Int. J. Cancer: **122,** 244–246 (2008)

SHORT REPORT

Human papillomavirus types in women with invasive cervical carcinoma by HIV status in Kenya

Hugo De Vuyst[1,2*], Peter Gichangi[3], Benson Estambale[4], Eliud Njuguna[5], Silvia Franceschi[1] and Marleen Temmerman[2]

[1]*International Agency for Research on Cancer, Lyon, France*
[2]*Department of Obstetrics and Gynecology, Ghent University, Ghent, Belgium*
[3]*Department of Human Anatomy and Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya*
[4]*Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya*
[5]*Radiotherapy Unit, Kenyatta National Hospital, Nairobi, Kenya*

To evaluate the fraction of invasive cervical carcinoma (ICC) that could be prevented in HIV-infected women by vaccines currently available against human papillomavirus (HPV)16 and 18, we conducted a cross-sectional study in women with ICC in Nairobi, Kenya. Fifty-one HIV-positive women were frequency-matched by age to 153 HIV-negative women. Cervical cells were tested for HPV DNA using polymerase chain reaction-based assays (SPF10-INNO-LiPA). Comparisons were adjusted for multiplicity of HPV types. As expected, multiple-type infections were much more frequent in HIV-positive (37.2%) than in HIV-negative (13.7%) women, but the distribution of HPV types was similar. HPV16 was detected in 41.2% *versus* 43.8% and HPV16 and/or 18 in 64.7% *versus* 60.1% of HIV-positive *versus* HIV-negative women, respectively. The only differences of borderline statistical significance were an excess of HPV52 (19.6% *versus* 5.2%) and a lack of HPV45 (7.8% *versus* 17.0%) in HIV-positive women compared to HIV-negative women, respectively. We have been able to assess an unprecedented number of ICCs in HIV-positive women, but as we did not know the age of HIV acquisition, we cannot exclude that it had occurred too late in life to affect the type of HPV involved in cervical carcinogenesis. However, if our findings were confirmed, they would suggest that the efficacy of current vaccines against HPV16 and 18 to prevent ICC is similar in HIV-positive and HIV-negative women, provided vaccination is administered before sexual debut, as recommended.

Key words: HIV; cervical cancer; human papillomavirus; polymerase chain reaction; Africa

The main cause of invasive cervical carcinoma (ICC) is infection with high-risk human papillomavirus (HPV), most notably HPV16 and 18, which have been detected in approximately 70% of ICC in all world regions.[1] Newly developed vaccines have been shown to be highly efficacious in preventing infection with these HPV types and routine vaccination of adolescent women is now being recommended.[2] Women infected with HIV are at increased risk of HPV infection[3] and development of precancerous and cancerous lesions of the cervix.[4] Furthermore, a large systematic review of HIV-positive women with normal cytology, low-grade and high-grade intraepithelial lesions has shown that HPV16 is relatively under-represented when compared to HIV-negative women.[3] This difference has been attributed to the fact that clearance of HPV16 is less dependent on an individual's immune status than clearance of other high-risk types, as shown in HIV-positive women.[5] Information on HPV types in ICC has been reported, however, in only 14 HIV-positive women.[3] We have therefore carried out a comparison of the distribution of HPV types in ICC in women with and without HIV in Kenya, a country hit hard by the HIV epidemic (www.unaids.org/en/Regions_Countries/Countries/kenya.asp).

Methods

Between January 2000 and March 2002, we identified and obtained informed consent from 367 women with ICC (*i.e.*, 96% of those eligible) who presented themselves for treatment at the Kenyatta National Hospital, Nairobi, Kenya.[6] HIV testing was performed using enzyme-linked immunosorbent assay (ELISA, Biochem Immuno Systems Kit, Montreal, Canada) and double ELISA (Biotech, Cambridge, Ireland) for confirmation. Exfoliated tumor cells were collected using a Cervex brush (Rovers Medical Devices, Oss, the Netherlands) and stored at −20°C in phosphate buffered saline. Fifty-three women (14.4%) were HIV-positive, of whom 51 (mean age: 40.1; standard deviation: 11.0) had an adequate cell sample and were included in the present analysis. CD4 count was available for 41 women, of whom 20 had a CD4 count <500/μL, and 7 a CD4 count <200/μL. HIV-positive women were frequency-matched by age to 153 HIV-negative women (mean age: 45.0; standard deviation: 12.4). Medical records and histological diagnosis were revised by one of the authors (Peter Gichangi). The vast majority of ICC was squamous cell carcinoma (95.1% and 92.6%, respectively, in women with and without HIV).

