Prevalence and multiplicity of HPV in HIV women in Minas Gerais, Brazil

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Summary

Objective: To detect the frequency and subtypes of HPV in the uterine cervix of HIV-infected women. **Methods:** Sample consisted of 288 HIV-infected women, recruited from the public health system of five cities of Minas Gerais, Brazil. Women were seen from August 2003 to August 2008. Cervical samples were collected for cytological analysis and for HPV DNA detection, using polymerase chain reaction (PCR). HPV DNA was classified according to its oncogenic potential in low risk (types 6, 11) and high risk (types 16, 18, 31, 33, 35). Colposcopy was performed, followed by cervical biopsy when necessary. Categorical variables were compared using the Chi-squared test, with a significance level established at the 5% level. **Results:** HPV prevalence was 78.8%. Most frequent genotypes were HPV-6 (63.9%) and HPV-16 (48.5%). High-risk HPV were observed in 70.5% of the women; low-risk in 71.4%; both high and low-risk HPV were detected in 55.1% of the patients. Multiple HPV genotypes were detected in 64.8% of the patients; two genotypes in 23.8%, and three in 18.9%. **Conclusion:** HPV prevalence was high among HIV-infected women. Multiple HPV genotypes were common in samples from the uterine cervix of HIV-infected women.

Keywords: DNA probes, HPV; HIV; polymerase chain reaction; cervix uteri; papillomavirus infections, prevalence.

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Submitted on: 12/18/2010
Approved on: 05/19/2011

Financial Support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)

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Conflict of interest: None.

Resumo

Prevalência e multiplicidade do HPV em mulheres infectadas pelo HIV em Minas Gerais

Objetivo: Detectar a frequência e os subtipos do HPV na cérvice uterina de mulheres infectadas pelo HIV. **Métodos:** A amostra era composta por 288 mulheres infectadas pelo HIV, recrutadas do sistema público de saúde de cinco cidades de Minas Gerais, Brasil. As mulheres foram avaliadas de agosto de 2003 a agosto de 2008. Amostras cervicais foram coletadas para análise citológica e para detecção do HPV DNA, usando a reação em cadeia de polimerase (PCR). O HPV DNA foi classificado de acordo com seu potencial oncogênico em baixo risco (tipos 6,11) e alto risco (tipos 16, 18, 31, 33, 35). Foi realizada colposcopia, seguida de biópsia cervical, quando indicada. Variáveis categóricas foram comparadas usando o teste do quiquadrado, com nível de significância estabelecido de 5%. **Resultados:** A prevalência do HPV foi 78,8%. Os genótipos mais frequentes foram HPV-6 (63,9%) e HPV-16 (48,5%). Genótipos de HPV de alto risco foram observados em 70,5% das mulheres; de baixo risco em 71,4%; HPV de alto e baixo risco foram detectados em 55,1% das pacientes. Múltiplos genótipos de HPV foram detectados em 64,8% das pacientes; dois genótipos em 23,8%, e três em 18,9%. **Conclusão:** A prevalência do HPV foi alta entre mulheres infectadas pelo HIV. Múltiplos genótipos de HPV foram comuns em amostras da cérvice uterina destas mulheres.

Unitermos: Sondas DNA HPV; **HIV; reação em cadeia da polimerase; colo do útero; infecções** por papillomavirus; prevalência.

Introduction

The association between cervical cancer and human immunodeficiency virus (HIV) is well established[1,2]. Several studies have shown that HPV infection is significantly more common among HIV positive women compared to HIV negative women[3-10].

The association between cervical cancer and human papillomavirus (HPV) has also been demonstrated, but the potentially modifying role of HIV on the HPV/cervical intraepithelial neoplasia association is still under investigation[11]. Current data suggest that HIV infection increases HPV persistence, with increased risk for intraepithelial cervical lesions[2,4].

Published data strongly suggest that HIV-infected women are more likely to have HPV DNA, as well as multiple viral types and a higher frequency of the oncogenic types[6-8]. Similar findings have been reported in different regions of Brazil[9,12-14] and HPV 16 was the most frequent oncogenic type[7-9].

