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Comparison of HPV DNA testing in cervical exfoliated cells and tissue biopsies among HIV-positive women in Kenya

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Abstract

HIV-positive women are infected with human papillomavirus (HPV) (especially with multiple types), and develop cervical intraepithelial neoplasia (CIN) and cervical cancer more frequently than HIV-negative women. We compared HPV DNA prevalence obtained using a GP5+/6+ PCR assay in cervical exfoliated cells to that in biopsies of 468 HIV-positive women from Nairobi, Kenya. HPV prevalence was higher in cells than biopsies and the difference was greatest in 94 women with a combination normal cytology/normal biopsy (prevalence ratio, PR=3.7; 95% confidence interval, CI: 2.4–5.7). PR diminished with the increase in lesion severity (PR in 58 women with high-grade squamous intraepithelial lesions (HSIL)/CIN2-3 =1.1; 95% CI: 1.0–1.2). When HPV-positive, cells contained 2.0-to-4.6 fold more multiple infections than biopsies. Complete or partial agreement between cells and biopsies in the detection of individual HPV types was found in 91% of double HPV-positive pairs. The attribution of CIN2/3 to HPV16 and/or 18 would decrease from 37.6%, when the presence of these types in either cells or biopsies was counted, to 20.2% when it was based on the presence of HPV16 and/or 18 (and no other types) in biopsies. In conclusion, testing HPV on biopsies instead of cells results in decreased detection but not elimination of multiple infections in HIV-positive women. The proportion of CIN2/3 attributable to HPV16 and/or 18 among HIV-positive women, which already appeared to be lower than that in HIV-negative, would then further decrease. The meaning of HPV detection in cells and random biopsy from HIV-positive women with no cervical abnormalities remains unclear.

Keywords

HIV; cervical neoplasia; human papillomavirus; cervical exfoliated cells; cervical tissue biopsy; Africa

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INTRODUCTION

Assignment of human papillomavirus (HPV) type to individual cervical lesions is essential to understand the biology of different HPV types; the efficacy of HPV vaccines, and which types should be included in HPV detection assays[1,2] In particular, high-risk (hr) HPV infection is detected in the vast majority of high-grade cervical intraepithelial neoplasia (CIN) 2 and 3, and HPV16 and 18 are implicated in over half of HPV-positive CIN2/3.[1] Genital multiple-type infections are however frequent in women with and without precancerous and cancerous lesions[1,2], notably in HIV-positive women who are at increased risk to acquire HPV infection, develop a persistent HPV infection, and progress to CIN2/3 or invasive cervical carcinoma (ICC).[3,4]

Comparison of cytological and histological samples can help to distinguish the broad range of HPV types, including low-risk types, that are present in exfoliated cervico-vaginal cells from the fewer that are detected in cervical tissue biopsies and are likelier to include the causal type.[5] Colposcopy-guided biopsies can detect the presence of HPV types in the worst-looking area of the cervix[6] but random biopsies can also identify lesions and HPV infections in an apparently normal cervix.[7]

Here we describe the detection of different HPV types in paired samples of cervical exfoliated cells and formalin-fixed paraffin-embedded tissue biopsies that were collected at the same study visit in HIV-positive women who participated in a cervical cancer screening study in Nairobi, Kenya. Our aims are to compare: 1) the prevalence and agreement in HPV type and multiple infections between cervical exfoliated cells and biopsies, and 2) the influence of reliance on either or both two types of samples on the assignment of a CIN2/3 lesion to HPV16 and/or 18.

MATERIALS AND METHODS

Participants and study procedures

HIV-positive women who attended the Coptic Hope Center for Infectious Diseases, Nairobi, Kenya in 2009 to receive HIV-related care were invited to participate in a study on cervical cancer screening methods. They were eligible if they were: between 18 and 55 years of age; not screened in the last year; and never treated for cervical cancer or pre-cancerous lesions.[8–10] In total, 498 women were included.

