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Kaposi Sarcoma-Associated Herpesvirus Serum DNA and Antibodies Not Associated With Subsequent Non-Hodgkin Lymphoma Risk

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Abstract

Kaposi sarcoma-associated herpes virus (KSHV) infects B-cells and is found in non-Hodgkin lymphoma (NHL) B-cell tumors and could therefore contribute to the occurrence of NHL. We performed a nested case-control study including 155 incident NHL cases and matched noncancer controls. Pre-NHL serum was tested for KSHV DNA and antibodies. Serum KSHV DNA was more common in cases than controls (14% versus 6%, $P = 0.03$), but after adjustment, the difference was not significant. Epstein-Barr virus serum DNA was similarly unassociated with NHL as were KSHV antibodies. KSHV is not a primary cause of NHL in HIV-infected men who have sex with men.

Keywords

Kaposi sarcoma-associated herpesvirus; non-Hodgkin lymphoma; MACS; human herpesvirus 8; DNA; AIDS cancer

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INTRODUCTION

Aggressive B-cell non-Hodgkin lymphoma (NHL) is the second most common cancer associated with HIV and is an AIDS-defining illness.^{1,2} The incidence of NHL in HIV-infected individuals has decreased with the introduction of effective antiretroviral therapy³ but remains elevated above the background incidence.^{4,5} Like Epstein-Barr virus (EBV), Kaposi sarcoma herpes virus (KSHV, also known as HHV8) is a B-cell-tropic virus thought to cause of a subset of AIDS-related NHL.^{6,7}

KSHV was originally discovered in studies of patients with Kaposi sarcoma (KS) and subsequently recognized to be associated with a rare subset of NHL, often presenting as lymphomatous effusions (primary effusion lymphoma).⁶ The prevalence of KSHV antibodies is low in the general US population (0–8%) but elevated in patients with HIV, particularly in men who have sex with men (MSM; 25–50%).^{6,8} Several studies have suggested a broader relationship between KSHV infection and AIDS-related NHL beyond that known for the clinically distinctive primary effusion lymphomas.^{9,10} However, initial studies did not identify an association between antibodies to KSHV and NHL risk in pre-¹¹ or postdiagnostic¹² NHL serum. However, KSHV serologic testing has limited sensitivity and specificity.¹³ KSHV DNA in serum is a more specific marker of KSHV infection. We investigated the association of KSHV DNA and KSHV antibodies with NHL risk within a well-established cohort of HIV-positive MSM.¹⁴

METHODS

Study Population And Study Design

We performed a nested case–control study of HIV-positive NHL cases identified within the Multicenter AIDS Cohort Study (MACS) between 1984 and 2003.¹⁵ The MACS is a multicenter cohort of HIV-infected and uninfected MSM that includes semiannual visits.^{14,16,17} Cases with sufficient banked serum available to perform viral DNA analyses in the 5 years before lymphoma diagnosis were studied. Each NHL case was individually matched by year of first HIV-positive visit (within 1 year) and duration of HIV infection at the time of NHL diagnosis to a HIV-positive man who did not develop NHL and had banked serum available. The questionnaires and protocols used in the MACS have been approved by the appropriate Institutional Review Boards, and informed consent was received for all participants.

For each participant, KSHV DNA was tested in stored serum from up to three time points before the date of NHL diagnosis: within 1 year, between 1.1 and 3 years, and between 3.1 and 5 years before diagnosis. Serum for the time point closest to diagnosis was evaluated for each participant, which included samples available from within 1 year before diagnosis in 45% of cases and 57% of controls. A second nested case–control analysis was performed among only cases who developed NHL after developing AIDS. For this second analysis, controls were selected among HIV-positive men who had also developed AIDS and were matched by time since AIDS diagnosis.

