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Research Article

PREVALENCE AND GENOTYPE DIVERSITY OF ANAL HPV INFECTION AMONG HIV-INFECTED WOMEN IN BELÉM, PARÁ, NORTH OF BRAZIL

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Abstract- The objective of this cross-sectional study was to determine the prevalence of anal HPV infection, genotype diversity and its relationship with demographic aspects of HIV-infected assisted in Belém, Pará, Brazil. A questionnaire regarding socio-demographic variables and sexual behavior was used and anal samples were collected from 108 women recruited from a HIV Counselling and Testing in the Belém, Pará, an important metropolis of the northern region of Brazil. A blood sample was also obtained to determine CD4+ T lymphocytes counts and viral load levels of HIV. HPV DNA was detected by PCR amplification of a conserved region of the HPV L1 gene and further genotyped with direct DNA sequencing. HPV infection was detected in 29 (26,8%). Twelve different types were found (HPV-16, 58, 59, 6, 11, 53, 61, 62, 66, 70, 71, 102) and low risk types were present in 72.4% of samples. HPV infection was positively correlated with CD4+ T lymphocytes counts. In conclusion, we found a low prevalence of HPV infection and low risk types in this cohort and we emphasize the relevance of implementation of screening program for anal cancer and HPV infection in the HIV-infected women population.

Keywords- HPV, Genotypes, Diversity, Anal, HIV.

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Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections (STIs) in the world and several studies have shown that people living with human immunodeficiency virus (HIV) have increased risk for HPV infection, cervical cancer, and anal cancer compared to HIV negative people [1]. HPV can be transmitted through direct or indirect contact and sexual transmission is one of the most important forms of HPV entry into the region of the cervix and the anal region [2], and transmission from woman to man seems to be more effective than from man to woman [3].

In Brazil is well documented the association between HPV and invasive cervical cancer in HIV-infected women compared to HIV-uninfected women, as well as high prevalence of HPV infection in HIV-infected women [4] but studies on anal HPV in this population are scarce. Several studies have shown that cervical persistent HPV infection predisposed abnormalities in anal region [5].

There are 51 mucosal HPV types that have been associated with anogenital infection and which are divided into high risk (HR) or low risk (LR) types due to their association with cervical cancer [6]. In Brazil, several studies have described a higher frequency of HR types related to a more persistent infection in HIV-infected women [7-8]. Additionally, studies comparing two groups of women, infected and HIV-uninfected, found higher incidence in later one. [9].

The screening for cervical cancer is recommended for women in various countries but very few studies of prevalence, genotype diversity and factors associated with anal HPV infection in HIV-infected women have been documented in Brazil, particularly in the North Region. There are no data about the prevalence and molecular epidemiology of HPV in anal specimen in the State of Pará and these findings can be used to elaborate prevention strategies for HIV-infected women population. To address this gap, the objective of the present study was to determine the type-specific presence of HPV and identify the factors associated with anal HPV infection in women infected by HIV assisted in Belém, Pará, Brazil.

Materials and Methods

Study population and Ethical aspects

We conducted a cross-sectional study in women HIV-infected assisted by Hospital Universitário João de Barros Barreto (HUJBB) and Unidade de Referência em Doenças Infecciosas e Parasitárias (URE-DIPE) in Belém, Pará, Northern Brazil. Between May and December of 2010, anal samples were collected from 108 women randomly chosen to be enrolled in the study while undergoing routine Pap smear testing. Inclusion criteria were: HIV infection confirmed; age ≥ 18 years; sexually active, consent to participate (signature of the approved informed consent form). Exclusion criteria were: pregnancy and history of hysterectomy. Socioeconomic and demographic data, as well as information on sexual history and sexual behavior, were obtained using a pretested standardized questionnaire. Blood samples were collected to obtain T CD4+ lymphocytes count and HIV viral load.

The Ethics and Research Committee of the HUJBB approved the study under protocol number 200856879, and written informed consent was obtained from all participants prior to enrollment.

CD4+ T lymphocytes counts and plasma HIV-1 viral load quantification

The counts of CD4+ T lymphocytes were performed by flow citometry using the BD

TrunCOUNT™ Tubes (Becton Dickinson and Company- Immunocytometry Systems, San Jose, U.S.A) in the BD FACSCalibur™ (BD Biosciences Immunocytometry Systems - Bdbio-IS, U.S.A, 2008) flow citometer and the BD MultiSET™ software.

