2017.12.008.
- [16]. Hernández-Márquez C.I., Salinas-Urbina AA, Cruz-Valdez A, Hernández-Girón C, Knowledge of human papilloma virus (HPV) and acceptance of vaginal self-sampling among Mexican woman Clara, *Rev. Salud Pública* 16 (2014) 697–708. doi:10.15446/rsap.v16n5.30071. [PubMed: 26120755]
- [17]. Allen-Leigh B, Uribe-Zúñiga P, León-Maldonado L, Brown BJ, Lörincz A, Salmeron J, Lazcano-Ponce E, Barriers to HPV self-sampling and cytology among low-income indigenous women in rural areas of a middle-income setting: A qualitative study, *BMC Cancer*. 17 (2017) 1–11. doi: 10.1186/S12885-017-3723-5. [PubMed: 28049525]
- [18]. Alaina J CLT. Brown, New Technologies for Cervical Cancer Screening, *Best Pr. Res Clin Obs. Gynaecol* 26 (2012) 233–242. doi:10.1080/10810730902873927. Testing.
- [19]. Bansil P, Wittet S, Lim JL, Winkler JL, Paul P, Jeronimo J, Acceptability of self-collection sampling for HPV-DNA testing in low-resource settings: A mixed methods approach, *BMC Public Health*. 14 (2014) 596. doi:10.1186/1471-2458-14-596. [PubMed: 24927941]
- [20]. Jentschke M, Chen K, Arbyn M, Hertel B, Noskiewicz M, Soergel P, Hillemanns P, Direct comparison of two vaginal self-sampling devices for the detection of human papillomavirus infections, *J. Clin. Virol* 82 (2016) 46–50. doi:10.1016/j.jcv.2016.06.016. [PubMed: 27434147]
- [21]. Catarino R, Vassilakos P, Bilancioni A, Bougel S, Boukrid M, Meyer-Hamme U, Petignat P, Accuracy of self-collected vaginal dry swabs using the Xpert human papillomavirus assay, *PLoS One*. 12 (2017) 1–12. doi:10.1371/journal.pone.0181905.
- [22]. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Palma PD, Del Mistro A, Ghiringhello B, Girlando S, Gillio-Tos A, De Marco L, Naldoni C, Pierotti P, Rizzolo R, Schincaglia P, Zorzi M, Zappa M, Segnan N, Cuzick J, Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial, *Lancet Oncol*. 11 (2010) 249–257. doi:10.1016/S1470-2045(09)70360-2. [PubMed: 20089449]
- [23]. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJF, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJLM, Cuzick J, Zappa M, Carozzi F,