Laboratory Methods

Serum was tested for KSHV and EBV DNA using real-time quantitative polymerase chain reaction as described previously.¹⁸ The assay uses primers to amplify sequences from the K8 open reading frame and reproducibly detects as few as 10 copies of viral DNA per sample. KSHV serology data (antibodies against lytic KSHV proteins determined by immunofluorescence) were available for all subjects at MACS entry with baseline seronegatives repeatedly tested to detect seroconversion.¹⁹ KSHV seropositivity was defined as the presence of antibodies at any time before NHL diagnosis.

Study Statistics

Demographic characteristics were examined in cases and controls using chi square tests for categorical variables and a test of medians for continuous variables. Univariate and multivariate conditional logistic regression models were used. Conditional odds ratios (ORs) and 95% confident intervals (CIs) for the association of KSHV DNA with NHL were reported. KSHV and EBV serum DNA were measured in serum at multiple time points, when available, and results in the serum sample at the time point closest to diagnosis for each individual were evaluated.

Factors included in the final multivariate model were: age at diagnosis, absolute CD4 T-cell count at MACS study baseline, change in CD4 T-cell count defined as change from study baseline until diagnosis or the beginning of the effective antiretroviral therapy era (March 1996), whichever came first. For controls, change in CD4 cell count was calculated from study baseline until matched time at risk. All statistical tests were two-sided and considered significant at the $\alpha = 0.05$ level. Statistical tests were performed using STATA 10.0 (Stata Corp, College Station, TX).

RESULTS

There were 179 incident cases of NHL identified in the MACS between 1984 and 2003, of which 160 had prediagnostic serum available for KSHV DNA assays. Because our intent was to study only AIDS-related B-cell lymphomas, two T-cell lymphomas and three lymphoplasmacytic lymphomas were excluded, leaving 155 lymphomas for this analysis. NHL cases included 50 (32%) primary central nervous system lymphoma, 30 (19%) diffuse large B-cell lymphoma, 23 (14%) immunoblastic lymphoma, 21 (15%) Burkitts lymphoma, and 31 (20%) lymphomas not otherwise specified. None of the cases were classified as primary effusion lymphoma. Characteristics of cases and their matched controls are compared in Table 1. NHL cases were significantly more likely to have had KS and to have more advanced HIV disease, as indicated by lower CD4 cell count at study baseline and greater reduction in CD4 cell count over time than their matched controls (each $P < 0.05$; Table 1).

Detection of KSHV DNA in prediagnostic serum was more common among NHL cases than controls (14% versus 6%, $P = 0.03$). However, among cases and controls who had detectable KSHV DNA, the median KSHV viral load (406 versus 325, $P = 0.39$) was comparable. Detection of KSHV DNA was no longer significantly associated with odds of NHL (OR, 1.0; 95% CI, 0.39–2.8) after controlling for age, CD4 cell count at baseline, and change in CD4 cell count. Similar results were seen when analysis was restricted to 65 case–control pairs with serum samples within 1 year before diagnosis (Table 2), when a higher KSHV DNA copy number threshold (5 or greater or 25 or greater) was considered as positive and when adjustment was made for HIV viral load and EBV serum copy number (data not shown).

When men were stratified by history of KS, serum KSHV DNA prevalence was significantly higher among NHL cases than controls among the 254 participants without a history of KS (11% versus 4%, $P = 0.04$) but was similar among the 54 men with a history of KS (24% versus 25%, $P = 0.92$). However, KSHV DNA was no longer associated with incident NHL in multivariate analysis among either the 108 case–control pairs without KS (OR, 1.5; 95% CI, 0.30–7.2; Table 2) or in multivariate unconditional logistic regression among the 116 cases and 138 controls without a history of KS (OR, 2.0; 95% CI, 0.60–7.2). When NHL subtype was considered, KSHV DNA was not significantly associated with elevated odds of NHL among the 21 Burkitts lymphomas, the 23 immunoblastic

lymphomas, the 50 primary central nervous system lymphoma, or the 30 diffuse large B-cell lymphoma (data not shown).