After obtaining a written informed consent, information on clinical and lifestyle characteristics of study women was collected. A medical examination was performed and biological specimens were taken. Briefly, cervical exfoliated cells (hereafter for brevity referred to as cells) were collected by a nurse using a Cervex-Brush (Rovers Medical Devices, Oss, the Netherlands) and smeared on a glass slide for conventional cytological examination. The brush was then placed in PreservCyt medium (Hologic, Marlborough, MA, USA) for HPV testing. A medical doctor performed a colposcopic examination and took a biopsy from all women, either from the colposcopically worst-looking area on the cervix or, if no lesion was visualized, at 12 o'clock (i.e., the most frequent location of cervical lesions). Biopsy tissues were immediately immersed in 10% buffered formalin and transported to the pathology laboratory the same day.

The study protocol was approved by the Ethical Review Committees of the Kenyatta National Hospital, Kenya; the University of Washington, USA; and the International Agency for Research on Cancer, France.

Cytology and histology

Cytological slides and biopsies were processed by staff under the supervision of the study pathologist (Dr. Farzana Rana) at the Aga Khan University, Nairobi, who also read all the cytological and histological slides. Cytological slides were processed within a week and reported according to the Bethesda 1991 revised classification.[11] Formalin removal and paraffin embedding of the biopsies were performed with strict adherence to the appropriate procedures within 24 hours after arrival in the laboratory.[12] The formalin-fixed and paraffin-embedded tissue blocks that remained after the preparation of histology slides were stored and sent to the Department of Pathology of the Vrije University Medical Centre, Amsterdam, The Netherlands, where new tissue cuts were made for HPV DNA testing and for a separate histological assessment. Fourteen blocks were missing, and hence these cases were excluded. The Amsterdam histological assessment was used for this report, except for 13 'indeterminate' results, of which 11 had a valid histological diagnosis on local biopsy slides (three normal results, five CIN1 and three CIN2). Two cases that were 'indeterminate' on both local assessment and in Amsterdam were excluded.

HPV DNA testing

HPV DNA testing was performed on cervical exfoliated cells and tissue biopsies at the Department of Pathology at the Vrije University Medical Center, Amsterdam, The Netherlands. DNA was extracted from the PreservCyt specimen using magnetic beads (Macherey-Nagel, Dueren, Germany) on a robotic system (Hamilton, Germany), according to the manufacturer's instructions. Biopsies were sectioned using a 'sandwich' approach, whereby inner sections were destined for HPV testing and outer sections for histological examination. One or more 5-μM sections representing approximately one cm2 of tissue were predigested with proteinase K after which DNA was extracted. Beta-globin PCR analysis was performed in order to assess the DNA quality. The presence of HPV DNA was first determined using general primer GP5+/6+-mediated PCR.[13] PCR products were hybridized using an enzyme immunoassay (EIA) that included two oligoprobes: one for hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 and another for low-risk HPV types 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 67, 69, 70, 71, 72, 73, 81, 82/mm4, 82/is39, 83, 84, 85, 86, 89 and 90. Subsequent HPV typing was performed by reverse-line blot hybridisation of PCR products, as described previously.[14] HPV types of IARC classification group 1 "carcinogenic to humans" (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and group 2A "probably carcinogenic to humans" (HPV68) were considered as high-risk types.[15] All other HPV types were considered low-risk types. Specimens that were positive at EIA, but did not reveal any known genotype, were classified as uncharacterized types. No cells specimens were beta-globin negative. Twenty-four biopsies were beta-globin negative but positive for HPV testing and were kept in the current analysis. Conversely, 12 biopsies that were negative on both tests were excluded from this report.