The HIV-1 plasmatic viral load was performed using the branched DNA methodology (bDNA) with the Versant® HIV-1 RNA 3.0 Assay bDNA test kit (Siemens Healthcare Diagnostics Inc., U.S.A) in the System 340 bDNA Analyzer & Data Management Software (Bayer Health Care, Tarrytown, U.S.A, 2006) equipment.

HPV typing and sequence analysis

Collection of anal specimens was made using a Rayon swab. The swab was inserted 1.5 to 2.0 cm into the anus and rotated 360° clockwise for five times. The swab was placed in 2.0 mL of Phosphate Buffered Saline (PBS), stored at -20ºC and subsequently used for molecular analysis.

Genomic DNA was extracted using the phenol-chloroform standard method [10]. A 450 bp fragment of the HPV L1 gene was amplified using a nested polymerase chain reaction (nested-PCR) with the primer pairs MY09/MY11, as described previously [11]. A fragment of TNF-α gene was amplified in a single round reaction according to standard conditions [12], as an internal control, for assessing the quality of DNA and validating viral PCR amplification. Amplified products were visualized in 1% agarose gel in TAE buffer with 3,0 µL of Syber-safe (Invitrogen, Oregon, USA) and viewed under ultraviolet light.

The positive samples were sequenced (in duplicate) by using ABI PRISM BigDye Terminator Cycle Sequencing v 3.1 Ready Reaction (Applied Biosystems) to obtain both the forward and reverse sequences with the ABI 3130 XL genetic analyzer (Applied Biosystems). Nucleotide sequences were edited using the BIOEDIT software version 5.0.9 [13]. Sequence comparisons were performed using the NCBI BLASTn software (ncbi.nlm.nih.gov) to identify the HPV type.

Statistical analysis

The clinical-epidemiological and laboratory data were entered into a database using Microsoft Access 2013 software. The HPV types were classified according to their phylogenetic characteristics. All variables were expressed as absolute and relative frequencies. The associations between HPV infection and socio-demographic factors were performed with the chi-square test, exact Fisher test or G test. A p-value < 0.05 was considered significant.

Results

Anal HPV infection was investigated among 108 HIV-positive women who were assisted between May and December of 2010 in Belém, Pará, Brazil. Among them, 78.7% (85/108) were currently under antiretroviral therapy. The mean age of these patients were 38.2 years (ranging from 19 to 66 years), 45.4% (49/108) were married and 51.8% (56/108) had less than eight years of education. Few women smoked (25/108; 23.1%), were users of illicit drug (8/108; 7.4%), and reported history of sexually transmitted infection (27/108; 25.0%). Almost half of the women were alcohol users (52/108; 48.1%) and reported anal sex practice (58/108; 53.7%). Moreover, most of them (37/58; 63.8%) do not use condoms in their anal sexual relation.

The median age of the first sexual intercourse was 17.3 years, whereas most of the women (55/108; 50.9%) were between 10 and 15 years old; 59.3% (64/108) were sexually active and 40.7% (44/108) had between 1 and 5 lifetime partners. Few women reported anal warts (25/108; 23.1%) and genital warts (21/108; 19.4%). Most HIV-infected women showed CD4⁺ T lymphocyte count > 200 cells/mm³ and HIV-1 viral loads < Log10 4,00 (10.000 copies/mL; [Table-1].

Of the 108 women that participated in the study, 29 (26.8%) presented positive HPV DNA detection results, and the mean age of these patients were 35.7 years (ranging from 19 to 47 years). Demographic and behavioral characteristics of the women co-infected with HPV were found to be similar to those of women without detectable HPV infection in relation to age (p = 0.0589), schooling level (p = 0.8406), age of first sexual intercourse (p = 0.8592), number of partners (p = 0.4459), history of sexually transmissible infection (p = 0.9002), regular use of condom (p = 0.9422), practice of anal sex (p = 0.9743), anal and cervical warts (p = 0.9127 and p = 0.5321, respectively) and HIV-1 viral load (p = 0.0583). Among 16 patients presenting T CD4⁺ lymphocytes count < 200 cells/mm³, 9 (56.3%) presented the HPV DNA. On the other hand, 92 patients had T CD4⁺ lymphocytes count ≥ 200 cells/mm³ and 20 (69.0%) of them presented HPV DNA. The differences were significant (p = 0.0112) [Table-1].

