The Kynurenine Pathway of Tryptophan Catabolism and AIDS-Associated Kaposi Sarcoma in Africa

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Background: Other than Kaposi sarcoma (KS)–associated herpesvirus and CD4$^+$ T-cell lymphopenia, the mechanisms responsible for KS in the context of HIV are poorly understood. One recently explored pathway of HIV pathogenesis involves induction of the enzyme indoleamine 2,3-dioxygenase-1 (IDO), which catabolizes tryptophan into kynurenine and several other immunologically active metabolites that suppress T-cell proliferation. We investigated the role of IDO in the development of KS in HIV disease.

Methods: In a case–control study among untreated HIV-infected Ugandans, cases were adults with KS and controls were without KS. IDO activity was assessed by the ratio of plasma kynurenine to tryptophan levels (KT ratio), measured by liquid chromatography–tandem mass spectrometry.

Results: We studied 631 HIV-infected subjects: 222 KS cases and 409 controls. Non-KS controls had a higher median plasma KT ratio (130, interquartile range: 90 to 190 nM/μM) than KS cases (110, interquartile range: 90 to 150 nM/μM) ($P = 0.004$). After adjustment for age, sex, CD4 count, and plasma HIV RNA level, subjects with the highest (fourth quartile) plasma KT ratios had a 59% reduction (95% confidence interval: 27% to 77%) in the odds of KS compared with those with the lowest (first quartile) levels. KS was also independently associated with lower CD4$^+$ count, higher plasma HIV RNA, and men.

Conclusions: Among HIV-infected individuals, greater activity of the kynurenine pathway of tryptophan catabolism, as evidenced by higher levels of plasma KT ratio, was associated with lower occurrence of KS. Some consequences of immune activation in HIV infection might actually suppress certain cancers.

Key Words: tryptophan, kynurenine, indoleamine 2,3-dioxygenase-1, HIV, Kaposi sarcoma, plasma HIV RNA, Africa

(*J Acquir Immune Defic Syndr* 2015;70:296–303)

Kaposi sarcoma (KS) was a harbinger of the HIV epidemic[1] and, despite the marked reduction in its incidence since the advent of effective antiretroviral therapy (ART),[2,3] remains the most common malignancy in people with HIV worldwide.[4] In sub-Saharan Africa, the extent of the HIV epidemic has resulted in KS becoming one of the most frequently reported cancers among all adults.[4] Although the discovery of the causative viral agent for KS, Kaposi sarcoma–associated herpesvirus (KSHV, also known as human herpesvirus 8), was a landmark accomplishment in the etiology of KS,[5] this virus is not sufficient to cause KS. In fact, other than KSHV[5] and CD4$^+$ T-cell lymphopenia,[3,6] the specific biological mechanisms responsible for the development of KS, particularly in sub-Saharan Africa, are poorly understood.

A potential mechanism for KS that has recently received attention in HIV pathogenesis is the kynurenine pathway of tryptophan catabolism.[7–10] The induction of indoleamine 2,3-dioxygenase-1 (IDO) in activated monocytes and dendritic cells by interferon-γ and other inflammatory mediators in HIV-infected individuals causes catabolism of tryptophan into kynurenine and several other downstream catabolites with immunologic properties.[11,12] Depletion of tryptophan[13] and the accumulation of the catabolites kynurenine and picolinic acid[14] directly inhibit T-cell proliferation, potentially contributing to immune deficiency. In addition to its role in HIV infection, the induction of the kynurenine pathway has been implicated in maternal tolerance of the fetal allograft[15] and in the evasion of host immune responses by several cancers.[16] In KS etiology, we hypothesized that the kynurenine pathway might promote KS by decreasing immune surveillance. Alternatively, given the inflammatory histopathologic nature of KS lesions, it is conceivable that the induction of the kynurenine pathway may inhibit emergence of KS by suppression of lymphocyte proliferation.

Received for publication January 1, 2015; accepted May 6, 2015.

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Supported by National Institutes of Health (NIH) grants D43 CA153717, R01 CA119903, R01 MH054097, U01 CA066529, U01 AI069911, and P30 AI027763.