- Confortini M, Dalla Palma P, Zorzi M, Del Mistro A, Gillio-Tos A, Naldoni C, Rijkaart D, Van Kemenade F, Bulkman N, Heideman D, Rozendaal R, Kenter G, Almonte M, Roberts C, Desai M, Sargent A, Ryd W, Naucier P, Efficacy of HPV-based screening for prevention of invasive cervical cancer: Follow-up of four European randomised controlled trials, *Lancet*. 383 (2014) 524–532. doi:10.1016/S0140-6736(13)62218-7. [PubMed: 24192252]
- [24]. Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL, Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first-line screening test, *Gynecol. Oncol* 136 (2015) 189–197. doi:10.1016/j.ygyno.2014.11.076. [PubMed: 25579108]
- [25]. Canfell K, Caruana M, Gebiski V, Darlington-Brown J, Heley S, Brotherton J, Gertig D, Jennett CJ, Farnsworth A, Tan J, Wrede CD, Castle PE, Saville M, Cervical screening with primary HPV testing or cytology in a population of women in which those aged 33 years or younger had previously been offered HPV vaccination: Results of the Compass pilot randomised trial, *PLoS Med*. 14 (2017) 1–21. doi: 10.1371/journal.pmed.1002388.
- [26]. Ogilvie GS CA, Krajden M, van Niekerk D, Smith LW, Cook D, Ceballos K, Lee M, Gentile L, Gondara L, Elwood-Martin R, Peacock S, Stuart G, Franco EL, HPV for cervical cancer screening (HPV FOCAL): Complete Round 1 results of a randomized trial comparing HPV-based primary screening to liquid-based cytology for cervical cancer., *Int. J. Behav. Med* 140 (2017) 440–448.
- [27]. and Sylvia Regina Vasconcellos de Aguiar JASQ, Elizabeth Villanova Fabiola, Caricio Martins Luisa, Silva dos Santos Milena, de Paula Maciel Juliana, Fábio Magno Falcão Luiz, Thais Fuzii Hellen, Human Papillomavirus: Prevalence and Factors Associated in Women Prisoners Population From the Eastern Brazilian Amazon, *J. Med. Virol* 86 (2014) 1528–33. doi:10.1002/jmv. [PubMed: 24838771]
- [28]. Duarte D, Vieira R, Brito E, Pinheiro M, Monteiro J, Valente M, Ishikawa E, Fuzii H, Sousa M, Prevalence of Human Papillomavirus Infection and Cervical Cancer Screening among Riverside Women of the Brazilian Amazon, *Rev. Bras. Ginecol. eObs./RBGO Gynecol. Obstet* 39 (2017) 350–357. doi: 10.1055/S-0037-1604027.
- [29]. do Amaral JLA, de A MV, Araújo GA da S. Dias, von Ledebur EICF, Quaresma JAS, Fuzii HT, Clinical and epidemiological study of human papillomavirus infection in women with systemic lupus erythematosus in eastern brazilian amazon., *Acta Reumatol. Port*. 42 (2016) 47–54.
- [30]. da Silva L, Miranda A, Batalha R, Ferreira L, Santos M, Talhari S, High-risk human papillomavirus and cervical lesions among women living with HIV/AIDS in Brazilian Amazon, Brazil, *Brazilian J. Infect. Dis* 19 (2015) 557–562. doi:10.1016/j.bjid.2015.07.001.
- [31]. Fonseca AJ, Taeko D, Chaves TA, Da Costa Amorim LD, Murari RSW, Miranda AE, Chen Z, Burk RD, Ferreira LCL, HPV infection and cervical screening in socially isolated indigenous women inhabitants of the amazonian rainforest, *PLoS One*. 10 (2015) 1–18. doi: 10.1371/journal.pone.0133635.
- [32]. Ranjeva SL, Baskerville EB, Dukic V, Villa LL, Lazcano-Ponce E, Giuliano AR, Dwyer G, Cobey S, Recurring infection with ecologically distinct HPV types can explain high prevalence and diversity, *Proc. Natl. Acad. Sci* 114 (2017) 13573–13578. doi:10.1073/pnas.1714712114. [PubMed: 29208707]
- [33]. Hanisch RA, Sow PS, Toure M, Dem A, Dembele B, Toure P, Winer RL, Hughes JP, Gottlieb GS, Feng Q, Kiviat NB, Hawes SE, Influence of HIV-1 and/or HIV-2 infection and CD4 count on cervical HPV DNA detection in women from Senegal, West Africa, *J. Clin. Virol* 58 (2013) 696–702. doi: 10.1016/j.jcv.2013.10.012. [PubMed: 24210330]
- [34]. Teixeira MF, Sabidó M, Leturiondo AL, Ferreira CDO, Torres KL, Benzaken AS, High risk human papillomavirus prevalence and genotype distribution among women infected with HIV in Manaus, Amazonas, *Virol. J* 15 (2018) 36. [PubMed: 29454382]
- [35]. Firnhaber C, Van Le H, Pettifor A, Schulze D, Michelow P, Sanne IM, Lewis DA, Williamson AL, Allan B, Williams S, Rinas A, Levin S, Smith JS, Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa, *Cancer Causes Control*. 21 (2010) 433–443. doi:10.1007/s10552-009-9475-z. [PubMed: 19949850]
- [36]. Unemo M, Bradshaw CS, Hocking JS, de Vries HJC, Francis SC, Mabey D, Marrazzo JM, Sonder GJB, Schwebke JR, Hoornenborg E, Peeling RW, Philip SS, Low N, Fairley CK,