In this HIV-infected population of MSM, the majority of subjects (79%) had detectable KSHV antibodies (Table 1) and seropositivity was not associated with NHL (Table 2). Among 253 men with KSHV antibody data, seropositivity was common among both the 27 men with and the 226 men without detectable KSHV serum DNA detected (93% and 77%, respectively). History of EBV infection was also common among these men, and having detectable serum EBV DNA was not more common in cases than controls (57% versus 53%, $P = 0.45$). Odds of KSHV serum DNA detection were not associated with lower CD4 cell count, higher HIV viral load, or serum EBV DNA detection.

Because the 155 NHL cases had more advanced HIV disease than controls matched on duration of HIV infection (Table 1), we performed a second case-control study on the subset of 76 NHL cases who developed NHL after AIDS diagnosis (AIDS-NHL) matched to AIDS controls by time since AIDS. Detectable serum KSHV DNA was not more common among AIDS-NHL cases than AIDS controls overall (14% versus 9%, $P = 0.32$) and was not associated with increased risk of AIDS-NHL (Table 2).

DISCUSSION

We found no significant independent association between KSHV or EBV DNA in prediagnostic serum with overall odds of NHL in this nested case-control study of HIV-infected MSM. KSHV DNA was detected in serum from a small number of both NHL cases and HIV infected who did not develop NHL. Although NHL cases were more likely to have KSHV serum DNA detected, this difference was no longer observed once differences in age and immune status were controlled for. The lack of association between KSHV DNA in prediagnostic serum and NHL risk in this study casts further doubt on any link between KSHV infection and overall NHL risk.

In previous studies among HIV-infected individuals, serum KSHV DNA has been shown to be virion (ie, encapsidated) DNA rather than free DNA released from NHL or KS tumor cells,^{18,20} consistent with active infection. Although KSHV is associated with risk of KS^{2,21} and rare NHL subtypes,^{22,23} case-control studies relying on KSHV antibodies^{11,12} as a marker of infection have not found an association with overall NHL. Several studies have also reported a possible association between EBV and AIDS-related NHL^{7,24}; however, EBV was not associated with NHL in this study.

This is one of the first studies to prospectively evaluate an association of KSHV with NHL specifically among individuals without a history of KS. The presence of KSHV DNA among individuals who have had KS is likely associated with KS disease, and therefore analyses that do not exclude subjects with a KS history may underestimate an association among individuals without KS. However, our study suggests KSHV is not associated with NHL risk irrespective of a history of KS.

Our study had several limitations. The majority of the men were HIV-seropositive on study entry, and because NHL cases had more advanced HIV disease at baseline than controls, we cannot exclude the possibility of residual confounding by stage of HIV disease. However, models were carefully adjusted for stage of disease including CD4 cell count at baseline as well as the change in CD4 cell count from baseline to diagnosis. In addition, similarly null results were observed in a second analysis that did control for stage of HIV disease because the cases and controls were matched by time since AIDS diagnosis. Serum was not available within 1 year of diagnosis for many of the cases, but results limited to those with serum from the year before diagnosis were similar to that observed overall. Although 155 NHL

cases were available, the analyses of NHL subtypes were likely underpowered to detect an effect of KSHV, and the lack of any patients with primary effusion lymphoma prevented us from addressing the relationship between that condition and KSHV infection or KSHV DNA markers.

Identification of a biomarker for NHL to assist in earlier diagnosis in high-risk populations is of considerable interest. Although this study does not exclude a role of KSHV in a small proportion of NHL cases, it suggests serum KSHV DNA does not have predictive use for NHL risk among MSM.