Samples were shipped to the Laboratory of Virology, Ghent University Hospital, Belgium, where HPV DNA was isolated by incubating the samples with proteinase K.[7] Broad-spectrum HPV DNA amplification was performed with a short polymerase chain reaction (PCR) fragment (SPF10) primer set.[8] The PCR product was analyzed and genotyped directly on an HPV genotype Line Probe Assay[8] (INNO-LiPA Version 2, Innogenetics, Ghent, Belgium), which detects 24 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74). A double-control line (generic probe) was present on the LiPA strip for confirmation of any mucosal HPV type. HPV amplimers which did not hybridize to any type-specific probe, were assigned HPV genotype X (uncharacterized type). Each experiment was also controlled with separate positive and negative PCR controls.

The ethics and research committee of the Kenyatta National Hospital, the University of Nairobi, and the International Agency for Research on Cancer approved the study.

Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of being positive for specific HPV type(s) by HIV status were computed separately among women positive for single-type and multiple-type infections, and overall, after adjustment for multiplicity of types, using the Mantel-Haenzel test.

Results

Figure 1 shows the distribution of HPV types in single- and multiple-type infections found among HIV-positive and HIV-neg-

Grant sponsor: Flemish Interuniversity Council (VLIR); Grant sponsor: Bill & Melinda Gates Foundation; Grant number: 35537.
***Correspondence to:** International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon cedex 08, France. Fax: +33-4-72-73-83-45. E-mail: devuysth@iarc.fr or hdvuyst@yahoo.co.uk.
Received 12 April 2007; Accepted after revision 14 June 2007
DOI 10.1002/ijc.23045
Published online 31 August 2007 in Wiley InterScience (www.interscience.wiley.com).