Sexually transmitted infections: challenges ahead, *Lancet Infect. Dis* 17 (2017) e235–e279. doi: 10.1016/S1473-3099(17)30310-9. [PubMed: 28701272]

- [37]. McCloskey JC, Martin Kast W, Flexman JP, McCallum D, French MA, Phillips M, Syndemic synergy of HPV and other sexually transmitted pathogens in the development of high-grade anal squamous intraepithelial lesions, *Papillomavirus Res.* 4 (2017) 90–98. doi:10.1016/j.pvr.2017.10.004. [PubMed: 29179876]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights:

- High acceptability of cervico-vaginal self collection (CVSC) in women living in the Tapajós region, Amazon, Brazil
- CVSC had significant agreement for any HPV and hrHPV detection when compared to cervical scraping (*CS*)
- HIV-uninfected women without cervical lesions have high prevalence of any HPV, HPV16 and HPV18
- HIV-infected women with or without cervical lesions have high prevalence of any HPV, HPV16, HPV51 and HPV59
- HIV-infected women with lower CD4+ T-cell counts carried a significantly higher number of distinct hrHPV types

Fig. 1.
Representative map of the Tapajós region, within the state of Pará, Northern Brazil.
Geographic demarcation of Northern Brazil and the Brazilian legal Amazon. Highlighted are
Santarém and the 22 municipalities in the Tapajós region.

Fig. 2. Diversity of HPV types isolated in cervico-vaginal self-collection and cervical scraping of HIV-uninfected and HIV-infected women. The columns are not organized by paired samples from the same women, but according to the oncogenic risk for both specimen types. The high-risk HPV types are in red, “probable” or “possible” carcinogenic are in yellow and low-risk are in blue. The samples of HIV-infected women with high grade squamous intraepithelial lesion (HSIL) are with black borders and those with low grade squamous intraepithelial lesion (LSIL) are with green borders.

Fig. 3. Correlation between the type number of high-risk HPV and CD4+ T-cell counts. Spearman's correlation test; $\hat{r} = -0.432$; $p = 0.005$. P -value < 0.05 was considered significant.

Table 1.

Sociodemographic characteristics of HIV-infected and HIV-uninfected women living in the Tapajos region in the Northern Brazil.

Variables	Total	HIV-uninfected	HIV-infected	p value
Sample size (n)	153 (%)	112 (%)	41 (%)	-
Age (years)	36.9 (± 12.9)	36.9 (± 13.1)	36.9 (± 12.4)	0.995
Housekeeper	84 (54.9)	60 (53.6)	24 (58.5)	0.855
Marital status				0.006
<i>Single</i>	59 (39.3)	36 (32.7)	23 (57.5)	
<i>Married/living together</i>	91 (60.7)	74 (67.3)	17 (42.5)	
Education up to high school	129 (84.9)	91 (82)	38 (92.7)	0.102
City of birth				0.191
<i>Santarém, Pará</i>	103 (67.3)	78 (69.6)	25 (61.0)	
<i>Other municipalities in the Tapajós region</i>	27 (17.6)	16 (14.3)	11 (26.8)	
<i>Outside the Tapajós region</i>	23 (15.0)	18 (16.1)	05 (12.2)	
City of residence				0.005
<i>Santarém, Pará</i>	146 (96.1)	111 (99.1)	35 (87.5)	
<i>Other municipalities in the Tapajós region</i>	06 (3.9)	01 (0.9)	05 (12.5)	
Number of pregnancies	3.4 (± 3.5)	3.0 (± 2.3)	4.3 (± 5.7)	0.050
Hormonal contraception	46 (30.1)	39 (34.8)	07 (17.1)	0.004
History of cancer	26 (19.7)	15 (16.5)	11 (26.8)	0.167
Alcohol use	46 (30.3)	32 (28.6)	14 (35.0)	0.447
Use of cigarettes	22 (14.5)	16 (14.4)	06 (14.6)	0.973
Illicit drug use	05 (3.3)	02 (1.8)	03 (7.3)	0.124
Age of first sexual intercourse	16.7 (± 2.9)	16.9 (± 3.2)	16.1 (± 1.9)	0.091
Number of sexual partners	7.4 (± 14.8)	6.6 (± 15.7)	9.9 (± 11.5)	0.230
Regular use of condoms	25 (16.4)	13 (11.7)	12 (29.3)	0.010

Table 2.

