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Age of Infection with Kaposi Sarcoma Associated Herpesvirus and Subsequent Antibody Values Among Children in Uganda

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Abstract

We investigated associations between KSHV seroconversion age and KSHV antibody values in Ugandan children. Every annual delay in KSHV seroconversion age was associated with a reduction of 19% ($p < 0.0001$) in K8.1 and 27% ($p < 0.0001$) in ORF73 antibody values at age 6. Early infection may be an important risk factor for KSHV pathogenesis and viral shedding in saliva, leading to transmission.

Keywords

Kaposi sarcoma associated herpesvirus; seroconversion age; antibody values; Uganda

Introduction

Transmission of Kaposi sarcoma associated herpesvirus (KSHV) is mainly through salivary exchange and can occur in childhood in endemic regions [1]; KSHV prevalence increases with age [2]. The impact of age of infection with KSHV on the pathogenesis and control of KSHV has not been investigated.

Early infection with Epstein-Barr virus (EBV), another gammaherpesvirus closely related to KSHV, is associated with higher subsequent viral load [3]. High viral capsid antigen (VCA) antibody titres and EBV viral load have been associated with risk of Burkitt lymphoma [4], the most common childhood malignancy in equatorial Africa, linked to both EBV and malaria [5].

High antibody titres to KSHV are an important predictor of risk of KS disease [6]; they are also a marker of KSHV reactivation [7]. This study was designed to determine the

association between the age at which children from Uganda become KSHV seropositive (KSHV seroconversion age) and subsequent KSHV-specific IgG antibody values.

Methods

Study population

Samples collected from children enrolled in the Entebbe Mother and Baby Study (EMaBS), were tested for KSHV IgG antibody responses retrospectively. EMaBS was initiated as a randomised controlled trial, designed to investigate the impact of helminth treatment during pregnancy on childhood responses to vaccines and infectious diseases. The trial protocol and results have been described elsewhere [8]. A total of 2507 pregnant women from Entebbe, Uganda, a semi-urban area, were recruited and their children have been followed from birth. Blood and other samples have been collected annually and stored.

Ethical approval

This study was approved by the Uganda Virus Research Institute - Research and Ethics Committee (UVRI-REC), the Uganda National Council for Science and Technology (UNCST) and the London School of Hygiene & Tropical Medicine. Informed consent was obtained from study participants' parents or guardians.

KSHV serologic testing

Plasma samples collected at age 6 years (annual 6) were tested for KSHV IgG antibodies to identify KSHV seropositive children. KSHV sero-positivity was defined by sero-positivity to either ORF73 or K8.1 antigen. Any seropositive child was then tested at age 5, and so on retrospectively, until a seronegative specimen was identified. To determine if the effect of age at infection on subsequent antibody values is sustained for a longer time period, we then tested the available plasma samples at age 9 from the children who were seropositive at age 6 for IgG antibody responses to KSHV. The estimated age of KSHV seroconversion was defined as the midpoint between the last seronegative and the first seropositive specimen. Because of missing specimens, these samples were not always from consecutive years.

An in-house multiplexed bead assay was used to measure KSHV-specific IgG antibody responses as previously described [9]. This assay has a wider dynamic range than an ELISA which is an important advantage when comparing antibody values. ORF73 and K8.1 recombinant proteins were coupled to fluorescent magnetic beads (Biorad, Hercules, CA) according to the manufacturer's protocol. Coupled beads were mixed with plasma samples at a sample dilution of 1/200 and a bead concentration of 2,000 beads per well in assay/wash buffer (1% BSA/bovine serum albumin in 1XPBS/phosphate buffered saline), to make a total volume of 100µl per well. The mixture was incubated for an hour under gentle agitation and washed with wash buffer thereafter. 100µl of 0.5µl/ml of detection antibody (goat F(ab')² anti-human IgG R-PE conjugate) was added and incubated for 30 minutes under gentle agitation. After washing, 100µl of assay buffer was added per well, agitated for 2 minutes and the plate read using a Bioplex200 machine (Luminexcorp, USA) to obtain the Median Fluorescence Intensities (MFI). Each plate contained 3 negative and 3 positive control wells

plus 2 blank wells. The cutoff MFI values for ORF73 and K8.1 were 968 and 741 respectively plus the mean values of the negative control per plate.