Polimerase chain reaction (PCR) has the highest sensitivity for detecting HPV DNA genotypes and discriminates multiples infections better than other biomolecular methods. It also has good specificity and results are quickly available[2].

The aim of this study was to estimate the prevalence of HPV DNA infection in HIV-infected women from different cities. We also aimed to investigate the prevalence of HPV genotypes, as well as the frequency of multiple types of HPV.

Methods

Patients

Women attending a Reference and Training Center on Infectious and Parasitic Diseases in Belo Horizonte (*Hospital das Clínicas, Universidade Federal de Minas Gerais - UFMG*) and public health units of general gynecological care of four other cities of Minas Gerais State (Betim, Barbacena, Conselheiro Lafaiete and Divinópolis) were randomly chosen to be enrolled in the study. This study was conducted from August 2003 to August 2008.

A standardized questionnaire was used for collecting demographic, social, and behavioral characteristics of the sample, as well as for obtaining the medical history. Gynecological exam was performed and cervical samples were collected for Pap smear, as well as for HPV-DNA, by doctors from the group study to guarantee a standardized collection. Blood samples were collected to obtain T CD4+ cells count and HIV viral load. Colposcopy, biopsy and adequate treatment of cervical lesions were performed as part of the routine medical care.

Inclusion criteria were: HIV infection diagnosed by ELISA and confirmed by indirect immunofluorescence or western blot, with or without AIDS; age ≥ 18 years; consent to participate (signature of the approved informed consent form). Exclusion criteria were: difficulties in obtaining information (language barrier, disorientation); non-analyzable samples; pregnancy; history of hysterectomy.

The study protocol was approved by the UFMG Research Ethics Committee.

Procedures

Cervical samples for DNA-HPV exams were obtained using an Ayre's spatula, and transferred to a sterile tube containing 2 mL of saline solution (0.09%). The tube was conditioned in a special box with cooled gel. The samples were sent to a reference laboratory (*Núcleo de Ações e Pesquisas em Apoio Diagnóstico,* Medical School, UFMG), within 24 hours of the procedure.

DNA was extracted using Chelex 100 chelating resin (BioRad), according to the manufacturer's protocol[15]. Specific primers for HPV DNA types 6, 11, 16, 18, 31, 33 and 35 were tested.

Polymerase chain reaction and analysis criteria

In order to control for DNA quality, the globin gene was amplified in all samples[16]. Globin-negative samples were excluded from the study. HPV detection by PCR was carried out in a nested-PCR system, using MY09/11[17] and GP5+/6+[18] primers. Positive samples were tested with specific primers for HPV DNA types 6, 11, 16, 18, 31, 33 and 35, through independent reactions[15]. All products were submitted to agarose gel electrophoresis 2%, treated with ethidium bromide and analyzed under UV light. DNA was classified according to its oncogenic potential in low risk (6, 11) and high risk (16, 18, 31, 33 and 35).

Statistical analysis

Data were transferred to a specially designed database using Excel 2003. Demographic and social variables included were: age (in years) at enrollment; age of first sexual intercourse; lifetime number of sexual partners; marital status; working status; smoking; condom use; route of transmission; and drug addiction. Clinical and laboratory variables were: CDC classification (1993)[19]; antiretroviral regimen use; colposcopy; CD4+ T-lymphocytes cell count; HIV-1 viral load. Frequency and type of HPV genotypes (6, 11, 16, 18, 31, 33 and 35) were also analyzed.

The Epi-Info software version 3.3.2 was used for the statistical analysis. Proportions were compared with the Chi-squared test. Statistical significance was established at the 5% levels. Sample size was determined based on published findings (prevalence of HPV in HIV-infected women ranging from 52 to 87%)[9,14]. The prevalence of 50% was used in order to obtain a minimum sample that might guarantee more precise statistical tests. To achieve 80% of power, we estimated the sample at 278 participants.