A total of 12 different HPV genotypes were detected. The HR-HPV was detected in 8 (27.6%) cases, while LR-HPV was seen in 21 (72.4%) cases. The HR-HPV genotypes found were HPV 16, 58 and 58 and the most commonly detected LR-HPV genotypes were HPV 6 [Table-2].

Table-1 *Demographic and behavioral characteristics of the 108 HIV-1 positive women assisted in Belém, Pará, from May to December 2010, considering the HPV positivity results.*

Variables	N (%)	PCR		p-valor
		Negative (n=79) n (%)	Positive (n=29) n (%)	
Age (years)				
19-34	38 (35.2)	24 (63.2)	14 (36.8)	
35-42	38 (35.2)	27 (71.0)	11 (29.0)	0.0589(§)
> 42	32 (29.6)	28 (87.5)	4 (12.5)	
Age at first sexual intercourse (years)				
10-15	55 (50.9)	39 (70.9)	16 (29.1)	
16-20	44 (40.7)	33 (75.0)	11 (25.0)	0.8592(§)
> 20	9 (8.4)	7 (77.8)	2 (22.9)	
History of STI				
Yes	27 (25.0)	20 (74.1)	7 (25.9)	0.9002(¶)
No	81 (75.0)	59 (72.8)	22 (27.2)	
Practice of anal sex				
Yes	58 (53.7)	43 (74.1)	15 (25.9)	0.9743(¶)
No	50 (46.3)	36 (72.0)	14 (28.0)	
Anal wart				
Yes	25 (23.1)	19 (76.0)	6 (24.0)	0.9127(¶)
No	83 (76.9)	60 (72.3)	23 (27.7)	
CD4⁺ T lymphocyte at HPV diagnosis (cells/mm³)				
<200	16 (14.8)	7 (43.7)	9 (56.3)	0.0112(¥)
≥200	92 (85.2)	72 (78.3)	20 (21.7)	
Viral load				
< Log_{10} 4,00	104 (96.3)	78 (75.0)	26 (25.0)	0.0583(¥)
≥ Log_{10} 4,00	4 (3.7)	1 (25.0)	3 (75.0)	

* Not used in statistical analysis. (¶) χ2- square test. (§) G test. (¥) exact Fisher test.

Table-2 *Distribution of HPV species isolated from specimens obtained from HIV-1 women, assisted in Belém, Pará, from May to December 2010.*

	N	%
Oncogenic risk		
HR	8	27.6
LR	21	72.4
Total	29	100.0
HPV type		
HR		
HPV-16	4	50.0
HPV-58	2	25.0
HPV-59	2	25.0
Total	8	100.0
LR		
HPV-6	6	28.6
HPV-11	1	4.8
HPV-53	3	14.3
HPV-61	2	9.5
HPV-62	2	9.5
HPV-66	2	9.5
HPV-70	2	9.5
HPV-71	1	4.8
HPV-102	2	9.5
Total	21	100.0

Discussion

The present study reported for the first time the prevalence and diversity of HPV in anal specimens in HIV-infected women assisted in Belém, Pará, Brazil. Interestingly, we found a low prevalence of anal HPV infection (26.8%) in the examined women, which proved to be much lower than previously reports of anal specimens evaluated in other cities of Brazil, such as Santos [14], Manaus [15], and São Luiz do Maranhão [16], which showed a prevalence of 63%, 79% and 77.7%, respectively. The research of HPV in anal specimen of women infected with HIV-1 is of great importance as the HIV / HPV co-infection may be considered a risk factor for the development of pre-neoplastic lesions in both uterine cervix [17] and the anus [18].

We noticed that most of the participants co-infected with HIV and HPV have had sexual initiation between 10 and 15 years of age (55.2%), similar to that observed in Pernambuco [19]. However, the prevalence of HPV infection was lower than that observed in cervical samples in other cities of Brazil, such as 73.2% [20] and 78.8% [21] in Minas Gerais and 77.7% in Maranhão [16].

We expected a greater prevalence of HIV and HPV co-infection, similar to that found in the city of Manaus (79%), Amazonas' capital, another State of the northern region of Brazil [7]. We could address possibly some reasons, one of them could be the methodology employed in the present study, the second is the fact that the majority of the women enrolled in the study related a non risk behavior since they reported a low number of sexual partners during their life. Even though, many others studies have described a high prevalence of co-infection HPV/HIV in women with low numbers of partners [8,16], which highlights the fact that the risk of HPV infection may be independent of the number of sexual partners, i.e., the chance of acquiring the infection does not seem to increase in a directly proportional ratio to the number of partners during their lifetime.

