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Open Forum Infectious Diseases

MAJOR ARTICLE

Detection and Concentration of Plasma Aflatoxin Is Associated With Detection of Oncogenic Human Papillomavirus in Kenyan Women

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Background. Cervical cancer is common in Kenyan women. Cofactors in addition to infection with oncogenic human papillomavirus (HPV) are likely to be important in causing cervical cancer, because only a small percentage of HPV-infected women will develop this malignancy. Kenyan women are exposed to dietary aflatoxin, a potent carcinogen and immunosuppressive agent, which may be such a cofactor.

Methods. Demographics, behavioral data, plasma, and cervical swabs were collected from 88 human immunodeficiency virus-uninfected Kenyan women without cervical dysplasia. Human papillomavirus detection was compared between women with or without plasma aflatoxin B1-lysine (AFB1-lys) and evaluated in relation to AFB1-lys concentration.

Results. Valid HPV testing results were available for 86 women (mean age 34.0 years); 49 women (57.0%) had AFB1-lys detected and 37 (43.0%) had none. The AFB1-lys detection was not associated with age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex. The AFB1-lys detection and plasma concentrations were associated with detection of oncogenic HPV types.

Conclusions. The AFB1-lys positivity and higher plasma AFB1-lys concentrations were associated with higher risk of oncogenic HPV detection in cervical samples from Kenya women. Further studies are needed to determine whether aflatoxin interacts with HPV in a synergistic manner to increase the risk of cervical cancer.

Keywords. aflatoxin; Kenyan women; oncogenic human papillomavirus.

Oncogenic types of human papillomavirus (HPV) ("high-risk" [HR-HPV]) are the causative agents of cervical cancer [1–3]. Cervical cancer is one of the most common malignancies in women living in sub-Saharan African countries, including Kenya [4]. The incidence rate (15 per 100 000 women per year) and mortality rate (12 per 100 000 women per year) of cervical cancer in Kenya far exceed rates for women living in the United States (4 and 1 per 100 000 women per year, respectively) [5]. The reasons why some but not all women develop malignant consequences of HR-HPV infection are poorly understood. Cofactors are likely to be important: women who are infected with human immunodeficiency virus (HIV) have a higher prevalence of HR-HPV infection and a higher incidence of HPV-associated malignancies compared with HIV-uninfected women [6–9]. However, although HIV may account for much of the high incidence of cervical cancer in Kenya, additional cofactors are likely to play a role, and HIV-uninfected Kenyan women continue to suffer from a high burden of cervical cancer.

Another potentially important cofactor for cervical cancer is chronic ingestion of aflatoxin. Contamination of corn crops by aflatoxin, a mycotoxin produced by *Aspergillus* species, is a food safety and security issue, particularly for people living in developing countries with temperate and tropical climates [10]. Chronic aflatoxin exposure is associated with a high incidence of hepatocellular carcinoma, one of the major cancers in men living in sub-Saharan Africa, Southeast Asia, China, and South Korea [11–14]. Little is known about the effects of aflatoxin on other human cancers.

Aflatoxins are also immunosuppressive agents. It is possible that the immunosuppression caused by aflatoxin ingestion could lead to poor immune control of oncogenic HPV infections and thus persistence and increased detection in cervical samples.

Received 15 May 2019; editorial decision 18 July 2019; accepted 5 August 2019.
Presented in part: 2019 ASCO Meeting, June 2019, Chicago, IL.
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DOI: 10.1093/ofid/ofz354

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Therefore, a study was conducted to determine whether aflatoxin exposure in Kenyan women was associated with increased detection of oncogenic HPV in cervical samples.