Statistical analysis

Data analysis was performed using Stata-13 software (STATA 13.0, Statacorp, College Station, USA). Antibody values (measured as mean fluorescent intensities, MFI) were log₁₀ transformed. Linear regression was used to examine the relationship between KSHV IgG antibody responses at ages 6 and 9 and seroconversion age while adjusting for sex. To investigate the association between seroconversion age and antibody values (KSHV IgG antibodies) at all timepoints/ages (1, 2, 3, 4, 5, 6, & 9), we used mixed models with random effects. Random effects modelling provided associations which, were independent of the duration of infection; and accounted for correlation of results from the same child at different time points. Geometric Mean Ratios (GMR) and their 95% confidence intervals (CI) were obtained by calculating the log₁₀ exponent of the regression coefficients and their 95% CI respectively. Antibodies to K8.1 and ORF73 were analysed using separate regression models.

Results

Age at KSHV seroconversion

The number of children who were KSHV seropositive at age 6 were 176/535 (33%), 128/535 (24%) were seropositive to K8.1, and 165/535 (31%) were seropositive to ORF73 proteins. The number of children with all 6 consecutive samples were 100/176. Therefore 76/176 children had at least 1 missing sample, 39, 11, 5, 3 and 18 had 1, 2, 3, 4 and 5 missing samples respectively. The 18 children with 5 consecutive missing samples were excluded from the analysis, leaving a total of 158 children for analysis. Results from 100 participants with all 6 consecutive samples (Table AII and CII) were comparable to those from 158 participants (Table AI and CI) at age 6 and at all ages combined. At age 9, the available samples from the 100 participants were 79 which might have reduced the power of the study to detect statistically significant differences (Table BII). Among the 158 KSHV seropositive 6 year old children analysed, 43, 50, 23, 14, 18 and 10 children were estimated to have seroconverted by ages 6, 5, 4, 3, 2 and 1 respectively. The proportions of seroconverters who were boys at the different seroconversion age bands were 22/43 (51%), 26/50 (52%), 14/23 (61%), 8/14 (57%), 10/18 (56%), 8/10 (80%) at 6, 5, 4, 3, 2 and 1 respectively. Antibody values increased with age. For every annual increase in age, we observed a 71% ($p<0.0001$) and 65% ($p<0.0001$) increase in K8.1 and ORF73 IgG antibody values respectively (Table 1CI). While median antibody values generally decreased with increasing age at infection (supplementary Figure 1).

We investigated the association between age at KSHV seroconversion and antibody values to K8.1 and ORF73 antigens at ages 6 and 9 years using sex-adjusted linear regression modelling. At age 6, antibody values decreased with increasing seroconversion age. For every year of delay in seroconversion age, we observed a 19% decrease in K8.1 antibody values, with an adjusted Geometric Mean Ratio/aGMR of 0.81, 95% CI (0.73 – 0.91), $p=0.001$ (Table 1AI). In addition, for every year of delay in seroconversion age, we observed a 27%

decrease in ORF73 antibody values, with an aGMR of 0.73, 95% CI (0.64 - 0.83), $p < 0.0001$ (Table 1AI).

At age 9, both K8.1 and ORF73 antibody values decreased with increasing seroconversion age. For every year of delay in seroconversion age, we observed a 33% decrease in K8.1 antibody values, with an adjusted Geometric Mean Ratio/aGMR of 0.67, 95% CI (0.57 – 0.79), $p > 0.0001$ (Table 1BI). Similarly, for every year of delay in seroconversion age, we observed a 25% decrease in ORF73 antibody values, with an aGMR of 0.75, 95% CI (0.66 - 0.85), $p < 0.0001$ (Table 1BI).