In this study, we identified 12 species of HPV and this number may be a reflection of the low prevalence of infection in the population studied and not the sample size, bearing in mind that other studies in Brazil have reported a wide variety of types of HPV in a sample similar to ours, such as in Rio de Janeiro [22], the Espírito Santo [23] and Maranhão [16].

An interesting finding in our study was the low prevalence of HPV of high oncogenic risk (27.6%), and the majority of infections found were caused by low risk HPV (72.4%). This fact contrasts with the results found in the majority of studies conducted both in anal specimens and uterine cervix in the population of women living with HIV in Brazil [4,7,16,21,22] and in the world [23,24], which demonstrate a high prevalence of high-risk HPV in women co-infected with HIV and HPV. This suggests a difference in the distribution of various types of HPV in Brazil, according to the geographical region evaluated and demonstrates that knowledge of the distribution of HPV types in different regions of Brazil and the world is of great importance, especially with regard to the composition of the HPV future vaccine.

Among the oncogenic high-risk HPV we find only the HPV-16, HPV-58 and HPV-59, not having been identified HPV-18, different from those found in annal samples from other regions of Brazil, like Rio de Janeiro [25], Maranhão [16], and also in cervical samples [4,8]. The fact that HPV-18 was not identified does not necessarily mean a low circulation of this type in Pará, which may have been underestimated due to the small sample size. However, some studies have already demonstrated the predominance of HPV-58 over HPV-16 and HPV-18 outside of Brazil [26].

Some studies have shown that T CD4+ lymphocytes count of less than 350 cells/mm^3 is a risk factor for HPV infection [7,21], including infection by high-risk types, which was also observed in the present study. However, we did not find any association between HPV infection and HIV viral load, similar to what has been observed in other studies (15,27].

The majority of studies on the prevalence of HPV infection in women living with HIV/AIDS in Brazil involves samples of uterine cervix and demonstrates the association of HPV with changes in the cytopathologic findings and with cervical cancer. Comparatively, the prevalence of HPV infecting the cervix of the uterus in women with HIV in Rio de Janeiro [28] and in Brasília [29], the Southeast and Center West of Brazil, was also much higher than that found in the present study. However, it is also necessary to study this agent in the anal region of these patients, with a view to the association of HPV with anal cancer, which is even more important in this specific population, since some studies indicate an association between the low count of T CD4+ lymphocytes and the progression of HPV infection [7].

We pose some limitations in the present study, first of all the non-detection of multiple infections by HPV due to the methodology used, in spite of that the achieved results had presented an overview of the types of HPV circulating in northern Brazil. In our study, the low prevalence of HPV infection observed has no relation with the small sample size, considering that several other studies in Brazil with anal specimen and uterine cervix had fewer participants and presented a higher prevalence of HPV infection than the one we described [2,16,30]. Accordingly, we may infer preliminarily that the prevalence of HPV in women living with HIV in the town of Belém is one of the lowest in Brazil, nevertheless, it is necessary a study with a greater sample size to ascertain this finding.

Conclusion

In conclusion, we observed a low prevalence of HPV infection in HIV-infected women in the city of Belém, Pará, with also low detection of HPV types of high oncogenic risk. The presence of co-infection HIV-HPV was associated with the number of T CD4+ lymphocytes. It is worth noting that anal HPV testing is not a routine practice in the public health system in the state of Pará and, in this way, it becomes necessary to access and the monitoring of HPV infection not only in samples of uterine cervix, but also in the anal specimens, which can be performed when the patient is performing the cervical cancer preventive exam on basic health units in the state, which can significantly contribute to the prevention of anal cancer and cervical cancer in this population.

Conflict of Interest: None declared.

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Author Contributions: All the authors equally contributed in this article.

Abbreviations: HPV: human papillomavirus, STIs: sexually transmitted infections, HIV: human immunodeficiency virus, HR: high risk, LR: low risk, HUJBB: Hospital Universitário João de Barros Barreto, URE-DIPE: Unidade de Referência em Doenças Infecciosas e Parasitárias, PCR: polymerase chain reaction.

Ethical approval: This study was approved by Ethics and Research Committee of Hospital Universitário João de Barros Barreto under protocol number 200856879. Written informed consent was obtained from all participants prior to enrollment.

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