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Characteristics of Incident Non-Hodgkin Lymphoma (NHL) Cases and Matched Controls Among HIV-Infected Men in the Multicenter AIDS Cohort Study (MACS) Between 1984 and 2003

TABLE 1

Characteristics	NHL Cases		Controls*		P [†]
	N = 155	Percent	N = 155	Percent	
Race					0.13
White, not Hispanic	128	83%	134	86%	
Black, not Hispanic	9	6%	9	6%	
Hispanic	18	12%	7	5%	
Other	0	0%	5	3%	
Age at diagnosis, in years: median (IQR)	155	40 (36–46)	155	40 (35–44)	0.42
NHL diagnosis date (and matched control follow-up)					
1984–1990	43	28%	43	28%	1.00
1991–1995	95	61%	95	61%	
1996–2003	17	11%	17	11%	
History of Kaposi sarcoma					0.001
No	116	75%	138	90%	
Yes	38	25%	16	10%	
CD4 cell count at MACS baseline					<0.001
<200 cells/ μ L	22	14%	1	1%	
200–499 cells/ μ L	62	40%	48	31%	
$\geq$ 500 cells/ μ L	66	43%	105	68%	
Missing	5	3%	1	1%	
Change in CD4 cell count [‡]					<0.001
$\geq$ 1 cell increase	10	7%	26	17%	
0–100 cell decrease	82	53%	99	64%	
>100 cell decrease	43	28%	20	13%	
Missing	20	13%	10	7%	
Serum KSHV copy number per 100 μ L, among those with DNA detected: median (IQR)	22	406 (161–2298)	10	325 (123–417)	0.39
KSHV antibodies ever detected					0.33
No	23	15%	30	19%	

Characteristics	NHL Cases		Controls*		P [‡]
	N = 155	Percent	N = 155	Percent	
Yes	102	66%	98	63%	
Missing/indeterminate	30	19%	27	17%	
Serum KSHV DNA detected [§]					0.03
No	133	86%	145	94%	
Yes	22	14%	10	6%	
Serum EBV DNA detected [§]					0.45
No	63	42%	70	47%	
Yes	86	57%	80	53%	

IQR, interquartile range; KSHV, Kaposi sarcoma-associated herpesvirus; EBV, Epstein-Barr virus.

* One control was individually matched to each case by year of first HIV-positive visit and duration of HIV-positive time at risk of NHL/AIDS (± 1).

[‡] The change in CD4 cell count was calculated from the first MACS study CD4 data available until case diagnosis or the effective antiretroviral therapy era (1996), whichever came first.

[§] P value tested using chi square for categorical and test of medians for continuous data.

[§] Serum KSHV and EBV results are from the serum available at the closest time point before diagnosis. Results were similar when KSHV and EBV detection at each of the three time points before diagnosis were considered.

TABLE 2

Comparison of Detectable KSHV Serum DNA and KSHV Antibodies Among HIV-Positive Incident NHL Cases Compared With HIV-Positive Matched Controls

Serum KSHV	Number of Men	OR (95% CI)	
		Univariate	Multivariate [†]
Serum KSHV DNA copy number			
In serum sample closest to diagnosis	310	2.3 (1.1–5.1)	1.0 (0.39–2.8)
In subset with serum within 1 year before diagnosis	130	1.2 (0.37–3.9)	1.0 (0.18–5.9)
In subset not diagnosed with Kaposi Sarcoma	216	4.0 (1.13–14)	1.5 (0.30–7.2)
Among those with AIDS*	152	2.0 (0.60–6.6)	2.0 (0.30–14)
Serum KSHV antibodies	252	1.3 (0.68–2.5)	0.91 (0.39–2.2)

KSHV, Kaposi sarcoma-associated herpesvirus; NHL, non-Hodgkin lymphoma; OR, odds ratio; CI, confidence interval.

^{*} In the subset of cases in which NHL diagnosis occurred after AIDS was already diagnosed, a second analysis was performed using a separate set of controls matched on time since AIDS diagnosis.

[†] Model was adjusted for age, CD4 cell count at baseline, CD4 change from baseline to diagnosis, or 1996 (whichever came first).