METHODS

Study Population

Women were enrolled from September 2015 to October 2016 at the Academic Model Providing Access to Healthcare (AMPATH) Cervical Cancer Screening Program (CCSP) at Moi Teaching and Referral Hospital (MTRH) in Eldoret in a prospective cohort study conducted to investigate biological, behavioral, and environmental factors that contribute to the risk of oncogenic HPV detection among women during 4 years of follow-up. Women aged 18 to 45 years living within 30 km of Eldoret presenting for screening at the CCSP were asked to participate if they had a normal visual inspection with acetic acid (VIA) that day. A total of 285 women were approached for participation in the overall project on which the current analysis was based; 223 gave consent and were enrolled. The HIV status was not available for 1 woman, and cervical samples from 2 women were inadequate based on negative β-globin control results. Therefore, these 3 women were excluded from the study, leaving 220 women in the cohort, including 115 HIV-infected and 105 HIV-uninfected women. Plasma obtained at enrollment was available for 88 of 105 HIV-uninfected women. No blood sample was available for the remaining 17 women. Two of these 88 women had inadequate swab samples for HPV testing (based on β-globin testing), leaving the final number of 86 women included in this analysis.

Interview and Questionnaire

Structured face-to-face interviews of enrollees by trained researchers were conducted at enrollment to capture social, behavioral, and biological information, including age, marital status, educational level, home ownership, walking distance to the local clinic, number of lifetime sexual partners, age of first sex, percentage of condom-protected coital events, number of lifetime pregnancies, and history of cervical cancer screening.

Sample Collection

At enrollment, a nurse or physician collected a cervical swab for HPV testing as part of the pelvic examination and inspection of the cervix. Swabs were placed in standard transport media and then frozen at −80°C in the AMPATH Reference Laboratory. Plasma was collected and frozen at −20°C at the same laboratory.

Human Papillomavirus Testing

Specimens were transported on dry ice to the Kenya Medical Research Institute (KEMRI)-University of Massachusetts Medical School laboratory for processing and subsequent genotyping. The Roche Linear Array was used to determine HPV types (Roche Molecular Systems, Inc., Branchburg, NJ) as previously described [15]. Human papillomavirus 16-positive, HPV 16-negative, and human β-globin (used to assess specimen adequacy) controls provided by the manufacturer were tested with each batch of samples.

Individual HPV types were determined, and, in addition, HPV types were grouped into HR-HPV and "low-risk" HPV based on (1) the designation in the Roche Linear Array instructions or (2) HR-HPV types as designated by the International Agency for the Research on Cancer [16]. Human papillomavirus types were further grouped into A9 and A7 types [17]. The specific HPV types included in each of these groups are detailed under Results.

Aflatoxin-Albumin Adduct (Aflatoxin B1-Lysine) Detection in Plasma Samples

Plasma aflatoxin B1-lysine (AFB_1-lys) was measured at the Department of Environmental Health and Engineering of the Johns Hopkins Bloomberg School of Public Health, using a minor variation of the method reported by McCoy et al [18]. Serum (150 μL) was spiked with an internal standard (0.5 ng of AFB1-d4-lysine in 100 μL), combined with Pronase (EMD Millipore, Billerica, MA) protease solution (3.25 mg in 0.5 mL phosphate-buffered saline), and incubated for 18 hours at 37°C. Solid-phase extraction-processed samples (Oasis MAX columns; Waters, Milford, MA) were analyzed with ultra-high pressure liquid chromatography (UHPLC)-isotope dilution mass spectrometry on a Thermo Fisher Scientific (San Jose, CA) system composed of a Vanquish UHPLC and a TSQ Quantis triple quadrupole mass spectrometer in positive electrospray ionization mode [19, 20]. The limit of quantification (<20% coefficient of variation) was 14 pg AFB1-lys/mL serum.

Statistical Analysis

Demographic and behavioral characteristics of participants were compared between women with and without detectable AFB_1-lys in plasma using *t* test, Wilcoxon rank test, or χ^2 test as appropriate. Logistic regression models were fit to examine (1) associations between HPV detections and plasma AFB_1-lys detection (yes vs no) and (2) associations between HPV detections and plasma AFB_1-lys concentrations. Demographic and behavioral characteristics including age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex (vaginal intercourse) were included in all logistic regression models as potential confounders. All analyses were performed using SAS (Cary, NC) version 9.4.

Ethics Considerations

Study approval was granted from the local review board at MTRH and Moi University (Eldoret, Kenya), the KEMRI's

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Scientific and Ethics Review Unit, and the Institutional Review Board of Indiana University.