Results

The sample consisted of 288 women from five different cities: A (n = 152; 52.8%); B (n = 36; 12.5%); C (n = 20; 6.9%); D (n = 44; 15.3%); and E (n = 36; 12.5%). As can be seen in Figure 1, general prevalence of HPV infection was high (78.8%) among these HIV-infected patients. There was no significant difference among HPV prevalence of women from these different cities (p = 0.19).

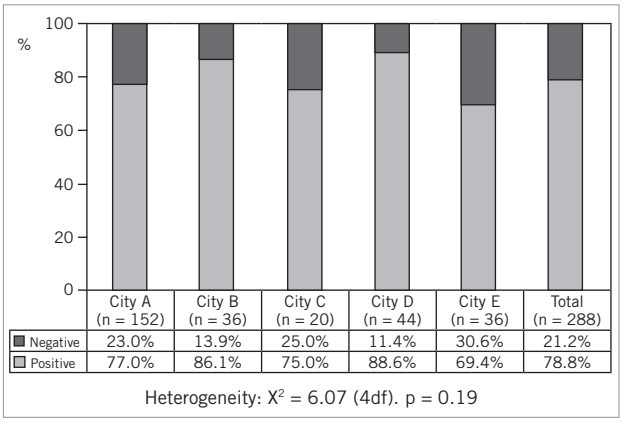

Figure 1 – DNA-HPV detection among women from five different cities in Minas Gerais, Brazil.

Median age at enrollment was 35 years; median age of first sexual intercourse was 17 years; median number of lifetime sexual partners was three. Regarding the clinical and behavioral characteristics, most participants were in a stable union (44.1%), worked at home (51.7%) and were infected through the sexual route (92.0%). Few women smoked (31.3%), used condom (42.7%) and were addicted to drugs (0.7%). Half of them had AIDS[19] and 67.7% were using antiretroviral drugs. Normal colposcopy was found in 63.2% of them. Table 1 summarizes some of the clinical and behavioral characteristics of these women.

It was detected that 151 patients presented a viral load higher than the established cut-off value of 400 copies/mL (minimum limit of detection of RNA HIV at the time of the study). Among them, in 123 (81.5%) was detected the DNA HPV. On the other hand, 119 patients had undetected viral load (≤ 400 copies/mL) and 90 (75.6%) of them presented the DNA HPV. The differences were not significant (p = 0,2441).

Furthermore, a cut in the T CD4+ cell count in 200 cells/mm³ was made based on the CDC classification of AIDS and non-AIDS[19]. Amongst 45 patients presenting T CD4+ cell count < 200 cells/mm³, 37 (82.2%) had the

Table 1 – Clinical and behavioral characteristics of women

Clinical and behavioral characteristics	City A (n = 152)	City B (n = 36)	City C (n = 20)	City D (n = 44)	City E (n = 36)	Overall (n = 288)	p*
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
CDC classification							0.46
AIDS	74 (48.7)	17 (47.2)	13 (65)	21 (47.7)	19 (52.8)	144 (50)	
Not AIDS	75 (49.3)	19 (52.8)	5 (25)	20 (45.5)	17 (47.2)	136 (47.2)	
Missing	3 (2)	0 (0)	2 (10)	3 (6.8)	0 (0)	8 (2.8)	
Antiretroviral drugs							0.67
Use	101 (66.4)	22 (61.1)	14 (70)	32 (72.7)	26 (72.2)	195 (67.7)	
Not use	51 (33.6)	14 (38.9)	5 (25)	11 (25)	10 (27.8)	91 (31.6)	
Missing	0 (0)	0 (0)	1 (5)	1 (2.3)	0 (0)	2 (0.7)	
Colposcopy							0.68
Normal	99 (65.1)	19 (52.8)	12 (60)	30 (68.2)	22 (61.1)	182 (63.2)	
Abnormal	50 (32.9)	16 (44.4)	8 (40)	14 (31.8)	13 (36.1)	101 (35.1)	
Missing	3 (2)	1 (2.8)	0 (0)	0 (0)	1 (2.8)	5 (1.7)	
Condom							0.23
Use	60 (39.5)	13 (36.1)	7 (35)	22 (50)	21 (58.3)	123 (42.7)	
Not use	84 (55.3)	23 (63.9)	13 (65)	22 (50)	15 (41.7)	157 (54.5)	
Missing	8 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)	8 (2.8)	
Route of HIV acquisition							0.23
Sexual	140 (92.1)	31 (86.1)	19 (95)	39 (88.6)	36 (100)	265 (92)	
Blood	0 (0)	1 (2.8)	0 (0)	1 (2.3)	0 (0)	2 (0.7)	
Missing	12 (7.9)	4 (11.1)	1 (5)	4 (9.1)	0 (0)	21 (7.3)	

*X² test (4 df).