Prevalence of HPV types in HIV-uninfected and HIV-infected women.

HPV types	HIV-uninfected women				HIV-infected women			
	CVSC HPV+		CS HPV+		CVSC HPV+		CS HPV+	
	N	%	N	%	N	%	N	%
	37	100.0	37	100.0	38	100.0	39	100.0
<i>Mono infection</i>	33	89.2	36	97.3	6	15.8	6	15.4
High-risk	15	45.4	22	61.1	0		2	33.3
16	3	9.1	7	19.4				
18	5	15.2	4	11.1				
31	1	3.0	4	11.1				
33	1	3.0	3	8.3				
45	1	3.0	1	2.8				
52	1	3.0	0					
56	1	3.0	1	2.8				
58	2	6.1	2	5.6				
Probable or possible carcinogenic	5	15.2	6	16.7	4	66.7	1	16.7
Low-risk	13	39.4	8	22.2	2	33.3	3	50.0
Total HPV	33	100.0	36	100.0	6	100.0	6	100.0
<i>Multiple infections</i>	4	10.8	1	2.7	32	84.2	33	84.6
High-risk	4	44.4	1	50.0	69	43.4	69	41.1
16	1	11.1	0		7	4.4	7	4.2
18	1	11.1	0		4	2.5	3	1.8
31	0		0		6	3.8	5	3.0
33	1	11.1	0		4	2.5	4	2.4
35	0		0		4	2.5	5	3.0
39	0		0		3	1.9	1	0.6
45	0		0		5	3.1	4	2.4
51	0		0		13	8.2	13	7.7
52	0		0		2	1.3	2	1.2
56	0		0		5	3.1	6	3.6
58	0		1	50.0	6	3.8	6	3.6
59	0		0		10	6.3	8	4.8
Probable or possible carcinogenic	4	44.4	1	50.0	31	19.5	40	23.8
Low-risk	1	11.1	0		59	37.1	59	35.1
Total HPV	9	100.0	2	100.0	159	100.0	168	100.0

Abbreviations: CVSC: Cervico-vaginal self-collection, CS: Cervical scraping

Table 3.

Univariate and multivariate analyses of risk factors for hrHPV-DNA detection in HIV-uninfected and HIV-infected women.

Variables	High-risk HPV infections				
	Total	Yes (%)	p value	OR (95% CI)	Adjusted OR (95% CI)
HIV-uninfected	112	32 (28.6)	<0.0001	1.00	1.00
HIV-infected	41	36 (87.8)		18.0 (6.48–49.98)	17.06 (5.9–48.8)
Marital status			0.118		
<i>Single</i>	59	31 (52.5)		1.69 (0.87–3.28)	-
<i>Married/living together</i>	91	16 (39.6)		1.00	-
City of residence			0.088		
<i>Santarém, Pará</i>	146	62 (42.5)		1.00	-
<i>Other municipalities in the Tapajós region</i>	06	05 (83.3)		6.77 (0.77–59.44)	-
Pap smear regularly			0.171		
<i>No</i>	20	12 (60.0)		1.95 (0.74–5.13)	-
<i>Yes</i>	115	50 (43.5)		1.00	-
History of HPV infection			0.216		
<i>No</i>	118	55 (46.6)		1.68 (0.74–3.76)	-
<i>Yes</i>	32	11 (34.4)		1.00	-
Alcohol use			0.017		
<i>No</i>	106	40 (37.7)		1.00	1.00
<i>Yes</i>	46	27 (58.7)		2.34 (1.16–4.75)	2.49 (1.09–5.7)
Number of sexual Partners			0.029		
<i>04</i>	87	32(36.8)		1.00	1.00
<i>05</i>	64	35(54.7)		2.07(1.07–4.00)	1.32(0.60–2.90)