Statistical analysis

The prevalence of HPV infection (any type; hrHPV types; and multiple types) in cells was compared with the prevalence in biopsies from the same woman using prevalence ratios (PRs) and corresponding 95% confidence intervals (CIs) of HPV. In order to account for the individual matching of the two samples, we used a generalized estimating equations (GEE) model for binary outcome with a log link (as opposed to the routine logistic model) and an exchangeable correlation structure. In order to take into account the strengths and weaknesses of different types of samples (i.e., the greater reliability of histological diagnosis but also severe dependence on sampling area), PRs were stratified by cervical status defined as a composite cytological and histological classification of study women into: a) normal findings in both cells and biopsy; b) any finding in cells and CIN1 in biopsy (or atypical

squamous cells of undetermined significance or worse with normal biopsy, n=98); c) cells <HSIL and biopsy CIN2/3; and d) cells HSIL and biopsy CIN2/3. Two cases were excluded due to undetermined cytology combined with CIN2/3 histology.

Agreement in the detection of individual HPV types in paired HPV-positive samples was classified as "complete" if all types detected in the two samples were the same, or "partial" if extra types were found in cells or biopsy. "Discordant" pairs were those in which cells and biopsy included no overlapping types. Finally, five possible strategies were used to establish the proportion of CIN2/3 attributable to HPV16 and/or 18. Assignment to HPV types was alternatively based on: a) cells; b) biopsy; c) biopsy or, in case of a HPV-negative biopsy, cells; d) either cells or biopsy regardless of whether any of the two was negative; and e) positivity for HPV16 and/or 18 infection in biopsy in the absence of other types.

RESULTS

The mean age of 468 women included in this report was 38.2 years (5%-95% percentiles: 27–51). Cervical biopsy was normal in 204 women (43.6%), CIN1 in 155 women (33.1%); CIN2 in 49 (10.5%); and CIN3 in 60 (12.8%). No ICCs were found.

Table 1 shows a comparison of the prevalence of HPV (any type; hrHPV types; and multiple types) in cervical exfoliated cells and biopsies by cervical status. HPV prevalence steadily increased from normal cytology/normal biopsy to HSIL/CIN2-3 women in both cells and biopsies. The difference in HPV prevalence between cells and biopsies was greatest in normal/normal women (PR = 3.7; 95% CI: 2.4–5.7) and any/≤CIN1 (PR = 2.3; 95% CI: 1.9–2.7). The similarity in HPV prevalence in cells and biopsies increased with the severity of cytological and histological findings, PR in cells *versus* biopsies was 1.4 (95% CI: 1.1–1.7) among <HSIL/CIN2-3 women and 1.1 (95% CI: 1.0–1.2) among HSIL/CIN2-3 women. Similar results were found when analyses were restricted to hrHPV (Table 1). HPV-positive cells contained 2.0-to-4.6 fold more multiple infections than HPV-positive biopsies depending on cervical status and the smallest difference was found among HSIL/CIN2-3 women (PR = 2.0; 95% CI: 1.4–2.9). Findings were similar in separate strata by CD4 count and combination antiretroviral therapy use (data not shown).

Table 2 shows the agreement for the presence of any HPV type in cells and biopsies by cervical status and overall. Overall agreement was 65.2% but it increased from 52.1% in normal/normal to 89.7% in HSIL/CIN2-3 women. The percent of double HPV-positivity increased from 16.0% in normal/normal women to 87.9% in HSIL/CIN2-3 women. The vast majority of discordant pairs contained HPV in cells only (n=157), whereas HPV in biopsy only was found in ≤2% in all cervical status strata.

HPV type-specific agreement was evaluated in 161 pairs in which both cells and biopsy were HPV-positive (Table 3). Complete agreement of HPV type detection was found in 55 pairs (34.2%). In 46.6% of pairs partial agreement with extra types in cells was found. In 8.7% of women there was a complete discordance in HPV type detection.

Table 4 shows the number and percentage of CIN2/3 attributable to HPV16 and/or 18 according to different strategies in descending order. Percentage was 37.6% based on the presence of HPV16 and/or 18 in either cells or biopsy. It slightly declined when only cells (35.8%) or biopsy and cells (in case of HPV-negative biopsy) (32.1%) were used. Relying only on the presence of HPV16 and/or 18 in biopsies or exclusively on biopsies in which no types other than HPV16 and 18 were detected led to fractions of 27.5% and 20.2%, respectively (Table 4).