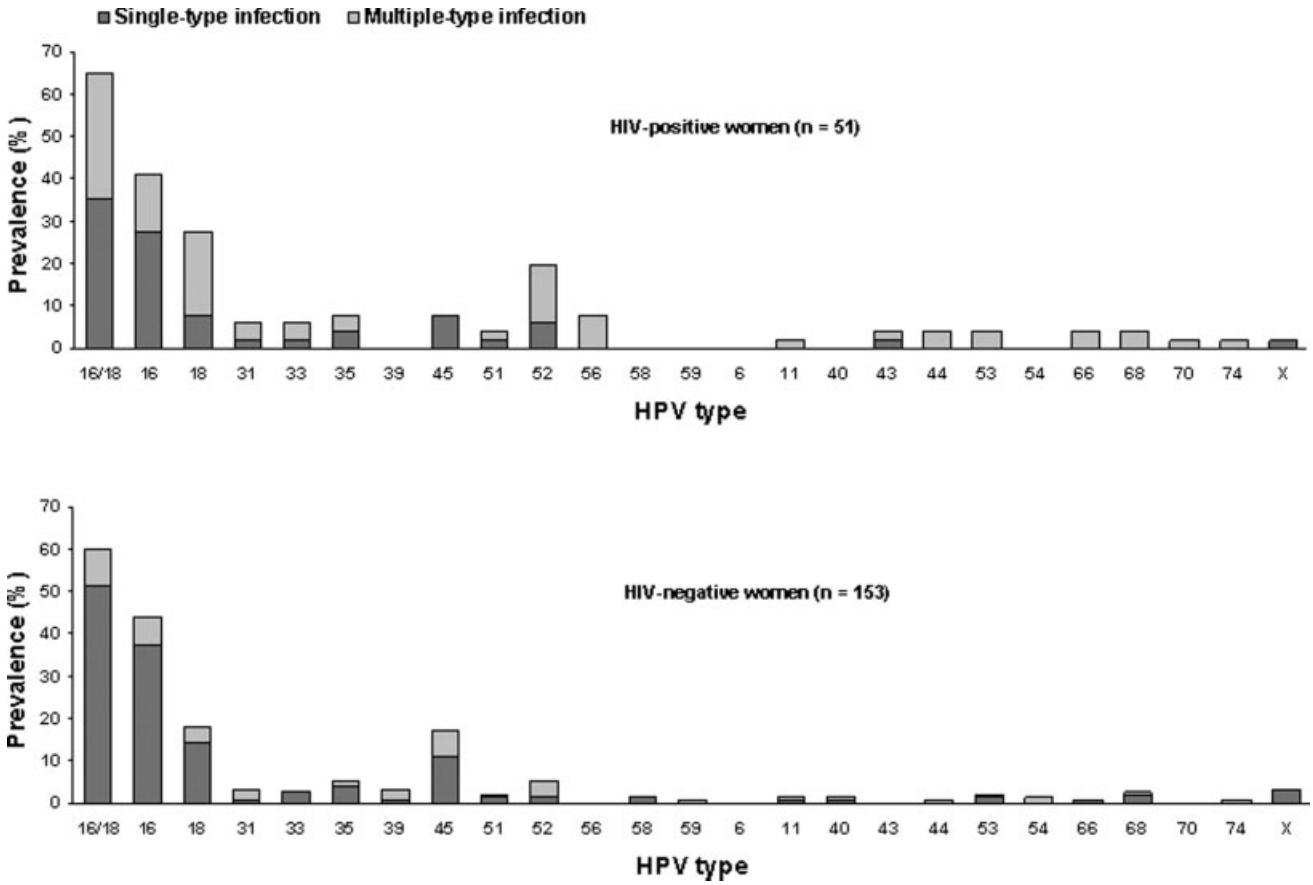

FIGURE 1 – Prevalence of HPV types in single- and multiple-type infections. Nairobi, Kenya, 2000–2002.

TABLE I – ODDS RATIOS (ORS) OF INFECTION WITH SPECIFIC HUMAN PAPILLOMAVIRUS (HPV) TYPE(S) AMONG HIV-POSITIVE COMPARED TO HIV-NEGATIVE WOMEN[1] BY MULTIPLICITY OF INFECTION, AND OVERALL (NAIROBI, KENYA, 2000–2002)

HPV type(s)	Percent of women infected						Overall OR (95% CI)[2]
	Single-type infections			Multiple-type infections			
	HIV+ (N = 32)	HIV− (N = 127)	OR (95% CI)	HIV+ (N = 19)	HIV− (N = 21)	OR (95% CI)	
16	43.8	44.9	1.0 (0.4–2.1)	36.8	47.6	0.6 (0.2–2.3)	0.9 (0.4–1.7)
18	12.5	17.3	0.7 (0.2–2.2)	52.6	23.8	3.6 (0.9–14.9)	1.3 (0.6–3.0)
16 and/or 18	56.3	62.2	0.8 (0.4–1.7)	79.0	61.9	2.3 (0.5–9.9)	1.0 (0.5–2.0)
31	3.1	0.8	4.1 (0.2–68.1)	10.5	19.1	0.5 (0.1–3.2)	0.9 (0.2–3.6)
39	0.0	0.8	0	0.0	19.1	0	0
35	6.3	4.7	1.3 (0.3–7.0)	10.5	9.5	1.1 (0.1–9.1)	1.2 (0.3–4.6)
45	12.5	13.4	0.9 (0.3–3.0)	0.0	42.9	0	0.4 (0.1–1.0)
52	9.4	1.6	6.5 (1.0–42.0)	36.8	28.6	1.5 (0.4–5.6)	2.3 (0.8–6.5)
X	3.1	3.9	0.8 (0.1–7.0)	–	–	–	–

CI, confidence interval.
[1]Five HPV-negative women are not included.–[2]Adjusted by multiplicity of HPV infection.

ative women. As expected, multiple-type infections were much more frequently detected in HIV-positive (37.2%) than in HIV-negative women (13.7%). More than 2 HPV types were detected in 7 HIV-positive and 5 HIV-negative women (data not shown). HPV16 was the type detected most frequently in both groups (41.2% of HIV-positive and 43.8% of HIV-negative women). HPV16 and/or 18 was found in 64.7% *versus* 60.1%, respectively. The next most frequent types were HPV52 (19.6%), 35, 45 and 56 (7.8% each) among HIV-positive women, and HPV45 (17.0%), 35 and 52 (5.2% each) among HIV-negative women. Type X was detected in 1 HIV-positive and 5 HIV-negative women. Five women were HPV-negative, all of whom were also HIV-negative.

Table I shows the ORs for the detection of the most common HPV types among HIV-positive when compared to HIV-negative women. No significant differences in HPV type distribution by HIV status emerged in either single- or multiple-type infections or overall. Differences of borderline statistical significance emerged for HPV52 (excess) and HPV45 (lack) among HIV-positive women. For HPV52, but not for HPV45, a difference clearly emerged between HIV-positive and HIV-negative women with single-type infections.

Discussion

Our present study, by far the largest comparison of HPV types in ICCs in women with and without HIV carried out to date,[3] suggests that there is no substantial difference in the relative importance of HPV16 and 18 by HIV status. The slight excess of

HPV52 among HIV-positive women with ICC is of some interest as it is clearly present in single-type infections and had already emerged in HIV-positive women with high-grade squamous intraepithelial lesions.[3]

The comparison of HPV type distribution by HIV status is substantially complicated by the greater frequency of multiple-type infections in HIV-positive women. We have, however, carried out our HPV testing using a validated assay that has been shown to be very sensitive in detecting multiple-type infections,[9] and we have confirmed our findings after stratification and adjustment for multiple infection. Nonetheless, half of HPV16 and/or 18 infections occur concurrently with another HPV type in HIV-positive women (Fig. 1), and it is not known whether the remaining high-risk HPV types would have caused invasive disease in the absence of HPV16 or 18.

A major weakness of our present study is the lack of information on year of seroconversion among HIV-positive women. CD4 count, although not always available, suggested that half had a CD4 count of $\geq$500/μL, which would suggest a relatively recent HIV infection. As the pathogenesis of persistent HPV into ICC takes at least a decade, we should ideally focus on women who have acquired an oncogenic HPV type after HIV infection and have survived long enough to develop ICC. It is thus possible that HIV infection had occurred in our study women too late in life to affect the HPV type involved in cervical carcinogenesis, and that, as a result, our present findings are biased towards a lack of difference. Therefore, confirmation on larger series of ICC among women who are known or likely to have been infected with HIV at an early age is needed.

Acknowledgements

We thank Dr. Salvatore Vaccarella for statistical analysis, Ms. Jayne Mbithi for performing the HPV testing and Ms. Regina Kilonso for HIV counselling.

References

1. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.
2. Saslow D, Castle PE, Cox JT, Davey DD, Einstein MH, Ferris DG, Goldie SJ, Harper DM, Kinney W, Moscicki AB, Noller KL, Wheeler CM, et al. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin 2007;57:7–28.
3. Clifford GM, Goncalves MA, Franceschi S, HPV and HIV Study Group. Human papillomavirus types among women infected with HIV: a meta-analysis. AIDS 2006; 20:2337–44.
4. Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst 2000;92: 1500–10.
5. Strickler HD, Palefsky JM, Shah KV, Anastos K, Klein RS, Minkoff H, Duerr A, Massad LS, Celentano DD, Hall C, Fazzari M, Cu-Uvin S, et al. Human papillomavirus type 16 and immune status in human immunodeficiency virus-seropositive women. J Natl Cancer Inst 2003;95: 1062–71.
6. Gichangi PB, Bwayo J, Estambale B, De Vuyst H, Ojwang S, Rogo K, Abwao H, Temmerman M. Impact of HIV infection on invasive cervical cancer in Kenyan women. AIDS 2003;17:1963–8.
7. Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, ter Harmsel B, Quint W. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;153:1731–9.
8. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel B, Burger M, Quint W. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.
9. Iftner T, Villa LL. Chapter 12: human papillomavirus technologies. J Natl Cancer Inst Monogr 2003;80–8.