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Sexually Transmitted Infections

Detection of four human polyomaviruses (MCPyV, HPyV6, HPyV7 and TSPyV) in cervical specimens from HIV-infected and HIV-uninfected women

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INTRODUCTION

Cervical cancer is the third most common female cancer worldwide and the fourth leading cause of cancer death in women. Persistent infection of the uterine cervix with oncogenic high-risk types of human papillomavirus (HR-HPV), particularly HPV16, is recognized as the cause for the development of precancerous cervical lesions that may progress to invasive cancer.¹ However, only a minority of women infected with HR-HPV develops premalignant and malignant lesions, suggesting that cofactors are involved in HR-HPV-associated carcinogenesis.

Polyomaviridae is a continuously growing family of viruses with up to 11 new human representatives recently added to the previously described BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV).² Human polyomaviruses (HPyVs) subclinically infect the general population and may induce disease in immunocompromised patients.² Moreover, these viruses have the potential capacity to induce tumors through their tumor antigen (T-Ag) oncoproteins.² The oncogenic capabilities of HPyVs were recently illustrated with the discovery of the Merkel cell polyomavirus (MCPyV), which is now commonly accepted as the etiologic agent of the Merkel cell carcinoma, a rare but aggressive skin cancer.³

Several studies have investigated the presence of HPyVs in different tissues, and their presence in the female genital tract may suggest an association between HPyVs and precancerous or cancerous cervical lesions, as illustrated by the detection of BKPyV, JCPyV and MCPyV genomic sequences in cervical cancer cases.⁴⁻⁷

In order to study more deeply the relationships between HPyVs, cervical lesions and immune status, we have investigated the presence of recently described HPyVs including MCPyV, HPyV6, HPyV7 and the Trichodysplasia spinulosa polyomavirus (TSPyV) in cervical samples from African and French women, in relation to HR-HPV co-infection, cervical disease stage, and HIV serostatus.

METHODS

A total of 190 cervical samples collected with the Digene cervical sampler and placed in the specimen transport medium (STM) (Qiagen, Gaithersburg, MD) were analysed. One hundred and forty samples were collected from a subset of women with a median age of 35.4 years (range, 21-47 years) enrolled in the HARP (HPV in Africa Research Partnership) study, which is conducted in two Sub-Saharan African countries, South Africa and Burkina Faso, with the aim to evaluate cervical cancer screening approaches in HIV-1 infected African women. In addition, 50 samples were collected from randomly selected HIV-seronegative women with a median age of 40.0 years (range, 25-68 years) attending a gynaecological outpatient department at the Montpellier University Hospital, France, who were tested for HR-HPV infection. Cervical biopsies were obtained from all of the African women who had a positive HR-HPV test and/or abnormal cervical cytology and/or abnormal visual inspection using acetic acid and Lugol's iodine, and these women were selected to provide similar proportions of women with high-grade lesions (CIN2+) and women with normal histological findings or low-grade lesions (\leq CIN1). In the group of French women, biopsies were performed only in case of abnormal cytological findings.

Written informed consent was obtained from the women. Ethical approval was granted from Ministry of Health in Burkina Faso (no. 2012-12-089), the Witwatersrand University in South Africa (no. 110707), and the London School of Hygiene and Tropical Medicine (no. 7400).

HR-HPV DNA was detected using the Hybrid Capture 2 assay (Qiagen) with subsequent HPV genotyping using the INNO-LiPA HPV Genotyping Extra assay (Furijebio, Les Ulis, France).

DNA was extracted from STM using the NucliSens® EasyMag® extraction kit (Biomérieux, Craponne, France) with the NucliSens® EasyMag® automated extractor. Detection of MCPyV, HPyV6, HPyV7 and TSPyV DNA was performed by real-time PCR using specific

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3 primers. Quantification of MCPyVs DNA was normalized to the amount of whole genomic
4 DNA as previously described.⁸ For the other viruses, semi-quantification was based on the
5 cycle threshold value (Ct) and samples with Ct>40 were considered as negative.
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9 Proportions were compared using the chi-square test or Fisher's exact test, and the MCPyV
10 DNA levels were compared using the Mann-Whitney U test.
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RESULTS

HR-HPV DNA was detected in cervical specimens from 124 (88.6%) HIV-1-positive African women and from 24 (48.0%) HIV-negative French women. HPV16 DNA was detected in cervical specimens from 36 (27.8%) African women and 6 (12.0%) French women. CIN2+ lesions were found in 65% (91/140) of the selected African women and 2% (1/50) of the French women.