DISCUSSION

Our study is the first to compare systematically the detection of HPV infection in paired samples of cervical exfoliated cells and biopsies in HIV-positive women. As expected,[4] the prevalence of HPV infection and cervical precancerous lesions among HIV-positive women was high. HPV prevalence was significantly higher in cells, compared to biopsies among women without CIN2/3 but similar among women with CIN2/3. Multiple infections were 2-to-5-fold more often detected in cells than biopsies. The smallest difference between the two samples, but also the highest prevalence of multiple infections in biopsies was found among women in whom high-grade lesions were detected in both cells and biopsy. Complete or partial agreement in the detection of individual HPV types was found in the majority of double HPV-positive pairs, with little variation by cervical status. Attribution of CIN2/3 to HPV16 and/or 18 ranged between 20.2% and 37.6% depending on the way HPV findings from cells and biopsies were used separately or in combination.

More frequent detection of HPV infection and multiple HPV types in cells than in biopsies is not surprising as exfoliated cells can come from a wide genital area including the whole cervix, vagina and vulvar opening whereas biopsies are restricted to a small portion of the cervix, i.e., the area supposed to contain the relevant lesion. The presence of CIN2/3 in a biopsy greatly diminished differences in HPV findings between the two samples especially if HSIL had been concurrently detected in cells. The especially strong disagreement in HPV-positivity and prevalence of multiple infections in women without CIN2/3 may be explained by a high proportion of transient rather than persistent infections in CIN1 lesions.[5] In addition, colposcopical identification of “the worst” cervical area is sometimes inaccurate and a single random biopsy in the absence of visible lesions is especially likely to miss HPV-infected areas.

HPV infection was detected in biopsies from 16 women (17%) in whom no abnormalities were found in either cells or biopsy. Among them, only one woman harboured HPV infection in random biopsy but not in cells. It is unclear whether the presence of HPV infection in normal cervical tissue is increased in HIV infection as similar data on HIV-negative women without cervical abnormalities are very limited. De Sanjose et al.[16] compared HPV findings obtained using GP5+/GP6+ in cells and frozen biopsies in 99 HIV-negative middle-age women with a normal cervix, but the majority of women (80%) were HPV-negative.[16] One cell-negative woman was HPV-positive at biopsy while two biopsy-negative women were HPV-positive in cells.

Other studies reporting on the agreement in HPV detection in cells and biopsies in HIV-negative women were restricted to women with cervical abnormalities.[5,17,18] In agreement with our study, multiple infections were always detected more frequently in cells than in biopsies. However, the studies used HPV assays with different sensitivity compared to the test we used, and, in some instances, used different HPV assays for cells and biopsies,[5,17] which had an impact on the comparison between cells and biopsies in those studies. Quint et al.[18] compared HPV-prevalence in exfoliated cells and formalin-fixed biopsies from 174 women with CIN1-3 using ultra-sensitive SPF10 PCR. They found no significant differences in HPV prevalence in exfoliated cells and biopsies (92% *vs.* 94%, respectively) and a similar distribution of individual HPV types. Gravitt et al.[17] compared paired exfoliated cells obtained with cervico-vaginal lavage and formalin-fixed biopsies from 146 women with CIN1-3. MY09/11 and SPF_{10} PCR assays were used, respectively, on cells and biopsies. They reported a slightly higher HPV prevalence by cells than biopsies (99.3% *vs.* 92.5%, respectively). The prevalence of HPV16 and/or 18, however, was similar.[17]

Laser capture micro-dissection (LCM) with high resolution genotyping is the most accurate technique for assigning HPV type to an area of CIN.[5,19] When multiple HPV types were present in the whole-tissue section, association of a single type with a discrete area of CIN was found for 93% (372/399) of LCM fragments analysed with SPF_{10} PCR/$LiPA_{25}$.[19] However, rarely detected colliding CIN lesions can be associated with multiple HPV types. In another study LCM was applied to 13 women with high-grade lesions and genotyping was repeated, in each woman, on cytology and four different areas of the cervix.[5] Four women had multiple HPV types in their biopsies but only one had two different types in morphologically distinct but colliding lesions on LCM. Interestingly, HPV16 was identified as the causal type in all women with HPV16 in cytology.[5]