Table 2 – Frequency and multiplicity of HPV genotypes in cervical samples of 227 HPV-infected women

HPV Frequency and multiplicity	City A (n = 117)		City B (n = 31)		City C (n = 15)		City D (n = 39)		City E (n = 25)		All cities (n = 227)	
	n	%	n	%	n	%	n	%	n	%	n	%
No genotyping	13	11.1	3	9.7	0	0.0	2	5.1	12	48.0	30	13.2
Genotyping	104	88.9	28	90.3	15	100	37	94.9	13	52	197	86.8
HPV-6	82	70.1	23	74.2	8	53.3	31	79.5	1	4.0	145	63.9
HPV-11	39	33.3	10	32.3	6	40.0	14	35.9	2	8.0	71	31.3
HPV-16	60	51.3	17	54.8	5	33.3	26	66.7	2	8.0	110	48.5
HPV-18	7	6.0	2	6.5	1	6.7	2	5.1	1	4.0	13	5.7
HPV-31	10	8.5	2	6.5	9	60.0	4	10.3	6	24.0	31	13.7
HPV-33	25	21.4	6	19.4	8	53.3	11	28.2	6	24.0	56	24.7
HPV-35	48	41.0	15	48.4	6	40.0	17	436	3	12.0	89	39.2
Simple infection	24	20.5	7	22.6	4	26.7	7	17.9	8	32.0	50	22.0
Multiple infection	80	68.4	21	67.7	11	73.3	30	76.9	5	20.0	147	64.8
2 types	32	27.4	6	19.4	4	26.7	9	23.1	3	12.0	54	23.8
3 types	23	19.7	8	25.8	2	13.3	9	23.1	1	4.0	43	18.9
4 types	13	11.1	4	12.9	2	13.3	8	20.5	1	4.0	28	12.3
≥ 5 types	12	10.3	3	9.7	3	20.0	4	10.3	0	0.0	22	9.7

HPV, human papillomavirus; PCR, polimerase chain reaction; HIV, human immunodeficiency virus.

DNA HPV. On the other hand, 226 patients had T CD4+ cell count ≥ 200 cells/mm³ and 177 (78.3%) of them presented the DNA HPV. The differences were not significant either (p = 0.5574).

High-risk HPV (patient showing at least one high-risk HPV type) was observed in 70.5% (160/288) of patients; low-risk HPV (patient showing at least one low-risk HPV type) was found in 71.4% (162/288) of women; and both high and low-risk HPV were detected in 55.1% (125/288) of them. The prevalence of HPV was higher (61.7%) in patients who had three or more sexual partners. Nevertheless, this result was not significant (p = 0.8431).

Table 2 displays the prevalence of each genotype as well as the proportion of women with multiple HPV genotypes. Most prevalent genotypes were HPV-6 (63.9%) and HPV-16 (48.5%). Differences emerged among cities. HPV-31 and HPV-33 genotypes were the most prevalent in City C (60% and 53.3%) and City E (24% for both). Multiple genotypes were present in 64.8% of women: two (23.8%) and three (18.9%) different genotypes were common. This table also shows that the percentage of patients with HPV which could not be typed varied from 0% to 11.1%. One of the cities (City E) presented the highest percentage of non-identified HPV (48%).

Discussion

Prevalence of HPV infection was high (78.8%) among our sample of HIV-infected patients recruited at five different cities. These findings are consisted with other studies[6,14,20].

Median age (35 years old) was similar to the age of a distinct sample in Brazil[14,21]. That suggests that HPV/HIV coinfection is common among women of reproductive age.