Presented in part at the 14th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies, November 12–13, 2013, Bethesda, MD and at the 21st Conference on Retroviruses and Opportunistic Infections, March 3–6, 2014, Boston, MA.

The authors have no conflicts of interest to disclose.

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To begin to address the role of the kynurenine pathway in the development of KS, we studied it directly in HIV-infected adults newly diagnosed with KS in sub-Saharan Africa. IDO activity can be quantified by measuring the ratio of plasma kynurenine to tryptophan (KT ratio), where a high KT ratio reflects heightened IDO induction. We also took this opportunity to formally investigate, in Africa, the relationship between KS and other factors that have been studied in other populations, such as $CD4^+$ T-cell count, plasma HIV RNA, sex, and age. The overarching objective was to identify causal determinants of KS to inform both the development of interventions to prevent KS and target groups for earlier ART initiation.

METHODS

Overall Design

We conducted a case–control study of untreated HIV-infected adults (≥18 years old) in Uganda to investigate the relationship between plasma KT ratio and KS independent of known confounders.

Study Population

Cases were individuals with KS sampled between April 2007 and February 2011 throughout Uganda, who were enrolled in the Antiretrovirals for Kaposi Sarcoma (ARKS) study based at the Infectious Diseases Institute, Kampala, Uganda.[17] To be eligible for ARKS, subjects had to have histologically confirmed KS, with the exception of a few patients with highly characteristic oral lesions that could not be easily biopsied. No subjects had what was considered advanced KS by virtue of having an urgent indication for chemotherapy. Specifically, no individuals had any functionally disabling complication of KS. Subjects also could not have evidence of current active untreated opportunistic infection [eg, tuberculosis (TB) or cryptococcosis] or other malignancy. Although ARKS was a randomized trial of different ART regimens for the treatment of KS, the patients in the current case–control study were studied at baseline before initiating ART. All ARKS enrollees with available plasma specimens were included as cases in the present analysis.

Controls were individuals without KS enrolled in the Uganda AIDS Rural Treatment Outcomes (UARTO) cohort. UARTO is a consecutive sample of ambulatory HIV-infected adults residing within 60 km of and starting ART at the Immune Suppression Syndrome (ISS) Clinic at Mbarara Regional Referral Hospital in southwestern Uganda. ART at this clinic is prescribed on the basis of $CD4^+$ T-cell count or clinical conditions, according to the Uganda National Antiretroviral Treatment Guidelines.[18] For this study, we included all UARTO participants without KS who were consecutively enrolled between June 2005 and April 2010 and who had available plasma specimens, and we studied them before ART initiation. The absence of KS in the controls was based on patient self-report and review of medical records at the ISS clinic. The ISS clinic has an active skin punch biopsy service for histological confirmation of KS sponsored by the International Epidemiologic Databases to Evaluate AIDS (IeDEA) consortium.

Among both cases and controls, we excluded any woman with a history of pregnancy in the prior 12 months given the influence of pregnancy on the kynurenine pathway[19] and the fact that pregnant women were excluded from the ARKS trial.

Measurements

Questionnaire Based

The ARKS and UARTO studies used the same instruments for all measurements of sociodemographic characteristics, medical history, and clinical assessment. Within these instruments, we used the Medical Outcomes Study to determine physical health status and mental health status[20] and the Filmer–Pritchett asset index, as previously described,[21] to characterize socioeconomic status.

Laboratory

All biological tests for the cases and controls were performed in the same laboratories on samples obtained before the start of ART. Tryptophan and kynurenine levels were measured on cryopreserved plasma samples by liquid chromatography–tandem mass spectrometry as previously described.[22] Plasma HIV RNA level was measured using the Amplicor HIV Monitor version 1.5 or the Cobas Taqman HIV-1 version 1.0 assays (Roche, Branchburg, NJ). $CD4^+$ T-cell counts were assessed using the FACSCalibur system (Becton Dickinson, San Jose, CA).

Two enzyme immunoassays[23,24] and 1 direct immunofluorescence assay[25,26] were used to detect antibodies to KSHV, and the results of these 3 assays were interpreted according to a previously described algorithm.[27]

Statistical Analysis

Multivariable logistic regression was used to examine the relationship between plasma KT ratio and KS. In our base-case analysis, we were guided by our prespecified perception of the biological system and hence adjusted for $CD4^+$ T-cell count, plasma HIV RNA, age, and sex.[3,6,28,29] Because of ample sample size and number of cases, we did not attempt to reduce the number of covariates. In additional analyses, we also adjusted for other proxies of HIV disease progression not captured by the aforementioned variables, including body mass index (BMI), hemoglobin, mental health status, physical health status, and socioeconomic status.[30–32] In sensitivity analyses, we first restricted KS cases to those recruited from areas with moderate to high malaria endemicity and then we also separately restricted the non-KS controls to those without suspicion of TB within the first 6 months after the initiation of ART. Finally, we also performed an analysis in which we restricted the non-KS control group to subjects who were KSHV antibody positive.

We assessed for nonlinearity in continuous predictor variables using splines, and variables were transformed or categorized where necessary. Influential observations were checked using postestimation plots of dfbetas, followed by

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sensitivity analyses on data points with high dfbetas. We also assessed for possible interactions with the primary predictor variable, KT ratio, and used a P value of 0.05 to guide reporting of interaction. The Hosmer–Lemeshow test was used to examine goodness of fit. We used multiple imputation with iterative chained equations for missing values.[33] All analyses were performed using STATA 13 (College Station, TX).

RESULTS

Characteristics of the Study Population

A total of 631 untreated HIV-infected Ugandans were examined: 222 KS cases and 409 non-KS controls. Men comprised 56% of the cases and 33% of the controls (Table 1). Compared with the controls, cases had a slightly higher median BMI (21.4 versus 21.1 kg/m^2), lower hemoglobin (11.6 versus 12.2 g/dL), and higher plasma HIV RNA levels (5.3 versus 5.1 copies/mL). Sixty-nine percent and 75% of cases and controls had a CD4$^+$ T-cell count ≤200 cells per microliter, respectively; 13% and 3% of cases and controls had CD4$^+$ T-cell count >350 cells per microliter, respectively. The cases had a wide spectrum of mucocutaneous KS ranging from oral lesions only to widespread cutaneous dissemination.

TABLE 1. Characteristics of the Cases and Controls

Characteristic	KS Cases (n = 222)	Non-KS Controls (n = 409)
Age, yrs	34 (28 to 40)*	35 (29 to 40)
Male sex	56%	33%
Physical health status†	53.3 (36.4 to 58.2)	54.0 (45.0 to 58.6)
Mental health status†	48.6 (37.7 to 56.7)	52.6 (45.9 to 58.7)
Asset index‡	0.18 (−1.80 to 1.78)	−0.58 (−1.82 to 0.86)
BMI, kg/m^2	21.4 (19.4 to 23.1)	21.1 (19.4 to 23.4)
Hemoglobin, g/dL	11.6 (10.3 to 13.2)	12.2 (10.6 to 13.7)
HIV RNA, $\log_{10}$ plasma copies/mL	5.3 (5.0 to 5.6)	5.1 (4.6 to 5.6)
CD4$^+$ T cells, count/μL		
<50	35%	16%
51–100	11%	18%
101–200	23%	41%
201–350	18%	21%
>350	13%	3%
Plasma KT ratio, nM/μM, by quartile		
Quartile 1 (34–89)	27%	24%
Quartile 2 (90–120)	28%	23%
Quartile 3 (121–179)	29%	23%
Quartile 4 (180–1369)	16%	30%

*Median (interquartile range) unless indicated.

†Mental and Physical Health Status scores were derived using the Medical Outcomes Study-HIV survey. Responses to questions on 5 scales regarding vitality, cognitive function, quality of life, health distress, and mental health are summarized in the Mental Health Status score and responses to questions on 6 scales regarding general health, vitality, pain, physical, role, and social function are summarized in the Physical Health Status score. Responses to individual questions are aggregated, and scores are converted to a 0–100-point scale, with 100 representing the best mental or physical health status.[20]

‡The Filmer–Pritchett index is a measure of socioeconomic status based on self-reported asset ownership and housing characteristics. Appropriate weights for individual questions are determined using the statistical method of principal components, and responses are aggregated to create the asset index score, which may be positive or negative with a median of 0. A higher score represents a higher socioeconomic status.[21]

Relationship Between Plasma KT Ratio and KS

The median plasma KT ratio among all subjects was 120 nM/μM [interquartile range (IQR): 90–180 nM/μM], which is higher than that in the HIV-uninfected individuals, at least in resource-rich settings.[34] The distribution of plasma KT ratio was more markedly skewed to the right in the controls compared with the cases (Fig. 1). In an unadjusted analysis, the non-KS controls had a higher median plasma KT ratio (130, IQR: 90–190 nM/μM) than the KS cases (110, IQR: 90–150 nM/μM) (P = 0.004). Specifically, there was a nonlinear "threshold" relationship between plasma KT ratio and KS (Table 2). Compared with those with KT ratio in the lowest quartile (ie, lowest values, KT ratio <90 nM/μM), there was no association with KS with increasing values of KT ratios until the highest values were reached. Those with KT ratio in the highest quartile (ie, highest values, KT ratio >179 nM/μM) had a 50% reduction in the odds of KS. We observed similar results in a multivariable logistic regression model adjusting for age, sex, CD4$^+$ T-cell count, and plasma HIV RNA level (Table 2). Compared with individuals with KT ratio in the lowest quartile, there was 59% reduction [95% confidence interval (CI): 27% to 77%; P = 0.002] in the odds of KS for those with KT ratio in the highest quartile. In an additional analysis, to attempt further adjust for the confounding effect of HIV disease progression, we also adjusted for BMI, hemoglobin, mental health status, physical health status, and asset index. In this analysis, we observed an even a stronger and more linear association between plasma KT ratio and KS. The association between KT ratio and KS was not modified by CD4$^+$ T-cell count, plasma HIV RNA level, sex, or age (P value for interaction = 0.61, 0.84, 0.54, and 0.19, respectively).

Effects of Malaria and TB on the Relationship Between KT Ratio and KS

Given that *Plasmodium* species may induce IDO activity[35] and that all the non-KS controls were enrolled from areas with moderate to high malaria endemicity, whereas 31% of the cases were enrolled from areas of low malaria endemicity,[36] we examined whether the association between KT ratio and KS might be due to differences in malaria comorbidity between the cases and controls. In analyses restricted to the 153 KS cases recruited from areas with moderate to high malaria endemicity,[36] we again observed, after adjustment for age, sex, CD4$^+$ T-cell count, and plasma HIV RNA viral load, that individuals with KT ratio in the highest quartile had a reduction in the odds of KS compared with those in the lowest quartile (odds ratio = 0.39; 95% CI: 0.20 to 0.77; Table 3). When we also adjusted for BMI, hemoglobin, mental health status, physical health status, and asset index, a dose–response relationship was again observed. Imbalance in concurrent TB was also a concern because Uganda has a high prevalence of TB among patients initiating

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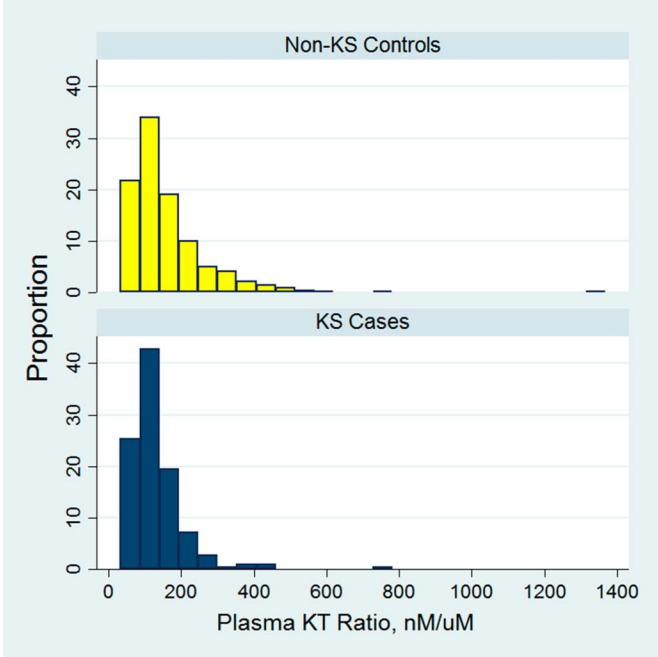

FIGURE 1. Distribution of plasma KT ratio in KS cases and non-KS controls.

ART,[37] *Mycobacterium tuberculosis* may induce IDO,[38] and the ARKS study excluded persons with untreated TB, whereas UARTO did not. To address this, we restricted our analyses to the non-KS controls without TB by excluding all subjects who were receiving any anti-TB medications at enrollment or who were subsequently treated for TB within the first 6 months after ART initiation (n = 62). Our findings of an independent inverse association between plasma KT ratio and KS remained consistent (Table 3).

Effect of KSHV Infection on the Relationship Between KT Ratio and KS

Consistent with prior data from this region, 40% of the 409 subjects in the non-KS control group were KSHV antibody positive. In an additional analysis in which we restricted the non-KS control group to only participants who were KSHV antibody positive and, again, adjusted for age, sex, plasma HIV RNA level, and CD4 count, we observed a somewhat stronger negative association between KT ratio and KS than in our analysis including all 409 non-KS controls. Compared with individuals with KT ratio in the lowest quartile, there was 71% reduction (95% CI: 42% to 86%; $P = 0.002$) in the odds of KS for those with KT ratio in the highest quartile.

Other Determinants of KS

We observed that KS was also independently associated with male sex, higher BMI, lower hemoglobin, lower mental health status score, lower $CD4^+$ T-cell count, and higher plasma HIV RNA level (Table 2). The correlation with plasma HIV RNA was most notable with a dose–response relationship seen throughout most of the range of exposure and over a 5-fold greater odds of KS observed among those with RNA >100,000 copies per milliliter compared with those with <10,000 copies per milliliter.

DISCUSSION

Other than KSHV infection and $CD4^+$ T-cell lymphopenia, the specific biological mechanisms responsible for the development of AIDS-associated KS remain poorly understood. In this case–control study of untreated HIV-infected Ugandans, we have described a potentially new mechanism affecting the occurrence of KS. Specifically, greater activity of the kynurenine pathway of tryptophan catabolism was associated with a lower occurrence of KS. These data provide the first evidence suggesting a role of the kynurenine pathway of tryptophan catabolism in KS pathogenesis and may explain why some KSHV-infected individuals with advanced AIDS fail to develop KS. We have also added to the knowledge base for how HIV infection causes KS by finding a strong independent association between plasma HIV RNA level and KS.

Our findings add to the growing literature regarding the kynurenine pathway of tryptophan catabolism in carcinogenesis.[16] In the majority of work to date, increased kynurenine pathway activity has been positively associated with the occurrence of cancer, which is generally attributed to the escape of tumor from immune surveillance.[16] IDO is expressed in both tumor cells and antigen-presenting cells in tumor-draining lymph nodes, and by fostering immune suppression, IDO activity facilitates the survival and growth of tumor cells expressing unique antigens that would normally be recognized as foreign.[39,40] Indeed, increased serum KT ratio has been shown to correlate with disease progression and poor prognosis in certain cancers.[41,42] In contrast, in our study, higher plasma KT ratio (reflecting higher levels of IDO activity) appeared protective against KS.

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TABLE 2. Unadjusted and Adjusted Logistic Regression Evaluating Factors Associated With KS

	Unadjusted		Adjusted Model 1		Adjusted Model 2	
Characteristic	**Odds Ratio (95% CI)**	***P***	**Odds Ratio* (95% CI)**	***P***	**Odds Ratio* (95% CI)**	***P***
Age, per 10 yrs	0.98 (0.97 to 1.01)	0.08	0.79 (0.62 to 1.01)	0.059	0.84 (0.64 to 1.09)	0.19
Sex						
Women	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Men	2.69 (1.92 to 3.76)	<0.001	2.42 (1.63 to 3.58)	<0.001	4.50 (2.60 to 7.79)	<0.001
Physical health status†	0.98 (0.97 to 0.99)	<0.001	—	—	1.00 (0.98 to 1.03)	0.83
Mental health status†	0.96 (0.95 to 0.98)	<0.001	—	—	0.97 (0.95 to 1.00)	0.041
Asset index‡	1.06 (1.01 to 1.14)	0.044	—	—	1.04 (0.95 to 1.14)	0.42
BMI, kg/m^2§	1.01 (0.97 to 1.04)	0.66	—	—	1.11 (1.04 to 1.18)	0.002
Hemoglobin, g/dL§	0.90 (0.85 to 0.96)	0.001	—	—	0.73 (0.60 to 0.89)	0.002
HIV RNA, plasma copies/mL						
≤10,000	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
10,001–50,000	1.95 (0.73 to 5.23)	0.18	2.61 (0.84 to 8.08)	0.096	2.43 (0.75 to 7.92)	0.14
50,001–100,000	3.05 (1.18 to 7.91)	0.021	4.07 (1.34 to 12.3)	0.013	4.05 (1.27 to 12.8)	0.018
100,001–500,000	7.37 (3.01 to 18.1)	<0.001	9.64 (3.35 to 27.7)	<0.001	9.26 (3.09 to 27.8)	<0.001
>500,000	2.91 (1.14 to 7.43)	0.026	5.55 (1.80 to 17.1)	0.003	5.17 (1.60 to 16.8)	0.006
CD4$^+$ T cells, count/μL						
<50	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
51–100	0.23 (0.15 to 0.37)	<0.001	0.30 (0.16 to 0.55)	<0.001	0.25 (0.13 to 0.49)	<0.001
101–200	0.24 (0.17 to 0.34)	<0.001	0.33 (0.21 to 0.54)	<0.001	0.34 (0.20 to 0.57)	<0.001
201–350	0.34 (0.23 to 0.50)	<0.001	0.54 (0.31 to 0.94)	0.029	0.52 (0.28 to 0.95)	0.033
>350	1.50 (0.96 to 2.35)	0.075	2.60 (1.15 to 5.87)	0.021	2.55 (1.08 to 7.04)	0.033
Plasma KT ratio, nM/μM, by quartile						
Quartile 1 (34–89)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quartile 2 (90–120)	1.10 (0.77 to 1.58)	0.61	1.02 (0.61 to 1.72)	0.93	0.74 (0.41 to 1.36)	0.34
Quartile 3 (121–179)	1.11 (0.77 to 1.58)	0.58	0.86 (0.51 to 1.45)	0.58	0.51 (0.26 to 1.00)	0.049
Quartile 4 (180–1369)	0.50 (0.33 to 0.76)	0.001	0.41 (0.23 to 0.73)	0.002	0.21 (0.09 to 0.46)	<0.001

*All variables adjusted for all other variables in column.
†Per 1 unit increase in score derived from Medical Outcomes Study-HIV survey.[20]
‡Per 1 unit increase in Filmer–Pritchett index.[21]
§Per 1 unit increase in respective native scale.

Our results suggest that intact lymphocyte proliferation is necessary for the development of KS. The in vitro observation that malignant B lymphocyte cell growth is suppressed by IDO activity also supports this hypothesis.[43] A protective effect of the highest levels of IDO activity against KS is also compatible with the observation that KS often occurs at local sites of inflammation[44] and the inflammatory nature histologically of KS lesions (ie, an immune cell infiltrate). In addition, several inflammatory cytokines are increased in KS lesions and are capable of increasing lytic replication of KSHV.[45] Th1 cytokines from activated infiltrating T and B cells may also trigger KS lesion formation by promoting spindle-cell proliferation and angiogenesis.[45,46] An inflammatory microenvironment fosters KS lesions suggests that IDO-derived local immune suppression could impair the development of KS. Alternatively, high levels of KSHV infection might suppress interferon gamma–induced IDO and lower KT levels as previously observed in other viral infections.[47] Experiments on KS tissue, and appropriate controls, to directly examine KT levels would be a natural next step to evaluate the biological plausibility of our finding.

We observed a stronger inverse association between plasma KT ratio and KS—in fact, a dose–response effect—when we also adjusted for other markers of HIV disease progression, namely BMI, mental health score, physical health score, asset index, and hemoglobin. Further interrogation of the regression models revealed that this change in the shape of the KT-KS relationship was caused solely by adjustment for hemoglobin (data not shown). We cannot, however, determine whether this further adjustment is warranted and hence whether the stronger association is valid. Specifically, because this is a cross-sectional study, we cannot distinguish between whether low hemoglobin is a common outcome of both KT ratio and KS (also known as a "collider" in epidemiologic parlance) or whether it is a proxy for a confounder (causally responsible for both KT ratio and KS) or a mediator (intermediary along the pathway) for the relationship between KT ratio and KS, and hence, we presented the results of both adjusted models 1 and 2. Although the latter 2 scenarios warrant adjustment for hemoglobin when trying to understand the direct causal effect of KT ratio on KS, the first scenario does not. In any case, whether hemoglobin is adjusted for, we consistently observed that having the highest level plasma KT ratio was associated with lower occurrence of KS.

As is often true in case–control studies, the principal limitation of our work concerns the selection of the controls.

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TABLE 3. Unadjusted and Adjusted Logistic Regression Evaluating Factors Associated With KS

	Unadjusted		Adjusted Model 1*		Adjusted Model 2†	
Characteristic	**Odds Ratio (95% CI)**	***P***	**Odds Ratio (95% CI)**	***P***	**Odds Ratio (95% CI)**	***P***
Restricted to subjects from moderate to high malaria endemic areas						
Plasma KT ratio, nM/μM, by quartile						
Quartile 1 (34–89)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quartile 2 (90–120)	1.12 (0.70 to 1.77)	0.64	1.14 (0.63 to 2.06)	0.67	0.72 (0.36 to 1.43)	0.34
Quartile 3 (121–179)	1.09 (0.68 to 1.74)	0.73	1.02 (0.57 to 1.83)	0.96	0.51 (0.24 to 1.07)	0.073
Quartile 4 (180–1369)	0.48 (0.28 to 0.81)	0.006	0.39 (0.20 to 0.77)	0.006	0.15 (0.06 to 0.38)	<0.001
Restricted to subjects with no suspicion of TB						
Plasma KT ratio, nM/μM, by quartile						
Quartile 1 (34–89)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quartile 2 (90–120)	1.22 (0.73 to 2.03)	0.45	1.08 (0.64 to 1.85)	0.77	0.75 (0.40 to 1.40)	0.37
Quartile 3 (121–179)	1.26 (0.75 to 2.09)	0.38	0.86 (0.50 to 1.49)	0.59	0.50 (0.25 to 0.99)	0.046
Quartile 4 (180–1369)	0.43 (0.24 to 0.78)	0.006	0.43 (0.23 to 0.79)	0.007	0.22 (0.10 to 0.50)	<0.001

*Adjusting for plasma HIV RNA, $CD4^+$ T-cell count, age, and sex.
†Adjusting for plasma HIV RNA, $CD4^+$ T-cell count, age, sex, BMI, hemoglobin, physical health status, mental health status, and asset index.