Major strengths of our study include the large number of women whose diagnosis ranged from normal cervix to CIN3 and availability for all women of a biopsy taken at the same time of cells collection. A PCR assay more sensitive than GP5+/GP6+ and more robust to DNA degradation in formalin-fixed paraffin-embedded tissue blocks (e.g., SPF10),[5,17,18] would have detected more infections and multiple infections in biopsies. The use in our study of a strict protocol for tissue biopsy preparation and the exclusion of beta-globin negative/HPV negative biopsies should have improved, however, the quality of biopsy findings. Although we found a substantial agreement for the presence of at least one common HPV type in HPV-positive pairs of cells and biopsies (93.8%) among women with CIN2/3, conclusions about the attribution of CIN2/3 to HPV16 and/or 18 were influenced by different reliance on results from cells and biopsies. Attribution to HPV16 and/or 18 ranged from 20.2% when it was based on the detection of HPV16 and/or 18 in biopsies in the absence of any other type to 37.6% when detection in either cells or biopsy was taken into account. Of note, our upper threshold is somewhat lower than the pooled prevalence of HPV16 and 18 in a meta-analysis of HIV-positive women with HSIL or CIN2/3 (31.9% and 12.9%, respectively), but no information on whether both types were present.[4] Our upper threshold is, however, similar to the proportion of cervical high-grade lesions (HSIL and/or CIN2/3) associated with HPV16 and/or 18 in African studies that excluded women known to be HIV-positive (36.9%, the lowest among all world regions).[1]

In conclusion, our present study shows that testing HPV on biopsies instead of cells results in decreased detection but not elimination of multiple infections in HIV-positive women. Relying exclusively on biopsies decreases the proportion of CIN2/3 presumably attributable to HPV16 and/or 18 among HIV-positive women, but accurate assignment of HPV type would require a LCM-based study. The meaning of HPV detection in cells and random biopsy from HIV-positive women with no evidence of cervical lesions is also, for the moment, unclear.

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Abbreviations used

CI confidence interval

CIN	cervical intraepithelial lesion
EIA	enzyme immunoassay
GEE	generalized estimating equations
HPV	human papillomavirus
hr	high-risk
HSIL	high-grade squamous intraepithelial lesion
ICC	invasive cervical carcinoma
PR	prevalence ratio

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Novelty and impact of the paper

Attribution of cervical lesions to an individual HPV type in HIV-positive women is hampered by the high proportion of multiple infections. Our study is the first to compare systematically HPV detection in paired cervical exfoliated cells and biopsies samples. HPV testing on biopsies instead of cells diminished but did not eliminate the high prevalence of multiple infections. Exclusive reliance on biopsies decreased the proportion of CIN2/3 attributable to vaccine-preventable HPV16 and/or 18 in HIV-positive women.

TABLE 1

Prevalence ratio (PR) of human papillomavirus (HPV) infection (any, high-risk and multiple types) in cervical exfoliated cells *versus* biopsies by cervical status. Kenya 2009

Cervical status		N	Any HPV		hrHPV		Multiple types	
Cyto	Histo		Cells: biopsy N (%): N (%)	PR	Cells: biopsy N (%): N (%)	PR	Cells: biopsy N (%): N (%)	PR
Norm	Norm	94	59 (62.8): 16 (17.0)	3.7 (2.4–5.7)	37 (39.4): 11 (11.7)	3.4 (2.0–5.6)	30 (50.8): 2 (12.5)	3.9 (1.1–14.4)
Any	≤CIN1[a]	265	167 (63.0): 74 (27.9)	2.3 (1.9–2.7)	120 (45.3): 49 (18.5)	2.5 (2.0–3.1)	92 (55.1): 16 (21.6)	2.7 (1.7–4.2)
<HSIL	CIN2/3	51	42 (82.4): 31 (60.8)	1.4 (1.1–1.7)	37 (72.6): 26 (51.0)	1.4 (1.1–1.8)	28 (66.7): 5 (16.1)	4.6 (2.0–10.6)
HSIL	CIN2/3	58	56 (96.6): 52 (89.7)	1.1 (1.0–1.2)	52 (89.7): 47 (81.0)	1.1 (1.0–1.2)	39 (69.6): 18 (34.6)	2.0 (1.4–2.9)
Overall		468	324 (69.2): 173 (37.0)		246 (52.6): 133 (28.4)		189 (58.3): 41 (23.7)	