Although the median age for the first sexual intercourse was 17, dispersion was high and some women had first sexual intercourse as early as at the age of 10, as previously described[8].

Sexual transmission was the predominant route of HIV acquisition (92%), at higher rates than previously reported[22]. Condom use was not a common practice, consistently used by only 42.7% of the partners. Similar percentage has been previously reported in a sample of 75 HIV-infected patients[22].

Data obtained from a metanalysis from different countries of North America, Africa, Asia, Europe and South/Central America evaluated prevalence of HPV and genotypes among 5.578 HIV-infected patients. South and North America (Brazil and Mexico) contributed 7.8% of the sample. HPV prevalence was higher among women from Brazil and Mexico (57.3%), compared to overall findings (36.5%). According to this metanalysis, HPV prevalence was associated with advanced cervical lesion. Multiple infections were frequent, and HIV-positive patients with HPV-16 were more likely to have advanced cervical lesions[7].

In addition, studies conducted in Brazil[9] and India[8] found that HPV-16 was the most common genotype (30.9% and 33% respectively) among HIV-infected women. We found that HPV-6 was the most prevalent genotype (63.9%), followed by HPV-16 (48.5%). Other studies found percentages ranging from 52% [6] to 87%[9].

The proportion of patients with non-identified HPV was 13.2%, with the highest frequency from City E (48%). The small number of types investigated in addition to the great variety of types present in these patients may explain differences among reported prevalences[23,24]. We did not test all genotypes of HPV, what is one of the limitations of our study. Besides, comparison among cities was merely descriptive, as the sample was not calculated for this purpose and the goal was only to detect the frequency and subtypes of HPV in the uterine cervix of HIV-infected women from different cities of Minas Gerais.

Number of sexual partners and alcohol consumption were the most significant risk factors for HPV infection, followed by young age and lower income in a sample of 225 Greek women attending a gynecological outpatient clinic[25]. On the contrary, we did not find any association between HPV prevalence and risk factors, such as unprotected sexual intercourse, number of sexual partners, HIV viral load and T CD4+ cell count, likely reflecting power limitations. Furthermore, we did not have access to longitudinal data for immunological status and HIV viral load.

According to our results the higher the HIV viral load was the more prevalent (81.5%) the HPV. However, these findings were not statistically significant ($p > 0.05$). There was a trend among patients with T CD4+ cell count < 200 cells/mm^3, to present a higher rate of HPV infection (82.2%). Once more the result was not significant ($p > 0.05$). Most likely the follow-up of HIV-infected women – including PCR for detecting HPV on the cervix uteri associated with T CD4+ cells count and viral load quantification – could allow better evaluation between HPV cervical infection and low immunity in these patients[11].

An important finding was the high prevalence of multiple HPV types[7,9], which was found in 64.8% of women. Different percentages of multiple HPV genotypes were found by other authors – 36%[26], 45%[9] and 52%[6] – likely explained by different methods of HPV genotyping, as well as different primers used for detection.

Our results differ from the findings of Cuschieri *et al.*[26] These authors performed PCR in 3,444 patients, and HPV infection was found in only 20% of participants. Multiple infections with at least one high risk HPV genotype were found in 23.3% (164/705) of cases, low risk HPV in 0.8% (6/705) and both high and low risk HPV in 19.3% (136/705), rates much lower than ours. Nonetheless, the lack of stratification for HIV status may explain this discrepancy.

The presence of multiple HPV genotypes can be explained by the reactivation of latent HPV types with superimposed new infection, driven by unprotected sexual intercourse and also perhaps by the long period of data collection of our study. They may also reflect failure of immunological response. We evaluated the T CD4+ cell counts, but did not measure local immune response. However, the mechanism of the HIV/HPV association can be only speculated at this time.

Conclusion

Although our study assessed women from a single state, data may reflect the situation of HIV-infected women in Brazil overall. We confirmed the high prevalence of HPV and of multiple HPV infections in samples from the uterine cervix of HIV-infected women. Our data also demonstrated the importance of studying subpopulations in order to plan adequate preventive programs.

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