Because of the relative rarity of KS and the lack of a population-based surveillance system in Uganda for the identification of KS as it occurs in the community, we were unable to assemble unassailable case and control groups sampled from a primary study base.[48] Instead, we took advantage of a large group of recently diagnosed cases of KS, compared them with a representative sample of persons without KS who were similarly advanced in their HIV infection by virtue of an indication for ART, and accommodated for any differences between the groups by statistical adjustment. Importantly, our case and control groups were not independently constructed and compared in retrospect with a piecemeal assemblage of measurements. Rather, the ARKS and UARTO studies were prospectively performed in parallel with identical questionnaire-based and laboratory measurements in anticipation of comparative research like this study. Although it remains possible that the UARTO-based control group was systematically enriched for patients with higher values of KT ratio, we feel our various analyses render this unlikely. First, the most obvious difference between the groups, their geographic residence, was addressed by restricting analysis in the case group to those living in malarious areas. In this sensitivity analysis, our primary inference was unchanged. Second, the potential for the control group to harbor patients with a greater prevalence of coinfections that are stimulating IDO induction was addressed in our main analysis by extensive adjustment for determinants of coinfections (eg, $CD4^+$ T-cell count) and manifestations of coinfections (eg, BMI). Furthermore, when we limited the control group to those without TB, we again saw no change in our inference. Although we were unable to directly examine the effect of potential differences in prevalence of soil transmitted helminths, the species that may induce IDO[49,50] are more prevalent in the geographic areas from which the cases were recruited.[51] Therefore, we would expect a bias towards the null. Nonetheless, as with any observational study, our findings require replication.

Consistent with prior research from resource-rich settings,[29,52] we found in untreated HIV-infected Africans that higher plasma HIV RNA levels were strongly associated with KS even after adjustment for $CD4^+$ T-cell count and other factors. The independent role of plasma HIV RNA provides clinical augmentation of earlier laboratory research, suggesting a causative role of HIV per se in KS etiology.[53] $CD4^+$ T-cell lymphopenia was also independently associated with KS, as has been previously well established. Of note, that a $CD4^+$ T-cell count >350 cells per microliter appeared to carry a greater risk of KS than a $CD4^+$ T-cell count <50/μL is likely an artifact of the patient sample and not a biologic phenomenon. That is, the non-KS controls were HIV-infected patients about to initiate ART according to local ART guidelines, which, for much of the period of the study, required a $CD4^+$ T-cell count <200 cells per microliter or a World Health Organization stage IV condition.[18] Therefore, the majority of the controls had a $CD4^+$ T-cell count <200 cells per microliter. However, there was no restriction on the $CD4^+$ T-cell count for the KS cases, and as seen elsewhere,[3,54] a relevant proportion of KS cases had a $CD4^+$ T-cell count >350 cells per microliter. This therefore explains why the KS cases are enriched, in comparison with the controls, for high $CD4^+$ T-cell counts. This difference in $CD4^+$ T-cell count distribution between cases and controls, however, does not explain the association between KT ratio and KS because $CD4^+$ T-cell count was statistically controlled for when assessing the KT ratio and KS relationship, and furthermore, there was no statistical interaction between KT ratio and $CD4^+$ T-cell count. We also observed that lower hemoglobin was associated with KS, which we speculate reflects anemia of chronic inflammation driven by elevated levels of inflammatory cytokines[55,56] and/or KS involvement of the gastrointestinal tract with occult hemorrhage.[57] Similarly, we believe that the association between BMI and KS is also an example of reverse causality, explained by KS-induced lymphedema and associated weight gain. Like prior studies in this setting, we observed that male sex was independently associated with KS.[28]

We note that the explosion of human subjects-based biological discovery in HIV/AIDS (sometimes called

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"translational" research) has not occurred in the study of KS.[58] The absence of translational research in KS is particularly regrettable in Africa where KS is among the most common cancers in the entire general population. The differences between Africa and settings like the United States in terms of human host, causative viral pathogen, and environment suggest that African KS science cannot simply survive by extrapolating from the translational work performed in resource-rich settings. Now that biological measurement tools and research infrastructure such as ARKS, UARTO, and the AIDS Malignancy Consortium are in place in sub-Saharan Africa, we hope that studies like ours will spur further African-based translational work in KS etiopathogenesis.

In summary, we have demonstrated that higher IDO activity, as evidenced by higher plasma KT ratio, is associated with lower occurrence of KS among HIV-infected adults in Africa. The inverse relationship between KT ratio and KS is consistent with the inflammatory nature of KS lesions and suggests that intact lymphocyte proliferation is required for KS lesion development. Results from this study imply that some consequences of immune activation in HIV might actually suppress or mask certain cancers.

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