RESULTS

Overall Characteristics of Participants and Aflatoxin Detection

Of 86 women with an available plasma sample and valid HPV testing results, 49 (57.0%) had detectable AFB_1-lys in plasma and 37 (43.0%) had no detection of AFB_1-lys (Table 1). Detection of AFB_1-lys in plasma samples was not associated with age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex (Table 1).

Associations of Aflatoxin B1-Lysine (AFB_1-lys) Detection and Plasma AFB_1-lys Concentration With Human Papillomavirus Detections

Substantial variation existed in plasma AFB_1-lys concentrations among the 49 women with detected aflatoxin in plasma, ranging from 0.015 to 0.209 pg/μL (data not shown). The counts and percentages of HPV detections in women with and without plasma AFB1-lys detection, and N, median (interquartile range [IQR]) of plasma AFB1-lys concentration (pg/μL) for women with and without HPV detections are shown in Supplementary Table 1.

As shown in Table 2, logistic regression analysis indicated that detection of AFB_1-lys in plasma was associated with detection of A9 HPV types (HPV 16, 31, 33, 35, 52, and 58) as a group in cervical swabs (odds ratio [OR] = 15.66; 95% confidence interval [CI], 2.03–120.87; $P = .008$) after adjustment for age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex. Detection of AFB_1-lys in plasma was also associated with non-HPV 16 A9 detection (OR = 39.05; 95% CI, 2.34–650.82; $P = .011$) (Supplementary Table 2) but not with other groups of HPV types (Table 2 and Supplementary Table 2).

Plasma AFB_1-lys concentrations were associated with detection of A9 HPV types as a group (OR per 0.1 pg/μL increase = 8.19; 95%CI, 1.61–41.66; $P = .011$) after adjustment for age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex (Table 2). In addition, plasma AFB_1-lys concentrations were associated with detection of all HR-HPV types (OR per 0.1 pg/μL increase = 2.88; 95% CI, 1.01–8.24; $P = .048$) and non-HPV 16 A9 types (OR per 0.1 pg/μL increase = 11.21; 95% CI, 1.77–70.98; $P = .010$) after adjustment for age, being married, having more than secondary school education, home ownership, living at a walking distance to healthcare ≥60 minutes, number of lifetime sex partners, or age of first sex (Supplementary Table 3), but they were not associated with other groups of HPV types (Table 2 and Supplementary Table 3). In addition to plasma AFB_1-lys detection or plasma AFB_1-lys concentration, certain demographic and behavioral characteristics significantly associated with HPV detection were identified from regression models (Table 2 and Supplementary Tables 2 and 3).

All individual A9 types except HPV 16 were detected more often in women with plasma AFB_1-lys detection than in women without AFB_1-lys detection, although these differences were not statistically significant (Figure 1). The number of detections for any individual A9 HPV type were small. For example, there was 1 HPV 16 case in either of the plasma AFB_1-lys detected or undetected women (Figure 1). The AFB_1-lys plasma concentration was significantly associated with HPV 52 detection. The median AFB_1-lys plasma concentration for 4 women with HPV 52 detected was 0.088 pg/μL (IQR, 0.073–0.099). The median AFB_1-lys plasma concentration for 82 women with no HPV 52 detected was 0.030 pg/μL (IQR, 0.000–0.080; $P = .042$). Plasma AFB_1-lys concentration was not significantly associated with detection of other individual A9 or other HPV types (data not shown).

Table 1. Characteristics of Women With or Without Plasma AFB1-lys Detection

	Plasma AFB1-lys Detection		
Characteristics	No N = 37	Yes N = 49	*P* Value
Median age (IQR)	34.0 (29.0–38.0)	34.0 (30.0–38.0)	.818[a]
Married	24 (65%)	36 (73%)	.390[b]
More than secondary school education	4 (11%)	9 (18%)	.333[b]
Home ownership	9 (24%)	15 (31%)	.520[b]
Walking distance to healthcare ≥60 minutes	2 (5%)	6 (12%)	.457[c]
Median number of lifetime sex partners (IQR)	2.0 (1.0–4.0)	3.0 (1.0–4.0)	.660[d]
Median age of first sex (IQR)	18.0 (17.0–20.0)	17.0 (16.0–20.0)	.506[a]

Abbreviations: AFB1-lys, aflatoxin B1-lysine; IQR, interquartile range.

[a] *P* value from *t* test.

[b] *P* value from χ^2 test.

[c] *P* value from Fisher's exact test.

[d] *P* value from Wilcoxon rank-sum test.

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Table 2. Logistic Regression Analysis of Associations of IARC HR-HPV, A9 HPV, A7 HPV, and ≥2 HR-HPV Types Detection With Plasma AFB1-lys Detection, Plasma AFB1-lys Concentration, and Characteristics of Women

	IARC HR-HPV[a]		A9 HPV[b]		A7 HPV[c]		≥2 HR-HPV Types[d]	
Variables	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
In Models With Plasma AFB1-lys Detection								
Plasma AFB1-lys detection	1.47 (0.48–4.52)	.505	15.66 (2.03–120.87)	.008	0.23 (0.03–1.86)	.170	1.76 (0.11–28.89)	.693
Age	1.00 (0.90–1.12)	.934	1.06 (0.91–1.23)	.441	0.90 (0.74–1.09)	.266	0.83 (0.65–1.07)	.158
Married	0.46 (0.14–1.59)	.220	0.20 (0.04–1.10)	.064	7.84 (0.46–134.09)	.155	0.09 (0.005–1.82)	.117
More than secondary school education	1.79 (0.40–7.96)	.446	4.64 (0.60–36.13)	.143	1.33 (0.09–19.04)	.834	9.77 (0.61–157.25)	.108
Home ownership	0.45 (0.10–2.08)	.309	0.04 (0.002–0.82)	.037	1.52 (0.18–12.33)	.696	0.48 (0–2.90)	.531
Walking distance to healthcare ≥60 minutes	1.39 (0.25–7.73)	.707	0.93 (0.09–9.51)	.954	2.17 (0.09–51.93)	.633	1.86 (0–13.14)	.999
Number of lifetime sex partners	1.26 (0.98–1.62)	.071	1.06 (0.73–1.52)	.774	1.67 (1.08–2.58)	.021	1.00 (0.57–1.76)	.990
Age of first sex	1.14 (0.96–1.36)	.135	1.23 (0.98–1.54)	.074	1.31 (0.97–1.77)	.078	0.96 (0.62–1.49)	.858
In models With Plasma AFB1-lys Concentration								
Plasma AFB1-lys concentration (pg/μL)[e]	1.76 (0.57–5.45)	.331	8.19 (1.61–41.66)	.011	0.26 (0.02–2.86)	.268	1.12 (0.04–30.43)	.947
Age	1.00 (0.90–1.12)	.935	1.05 (0.92–1.21)	.471	0.90 (0.74–1.09)	.268	0.78 (0.59–1.03)	.082
Married	0.45 (0.13–1.54)	.202	0.21 (0.04–1.16)	.074	7.19 (0.41–125.35)	.176	0.05 (0.001–1.71)	.096
More than secondary school education	1.92 (0.43–8.56)	.393	5.05 (0.70–36.50)	.108	0.88 (0.07–11.57)	.924	23.22 (0.92–585.46)	.056
Home ownership	0.47 (0.10–2.15)	.334	0.08 (0.006–1.15)	.063	1.37 (0.17–11.26)	.771	0.48 (0–2.63)	.515
Walking distance to healthcare ≥60 minutes	1.24 (0.21–7.23)	.809	0.60 (0.05–7.12)	.689	2.02 (0.08–54.48)	.676	13.97 (0–265.48)	.999
Number of lifetime sex partners	1.28 (0.99–1.64)	.056	1.16 (0.81–1.65)	.415	1.61 (1.05–2.46)	.028	0.86 (0.43–1.74)	.678
Age of first sex	1.15 (0.96–1.38)	.122	1.23 (0.98–1.53)	.070	1.31 (0.98–1.76)	.076	1.01 (0.65–1.55)	.982

Abbreviations: AFB1-lys, aflatoxin B1-lysine; CI, confidence interval; IARC, International Agency for the Research on Cancer; HPV, human papillomavirus; HR, high risk; OR, odds ratio.

[a]IARC HR-HPV: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66.

[b]A9 HPV: HPV 16, 31, 33, 35, 52, and 58.

[c]A7 HPV: HPV 18, 39, 45, 59, and 68.

[d]HR-HPV: HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and IS39.

[e]OR (95% CI) per 0.1 pg/μL increase of plasma AFB1-lys concentration.

DISCUSSION

Aflatoxins are produced by *Aspergillus* species during growth or after harvesting of crops such as corn and ground nuts. More than 4 billion people are exposed to aflatoxins in their foods, mainly corn and ground nuts [21–23]. In many sub-Saharan countries, corn is the major source of calories for most people. The poorest families are the most likely to be exposed: Leroy et al [24] showed that higher serum

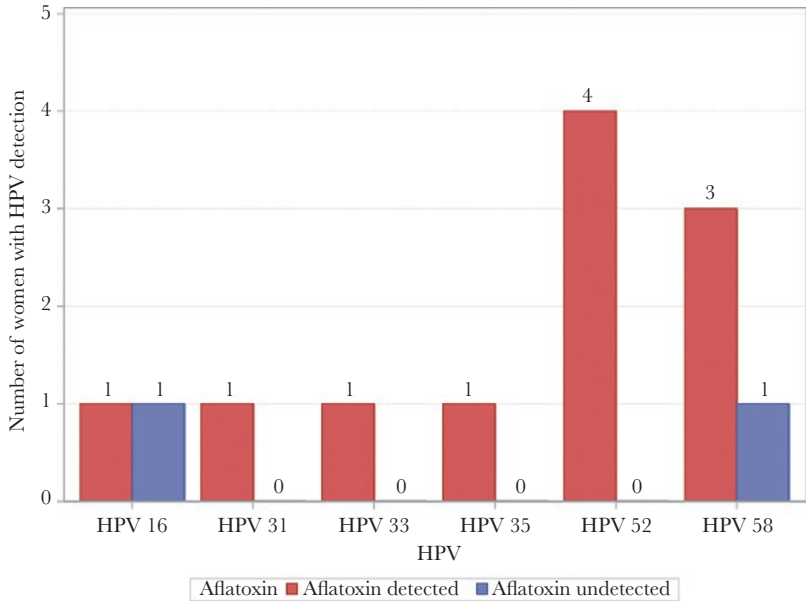

Figure 1. Associations of individual A9 human papillomavirus (HPV) detection and plasma aflatoxin B1-lysine (AFB1-lys) detection.

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aflatoxin levels in plasma from adult women from Eastern Province in Kenya were associated with lower household socioeconomic status.

The incidence of cervical cancer is extremely high in women living in sub-Saharan Africa, where screening programs as well as vaccination against HPV are available to very few [25, 26]. Although HIV infection greatly accelerates the natural history of cervical cancer, other cofactors are likely to be important, because only a small percentage of women infected with oncogenic HPV will ever develop cervical cancer, whether HIV-infected or not. What factors in addition to HIV are likely to be important?

In the current study of HIV-uninfected adult Kenyan women, more than half had detectable aflatoxin in plasma. This is consistent with other studies in sub-Saharan Africa demonstrating a high percentage of children and adults with aflatoxin detected in blood samples [27–29]. This is in stark contrast to the situation in the United States where less than 1% of adults have detectable aflatoxin in blood [30]. In the current study, aflatoxin detection was associated with an increased risk of detection of oncogenic HPV types in cervical swabs from these women. Aflatoxin plasma concentrations were also associated with an increased risk of oncogenic HPV detection, and although median differences between plasma aflatoxin concentrations are seemingly small, other studies indicate that such differences may have an impact in cancer risk. There have been several studies in humans to reveal the relationship between exposure to aflatoxin and the formation of the aflatoxin albumin adduct measured in the study. Based upon the work of Skipper and Tannenbaum [31], the accumulation of aflatoxin adduct in albumin would be 30 times the single daily exposure given the 28- to 30-day lifetime of the protein. When human volunteers were exposed to low levels of C-14-radiolabeled aflatoxin in the diet, a measurement of aflatoxin albumin adduct formation was made by accelerator mass spectrometry [32]. These findings, when combined to the work in Gambia of Wild et al [33], also showed a relationship between exposure and albumin addict formation. These data, taken together, indicate that at the limit of detection found in this study the daily exposure to aflatoxin was approximately 100 ng per day. Our available epidemiologic studies from a number of different populations at risk for liver cancer indicate that exposures up between 2 and 10 μg per day equate to substantial risk of liver cancer [34].

In addition to its oncogenic properties, aflatoxin is a potent immunosuppressive compound [35, 36]. Human papillomavirus infections occur more frequently in immunosuppressed people, including those infected with HIV and other conditions than in otherwise healthy people, as do HPV-associated cancers [1, 37, 38]. A possible mechanism for increased A9 HPV detection in women with detectable plasma aflatoxin may be aflatoxin-induced immunosuppression, leading to suboptimal immunological control of HR-HPV, the causative agents of cervical cancer. In vitro studies indicate a potent effect of aflatoxin on markers of immunity, even at very low doses [39]. Documented effects include (1) reduced phagocytosis of monocytes against *Candida albicans* and (2) decreased secretion of interleukins and tumor necrosis factor [39]. Aflatoxin exposure is associated with reduced salivary immunoglobulin A and a higher prevalence of malarial parasitemia [39]. In addition, studies of HIV-infected individuals in Ghana show an association of aflatoxin detection with higher HIV viral loads [40].

Aflatoxins are potent carcinogens that contribute heavily to the worldwide burden of hepatocellular carcinoma [41, 42]. For viral pathogens such as the hepatitis viruses B and C viruses, a synergistic effect with aflatoxin on development of hepatocellular carcinoma is well established [43, 44]. Although the relationship between aflatoxin exposure and other cancers is not well established, it is possible that oncogenic HPV types (such as the A9 HPVs) and dietary aflatoxin act synergistically in increasing the risk of cervical cancer in Kenyan women. In addition, aflatoxins have been detected in cervical tissue and could potentially act directly on cervical cells in the carcinogenic process [45].

If aflatoxin is involved in cervical cancer development, what can be done? Several specific strategies have been proposed and used to reduce aflatoxin exposure [1, 23, 46–49]. Some of these strategies can be applied before harvest and some after harvest. Some of these measures include biocontrol using atoxicigenic *Aspergillus* species, enhancement of host plant resistance by genetic manipulation, and integrated management systems at the level of the farm itself.

Some limitations of the present study need to be considered. Our reported results were based on a modest sample size that may give us suboptimal power for the data analysis. However, we have obtained statistically significant associations between circulating levels of aflatoxin and the risk of cervical detection of oncogenic HPV. Another limitation is that dietary factors were not adjusted as a potential confounder in our data analysis, which might have distorted the findings of the present study to some extent. This confounding could arise because animal and human studies have revealed that malnourished subjects exhibit suppressed immunity and thereby may be susceptible to aflatoxin exposure, aflatoxin adduct formation, and persistent HPV infection [22, 50]. In addition, the results of our study may be subject to multiple comparisons due to a relatively large number of the models presented. However, this problem is unlikely to occur because all exposure and outcome variables included in the constructed models were carefully selected in terms of the findings of previous studies and biological relevance.

CONCLUSIONS

In summary, AFB_1-lys was detected in plasma samples from 57% of HIV-uninfected Kenyan women without cervical

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dysplasia. The AFB_1-lys plasma detection and concentration of aflatoxin were associated with increased detection of the A9 group of oncogenic HPV types in cervical samples from HIV-uninfected Kenyan women who had normal VIA examinations. Further studies are needed to determine whether exposure to aflatoxin interacts with HPV infection (and possibly HIV coinfection) to modulate the risk of cervical cancer in women in Kenya and other developing countries in which aflatoxin exposure is frequent.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Financial support. This work was funded by the National Cancer Institute (Grant 1U54CA190151-01), Academic Model Providing Access to Healthcare-Oncology Institute: HPV and Cervical Cancer in Kenyan Women with HIV/AIDS (to P. L., O. O., and D. R. B.), and the internal pilot grant mechanism of the Indiana University Simon Cancer Center (to J. Z.).

Potential conflicts of interest. D. R. B. currently receives research funding and has received consulting fees in the past from Merck and Co., Inc. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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