[a] Including normal biopsies only in combination with any cytological abnormality.

CIN: cervical intraepithelial neoplasia; hr: high-risk; HSIL: high-grade squamous intraepithelial lesion.

TABLE 2

Agreement in human papillomavirus (HPV) positivity (any type) between paired cervical exfoliated cells specimens and biopsies by cervical status.Kenya 2009

Cervical status		N	Any HPV				
Cyto	Histo		Cells+/biopsy+ N (%)	Cells+/biopsy- N (%)	Cells-/biopsy+ N (%)	Cells-/biopsy- N (%)	Agreement (%)
Norm	Norm	94	15 (16.0)	44 (46.8)	1 (1.1)	34 (36.2)	52.1
Any	≤CIN1[a]	265	71 (26.8)	96 (36.2)	3 (1.1)	95 (35.8)	62.6
<HSIL	CIN2/3	51	30 (58.8)	12 (23.5)	1 (2.0)	8 (15.7)	74.5
HSIL	CIN2/3	58	51 (87.9)	5 (8.6)	1 (1.7)	1 (1.7)	89.7
Overall		468	167 (35.7)	157 (33.5)	6 (1.3)	138 (29.5)	65.2

[a]Including normal biopsies only in combination with any cytological abnormality.

CIN: cervical intraepithelial neoplasia; HSIL: high-grade squamous intraepithelial lesion.

TABLE 3

Agreement in detection of individual human papillomavirus (HPV) types between cervical exfoliated cells and biopsies[a], by cervical status. Kenya 2009

Cervical status		N	HPV, individual types				
Cyto	Histo		Complete agreement N (%)	Partial agreement (extra types in cells) N (%)	Partial agreement (extra types in biopsy) N (%)	Partial agreement (extra types in both) N (%)	All discordant N (%)
Norm	Norm	14	4 (28.6)	6 (42.9)	1 (7.1)	0 (0.0)	3 (21.4)
Any	≤CIN1[b]	67	21 (31.3)	31 (46.3)	2 (3.0)	7 (10.4)	6 (9.0)
<HSIL	CIN2/3	30	11 (36.7)	13 (43.3)	1 (3.3)	2 (6.7)	3 (10.0)
HSIL	CIN2/3	50	19 (38.0)	25 (50.0)	2 (4.0)	2 (4.0)	2 (4.0)
Overall		161[c]	55 (34.2)	75 (46.6)	6 (3.7)	11 (6.8)	14 (8.7)

[a] Only pairs in which both cells and biopsy were HPV-positive.

[b] Including normal biopsies only in combination with any cytological abnormality.

[c] 6 women with uncharacterized HPV types are not included.

CIN: cervical intraepithelial neoplasia; HSIL: high-grade squamous intraepithelial lesion.

TABLE 4

Number and percentage of CIN2/3 (N=109) attributable to HPV16 and 18 according to different strategies. Kenya 2009.

Strategy	CIN2/3 attributable to HPV16/18	
	No n (%)	Yes n (%)
Cells or biopsy	68 (62.4)	41 (37.6)
Cells only	70 (64.2)	39 (35.8)
Biopsy or, if biopsy was HPV-negative, cells	74 (67.9)	35 (32.1)
Biopsy only	79 (72.5)	30 (27.5)
Biopsy, no types other than HPV16/18	87 (79.8)	22 (20.2)

CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus.