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Human herpesvirus 8 load and progression of AIDS-related Kaposi sarcoma lesions

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Abstract

Introduction—Human herpesvirus 8 (HHV8) is necessary for Kaposi sarcoma (KS) to develop, but whether peripheral blood viral load is a marker of KS burden (total number of KS lesions), KS progression (the rate of eruption of new KS lesions), or both is unclear. We investigated these relationships in persons with AIDS.

Methods—Newly diagnosed patients with AIDS-related KS attending Mulago Hospital, in Kampala, Uganda, were assessed for KS burden and progression by questionnaire and medical examination. Venous blood samples were taken for HHV8 load measurements by PCR. Associations were examined with odds ratio (OR) and 95% confidence intervals (CI) from logistic regression models and with t-tests.

Results—Among 74 patients (59% men), median age was 34.5 years (interquartile range [IQR], 28.5-41). HHV8 DNA was detected in 93% and quantified in 77% patients. Median virus load was 3.8 $\log_{10}/10^6$ peripheral blood cells (IQR 3.4-5.0) and was higher in men than women (4.4 vs. 3.8 logs; p=0.04), in patients with faster (>20 lesions per year) than slower rate of KS lesion eruption (4.5 vs. 3.6 logs; p<0.001), and higher, but not significantly, among patients with more (>median [20] KS lesions) than fewer KS lesions (4.4 vs. 4.0 logs; p=0.16). HHV8 load was unrelated to CD4 lymphocyte count (p=0.23).

Conclusions—We show significant association of HHV8 load in peripheral blood with rate of eruption of KS lesions, but not with total lesion count. Our results suggest that viral load increases concurrently with development of new KS lesions.

Keywords

Africa; epidemiology

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Introduction

Human herpesvirus 8 (HHV8, also called Kaposi sarcoma-associated herpesvirus or KSHV) is a necessary, but insufficient, cause of Kaposi sarcoma (KS) [1]. High lytic anti-HHV8 antibody titers and detection of HHV8 DNA in peripheral blood are risk factors for KS, especially in persons with acquired immune deficiency syndrome (AIDS) [2] and in persons receiving immunosuppressive medication to prevent solid-organ transplant rejection [3,4]. Whether HHV8 load in peripheral blood of KS patients is indicative of the total count of KS lesions (i.e., KS burden) or of the rate new KS lesions erupt (i.e., KS progression) is unknown [5,6]. Some studies [5,6], but not others [7,8], have reported positive associations between HHV8 load in peripheral blood and clinical KS burden. Immunosuppression is a risk factor for KS [9] and is thought to increase the risk for KS by uncoupling immune control from viral replication in both peripheral blood and skin. Whether the extent of KS disease is a consequence of HHV8 load or the determinant of HHV8 load is not known. Characterizing the relationship between peripheral blood HHV8 load and KS burden, rate of KS progression and immunity may clarify the biological role of peripheral blood virus load in KS pathogenesis. We report a cross-sectional analysis of peripheral blood HHV8 load and KS burden and rate of KS progression among persons with AIDS.

Methods

The study subjects were newly diagnosed, biopsy-confirmed, patients with AIDS-related KS aged 20 years or older enrolled at Mulago Hospital in Kampala, Uganda, from December 2002 through September 2003 [10]. After informed consent, patients were evaluated by questionnaire to determine the site and date of onset of the initial KS lesion followed by a brief medical examination to count all KS lesions on the body (KS burden). Facilities were not available to determine if KS was present in internal organs. A venous blood sample was taken for a complete blood count, CD4 lymphocyte count, and testing for HIV serology, which was performed after pre-test HIV counseling. Laboratory results were returned to the patient's physician to provide treatment. HIV antiretroviral therapies were not routinely used in Uganda at the time the study was conducted, and they were not provided by our study because we had no means to sustain usage beyond the study period. Patients were referred to The AIDS Support Organization for HIV support care. The Uganda National Council of Science and Technology gave ethical approval to conduct the study.

Plasma anti-HHV8 antibodies to the K8.1 structural glycoprotein and to the ORF73 encoded latency associated nuclear antigen (LANA) were measured using enzyme immunoassays (EIA) as previously published [11]. HHV8 load was measured in DNA extracted from buffy coat samples, using the QIAamp DNA Blood Midi kit according to the manufacturer's instructions, by quantitative TaqMan PCR using the ABI Prism 7700 sequence detection system (Applied Biosystems, Forster City, CA). To minimize risk of contamination, reagent preparation, DNA extraction, and PCR amplification were conducted in separate rooms with dedicated equipment. Approximately 150 ng of input DNA measured by optical density was used for HHV8 K6 and human endogenous retrovirus 3 (ERV-3) TaqMan PCR [12-14]. Test and control samples were run in triplicate. ERV-3 PCR was used to determine the number of cell equivalents in the assay and subsequently calculate the viral load per million cells. The results of quantified HHV8 load were log-transformed (base 10) for analysis. The HHV8 PCR assay has a linear dynamic range of 5 logs and is calibrated to detect a single copy of viral DNA in 150ng genomic DNA. Samples with quantifiable ERV-3 and positive HHV8 PCR in some but not all triplicate wells were classified as detected, but not quantified.

Because measurement of KS burden in Uganda has not been standardized, we assessed KS burden using a simple lesion count and classified burden as mild when lesions were less than

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or equal to the median number of total KS lesions (≤20 KS lesions), otherwise as severe. Similarly, measurement of KS progression in Uganda has not been standardized, therefore, we determined the rate of KS progression using the ratio of the total number of countable KS lesions on the body and the duration in years since the patient noticed the first KS lesion. The rate of progression was classified, somewhat arbitrarily, as slowly progressive when the ratio was less than or equal to 20 new lesions per year, otherwise as rapidly progressive KS.

We examined for statistical associations between questionnaire variables and KS burden and rate of KS progression with frequency tables and Fisher exact tests. We used the unpaired Student's t-test to assess differences in means for continuous measures. HHV8 load was categorized as low or high; values less than or equal to the median of log transformed load (excluding the qualitatively positive samples) were considered low and took on a numeric value of 0, otherwise they were considered high and took on a numeric value of 1. This derived variable was used in logistic regression models to calculate odds ratio (OR) and 95% confidence interval (CI) for association with high viral load. Independent contribution of variables to high HHV8 load was determined by comparing the full multivariable logistic regression model versus nested models using the log-likelihood ratio test. Two-sided $p \leq 0.05$ were considered statistically significant. Records with missing values were excluded from analysis.

Results

Among 74 patients with AIDS-related KS, 59% were men and the median age was 34.5 years (inter-quartile range [IQR] 28.5-41). Most resided in central Uganda (67%) and most had received a secondary level or higher level of education (59%). Consistent with their AIDS diagnosis, median CD4 lymphocyte count was low (102 cells per mm^3, IQR 15-177). Anti-K8.1 HHV8 antibodies were detected in 69 (93%) and anti-ORF73 HHV8 antibodies were detected in 53 (71%) of the patients. Anti-K8.1 antibodies were detected in most patients who did not have detectable anti-ORF73 antibodies (18 of 21; 86%), whereas anti-ORF73 antibodies were detected in 3 of 5 (40%) patients who did not have detectable anti-K8.1 antibodies. Two patients did not have measurable anti-K8.1 or anti-ORF73 HHV8 antibodies.

The median number of KS lesions on the body was 20 (IQR 10-40) and the median duration since the first KS lesion appeared was 7.5 months (IQR 4-12). KS burden was classified as severe in 34 (46%) and the rate of KS progression was rapid in 47 (64%) patients. Patients with severe KS burden were more likely to have received no schooling or only primary level education compared to those with milder KS burden (p=0.03; Table 1). Furthermore, patients with severe KS burden had a higher percentage of eosinophils as a fraction of the total white cell count compared to those with milder KS burden (p=0.05). Regarding the rate of KS progression, patients with rapid progression were more likely to report no schooling or only primary (p=0.04), peasant occupation (p=0.02), and to have lower platelet count, compared to those with slower KS progression (p=0.03). Among these patients with AIDS, neither KS burden nor the rate of KS progression was related to CD4 count as stratified <50, 50-150, >150 cells/mm^3 (p_{trend}=0.43 and p_{trend}=0.32, respectively, Table 1).

HHV8 DNA was detected in peripheral blood in 93% of the patients (69 of 74) and it was quantified in 57 (77%). Median HHV8 genome copies were 3.8 $\log_{10}/10^6$ peripheral blood cells (IQR 3.4-5.0). Among these patients with AIDS-related KS, neither HHV8 DNA detection nor load was associated with the detection, or not, of anti-HHV8 K8.1 or ORF73 antibodies (not shown). Detection of HHV8 DNA in peripheral blood was positively associated with eosinophils as a percentage of the total white cell count (p=0.014), but it was not associated with total lymphocyte (p=0.6) or CD4 lymphocyte count (p=0.18). Among individuals with quantified HHV8 DNA in peripheral blood, viral load was higher in men than women (4.4 vs. 3.8 logs; p=0.04) and among patients who reported no schooling or only primary level

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education compared to secondary or higher level of education (4.5 vs. 3.9 logs; p=0.07). HHV8 load in peripheral blood was unrelated to CD4 lymphocyte count (p=0.23; Figure 1A), but it was inversely related to platelet count (p<0.001; Figure 1B) and with hemoglobin level (p=0.06; not shown). HHV8 load was non-significantly higher in patients with severe compared to those with milder KS burden (4.4 vs. 4.0 logs; p=0.16; Figure 1C), and significantly higher in patients with rapid versus slower rate of KS progression (4.5 vs. 3.6 logs; p<0.0007; Figure 1D). In multivariable models, higher peripheral blood HHV8 load was weakly associated with gender (OR 2.5, 95% CI 0.9-7.0) and significantly associated with rate of KS progression (OR 4.9, 95% CI 1.5-16), but it was not associated with KS burden (OR 1.4, 95% CI 0.2-2.4).

Discussion

In our cross-sectional analysis of well-characterized patients with AIDS-related KS from Uganda, HHV8 load in peripheral blood was positively and significantly associated with the rate of KS progression, and marginally associated with male gender, but not with KS burden or CD4 lymphocyte count. Our findings corroborate those from smaller studies of patients with AIDS in Italy [15] and in the U.S. [16-18] that have reported significant associations between peripheral blood HHV8 load and the development of new KS lesions. Together, these studies suggest that peripheral blood HHV8 load increases concurrently with, and perhaps may be the cause of, the development of new KS lesions.

Positive associations between HHV8 load in peripheral blood and KS progression have been reported in several studies conducted in Europe and in the U.S., but not in studies from Africa. In a study of 26 patients, Pellet and colleagues [19] observed that non-detection of HHV8 DNA in peripheral blood was associated with treatment-related improvement of AIDS KS. In their study of 17 patients, Marcelin and co-workers [8] reported that HHV8 load in peripheral blood was higher in patients with AIDS-related KS who did not respond to KS treatment compared to those who responded. Three studies looking at endemic and classical [6], post-transplant KS [20] or AIDS-related KS [18] have reported significant associations between HHV8 load in peripheral blood and KS progression, after controlling for KS burden. In the larger one conducted in the U.S., Laney and colleagues reported that higher HHV8 load in peripheral blood of patients with AIDS-KS was associated with eruption of new KS lesions [18]. Although these studies are small and were conducted in developed countries, they agree with our study that higher HHV8 load in peripheral blood may be associated with rate of KS progression. Higher HHV8 load in peripheral blood is likely a consequence of uncoupling of viral lytic replication from immune control in severely immunosuppressed patients with KS. Surprisingly, we did not find the expected positive association between HHV8 load and CD4 lymphocyte counts. However, similar results have been observed among U.S. patients [18]. Possibly, CD4 lymphocyte count is not be a sensitive indicator of the relationship between immunosuppression and peripheral blood HHV8 load or between HHV8 load and KS.

A notable observation in our study is that HHV8 DNA was detected more frequently in peripheral blood of our patients than is reported in KS patients from elsewhere. Specifically, HHV8 DNA was detected in peripheral blood of 82% of patients from Central African Republic [21], in 72% of patients from Zimbabwe (72%) [5] and is detected in ~60% of patients from Europe or the U.S. [3,6,18,22], as compared to 93% in our patients. Differences in the frequency of detection may be due to chance, KS burden, variation in assay sensitivity, input DNA, or virus load per infected cell [16]. Although unlikely, differences in degree of immunosuppression could also contribute, especially considering that our patients were not receiving highly active antiretroviral therapies (HAART). Nonetheless, we did not find an association between HHV8 load and CD4 lymphocyte count in our study. Moreover, no such association has been reported among persons with AIDS-KS in the U.S. [18], suggesting immunosuppression may not be the major or only explanation of these differences. HHV8

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DNA detection was associated with high eosinophil percentage; perhaps co-infection with parasites may contribute. HHV8 load was inversely related to platelet count and to hemoglobin level. The inverse association of platelet count with HHV8 load was previously reported among HHV8 seropositive persons without KS in Italy [22], but the biological reasons for the association are unknown. The inverse association of peripheral blood HHV8 load with hemoglobin level is intriguing. *In vitro* studies have reported that hypoxia triggers latent HHV8 to enter lytic replication [23,24]. It is possible that anemia, which is a marker for tissue hypoxia, may lead to higher viral load by triggering HHV8 lytic replication [25].

Our study has limitations. First, the rate of KS progression and KS burden were measured by questionnaire and medical examination at one point in time, which is imprecise and possibly biased. However, these are conservative limitations because the imprecision would attenuate the associations and recall bias would not influence HHV8 load measurements. Second, the cross-sectional design does not allow us to infer temporal relationships. Finally, we lacked data on HIV load, which may independently contribute to severity of immunosuppression and possibly HHV8 load [3]. The strengths of our study include our ability to simultaneously assess rate of KS progression and KS burden using a simple method, which is applicable and can be replicated easily in studies in resource-constrained settings.

To conclude, we observed significant association between HHV8 load in peripheral blood and rate of KS progression, independent of KS burden. These findings suggest that HHV8 load in peripheral blood may be a marker for development of new KS lesions.

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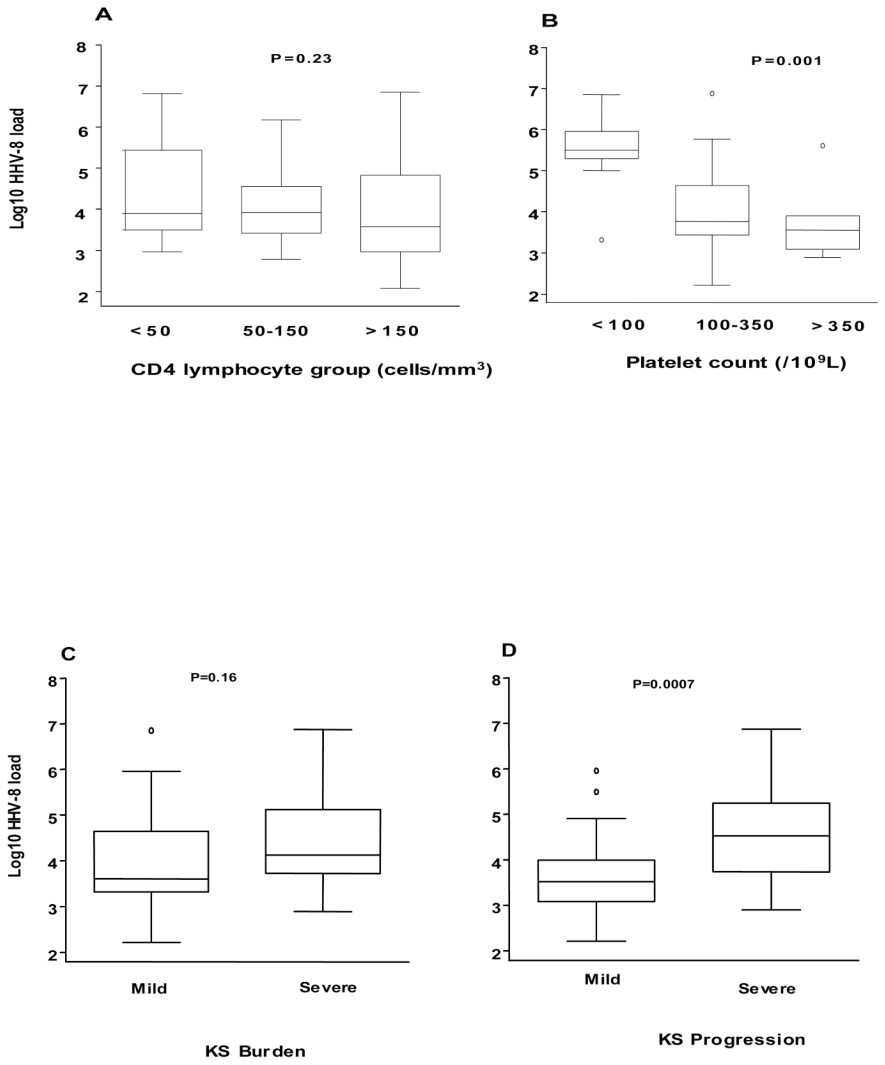