Overall, MCPyV, HPyV6, HPyV7 and TSPyV DNA was detected in 105 (55.3%), 6 (3.2%), 4 (2.1%), and 0 (0%) samples, respectively. MCPyV DNA loads were low, with a median MCPyV copy number of 80 copies/10⁶ cells (range 14 to 210 copies/10⁶ cells), the lower limit of detection (LLD) being 10 copies/10⁶ cells. Median MCPyV DNA load was 79 copies/10⁶ cells in women without CIN2+ lesions and 83 copies/10⁶ cells in women with CIN2+ lesions (p=0.75). HPyV6 and HPyV7 DNA levels were always weakly detected in the positive samples, with cycle thresholds ranging from 34 to 40.

As shown in Table 1, there was no association between the detection of MCPyV DNA and HIV serostatus, the detection of HR-HPV, or the presence of CIN2+ cervical lesions. HPyV6 (n=6) and HPV7 (n=4) were detected in 4 and 4 HIV-infected African women and 2 and 0 HIV-negative French women, respectively (p=1.00). The small sample size did not allow for sufficient statistical power to validly analyse the association of HPyV6 and HPyV7 detection with HR-HPV infection and cervical lesions.

Table 1 Associations between MCPyV detection and geographic origin/HIV serostatus, HR-HPV infection and cervical lesions

Parameter	MCPyV status		OR (95% CI)	P
	Positive	Negative		
	(N=105) n (%)	(N=85) n (%)		
HIV				
Positive* (n=140)	79 (56.4)	61 (43.6)	1.20 (0.60-2.40)	0.588
Negative (n=50)	26 (52.0)	24 (48.0)		
HR-HPV				
Positive (n=148)	81 (54.7)	67 (45.2)	0.91 (0.43-1.91)	0.781
Negative (n=42)	24 (57.1)	18 (42.9)		
HPV16				
Positive (n=45)	20 (44.4)	25 (55.6)	0.56 (0.27-1.17)	0.095
Negative (n=145)	85 (58.6)	60 (41.4)		
Cervical histology				
CIN2+ (n=92)	54 (58.7)	38 (41.3)	1.31 (0.71-2.42)	0.356
≤CIN1 (n=98)	51 (52.1)	47 (47.9)		

*All African women were HIV-1-positive and all French women were HIV-negative

CIN2+, cervical intraepithelial neoplasia grade 2 or more severe

DISCUSSION

This study, involved two very different populations of women in terms of geographic origin, HIV status, prevalence of HR-HPV infection and precancerous cervical lesions. Overall, MCPyV DNA was frequently detected at low levels in cervical specimens, whereas HPyV6 and HPyV7 were rarely detected, and TSPyV was not detected. MCPyV DNA detection was not associated with HIV status, HR-HPV infection or precancerous cervical lesions. Moreover, MCPyV DNA levels were not different between women with or without precancerous lesions. The sample size of HPyV6/HPyV7 was too small for useful statistical comparisons.

These results, which do not support a role of these human polyomaviruses in the development of cervical lesions, are in agreement with a recent study reporting a high frequency of MCPyV detection in cervical carcinomas but no statistically significant association between MCPyV infection and cervical cancer.⁷ These observations argue against a possible role of MCPyV as a cofactor of HPV-induced carcinogenesis. Interestingly, the detection rates of MCPyV, HPyV6, HPyV7 and TSPyV in cervical specimens observed in this study are similar to those reported in normal skin specimens, these viruses being considered as belonging to the skin microbiota.^{9,10} This observation suggests that MCPyV and, to a lesser extent, HPyV6 and HPyV7 might belong to the female genital tract microbiota. The transmission routes of these viruses and their mode of propagation to the female genital tract remain to be elucidated, and the hypothesis of genital mucosa contamination by skin viruses via hands or sexual activity cannot be excluded.

Key messages

- ▶ MCPyV is frequently detected at low levels in cervical samples, whereas HPyV6, HPyV7 and TSPyV are rarely or not detected.
- ▶ MCPyV detection is not associated with HIV status, HR-HPV infection or precancerous cervical lesions.
- ▶ Polyomaviruses might belong to the female genital tract microbiota.

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