Figure 1.

Graph shows log copies of HHV8 load in peripheral blood association and selected characteristics. The mid-point bars mark the median, while the bars at the end of the lines 25th (lower) and 75th (upper) interquartile range. Panel A: HHV8 load according to CD4 lymphocyte count. Panel B: HHV8 load according platelet groups. Panel C: HHV8 load according KS burden. Panel C: HHV8 load according to KS progression.

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Table

Demographic and clinical characteristics with severity of Kaposi sarcoma

Characteristic	KS burden*			KS progression†		
	Mild	Severe	P value±	Slow	Rapid	P value
Gender			0.40			0.60
Male	22 (50%)	223 (50%)		15 (33%)	29 (67%)	
Female	18 (60%)	12 (40%)		12 (40%)	18 (60%)	
Age, years			0.37			0.36
<26	4 (57%)	3 (43%)		4 (57%)	3 (43%)	
26-35	15 (44%)	19 (56%)		10 (29%)	24 (71%)	
36-45	13 (59%)	9 (41%)		10 (45%)	12 (55%)	
46+	8 (73%)	3 (27%)		3 (27%)	8 (73%)	
Education			0.05			0.05
None or primary	12 (40%)	18 (60%)		7 (23%)	23 (77%)	
Senior secondary/higher	28 (64%)	16 (36%)		20 (45%)	24 (55%)	
Occupation			0.15±			0.02±
Peasant/farmer	8 (44%)	10 (56%)		4 (22%)	14 (78%)	
Trader/sales	5 (33%)	10 (67%)		2 (13%)	13 (87%)	
Skilled manual laborer	9 (56%)	7 (44%)		6 (38%)	10 (62%)	
Professional	8 (67%)	4 (33%)		8 (67%)	4 (33%)	
Household	10 (77%)	3 (23%)		7 (54%)	6 (46%)	
Platelets			0.26			0.05
Low (<100)	3 (33%)	6 (67%)		2 (22%)	7 (77%)	
Normal (100-350)	31 (54%)	26 (45%)		19 (33%)	38 (67%)	
High (>350)	6 (75%)	2 (25%)		6 (75%)	2 (25%)	
Eosinophil %			0.05			0.20
Normal (<4%)	22 (67%)	11 (33%)		13 (39%)	20 (61%)	
High	12 (57%)	9 (43%)		10 (48%)	11 (52%)	
Very High (>10%)	6 (32%)	13 (68%)		4 (21%)	15 (79%)	
Lymphocyte count			0.19			0.13
Normal (<4.0)	38 (53%)	34 (47%)		25 (35%)	47 (65%)	
High (4.0-6.0)	2 (100%)	0 (0%)		2 (100%)	0 (0%)	
CD4 lymphocyte count (cells/mm^3)			0.42			0.32
< 50	17 (55%)	14 (45%)		11 (36%)	20 (64%)	
50-150	7 (41%)	10 (58%)		4 (24%)	13 (76%)	
> 150	16 (62%)	10 (38%)		12 (46%)	14 (54%)	
Time since initial KS lesion			0.51			<0.001
6 months or less	19 (58%)	14 (42%)		4 (12%)	29 (88%)	
> 6 months	21 (50%)	21 (50%)		23 (55%)	19 (45%)	

*KS burden: Mild ≤20 lesions, Severe > 20 lesions

†KS progression: Slow ≤20 lesions/ year, Rapid > 20 lesions/year

±p-value for trend, unless marked in which case it is p-value for heterogeneity