To determine the effect of age of infection on antibody titres which is independent of duration of infection, we then investigated the association between seroconversion age and antibody values at all ages/timepoints (1, 2, 3, 4, 5, 6 & 9) to K8.1 and ORF73 using random effects models adjusting for sex and age. Generally, antibody values to both K8.1 and ORF73 decreased with increasing seroconversion age. For every year of delay in seroconversion, we detected a 40% decrease in K8.1 antibody values, a GMR 0.60, 95% CI (0.55 - 0.65), $p < 0.0001$ (Table 1CI). Similarly, for every year of delay in seroconversion we observed a 41% reduction in ORF73 IgG antibody values, aGMR 0.59, 95% CI (0.53 – 0.64), $p < 0.0001$ (Table 1CI).

Discussion

In KSHV endemic areas, infection can occur early in life, but the importance of age of infection to subsequent transmission and disease risk has not been investigated before. Antibody responses to KSHV, and in particular, values of antibodies, have been associated with KS development, KSHV reactivation and KSHV transmission[6, 7]. In this study, we have observed very early seroconversions, and detected a strong association between age of KSHV seroconversion and subsequent antibody values to both K8.1 and ORF73. The earlier these children seroconverted, the higher their subsequent antibody values to both K8.1 and ORF73 proteins. To our knowledge, this is the first study to look at the effect of age of infection with KSHV on subsequent antibody responses. Antibody responses are a proxy measure of KSHV reactivation [6]. K8.1 is a glycoprotein expressed during the lytic phase of the virus life cycle and ORF73 encodes the Latently Associated Nuclear Protein (LANA), a structural protein expressed during the latent stage of the virus life cycle. Measurement of other parameters related to disease and transmission risk such as viral load in saliva and in blood in relation to KSHV age of infection would be of great interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Association between age of KSHV seroconversion, sex, age and KSHV antibody levels among children from Uganda

AI: Antibody levels at age 6 years from 158 children						
K8.1			ORF73			
Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Crude GMR [*] (95% CI)	Adjusted** GMR (95% CI)	P
Seroconversion age	0.81 (0.72 – 0.90)	<0.0001	0.81 (0.73 – 0.91)	<0.0001	0.73 (0.64 – 0.83)	<0.0001
AII: Antibody levels at age 6 years from 100 children with all consecutive samples						
K8.1			ORF73			
Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Crude GMR [*] (95% CI)	Adjusted** GMR (95% CI)	P
Seroconversion age	0.78 (0.69 – 0.89)	<0.0001	0.80 (0.70 – 0.37)	0.001	0.69 (0.59 – 0.81)	<0.0001
BII: Antibody levels at age 9 years from 158 children						
K8.1			ORF73			
Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Crude GMR [*] (95% CI)	Adjusted** GMR (95% CI)	P
Seroconversion age	0.67 (0.57 – 0.79)	<0.0001	0.67 (0.57 – 0.79)	<0.0001	0.75 (0.66 – 0.85)	<0.0001
BII: Antibody levels at age 9 years from 100 children with all consecutive samples						
K8.1			ORF73			
Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Crude GMR [*] (95% CI)	Adjusted** GMR (95% CI)	P
Seroconversion age	0.96 (0.78 – 1.18)	0.691	0.95 (0.77 – 1.17)	0.620	1.03 (0.88 – 1.19)	0.740
CII: Antibody levels at ages 1, 2, 3, 4, 5, 6 and 9 years from 158 children						
K8.1			ORF73			
Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Crude GMR [*] (95% CI)	Adjusted GMR** (95% CI)	P
Seroconversion age	0.72 (0.66 – 0.78)	<0.0001	0.60 (0.55 – 0.65)	<0.0001	0.71 (0.65 – 0.77)	<0.0001
Age	0.93 (0.85– 0.99)	0.018	1.70 (1.57 – 1.85)	<0.0001	0.82 (0.76 – 0.88)	<0.0001
CII: Antibody levels at ages 1, 2, 3, 4, 5, 6 and 9 years from 100 children with all consecutive samples						
K8.1			ORF73			

	Crude GMR [*] (95% CI)	P	Adjusted** GMR (95% CI)	P	Adjusted GMR** (95% CI)	P
Seroconversion age	0.80 (0.73 – 0.87)	<0.0001	0.73 (0.67 – 0.80)	<0.0001	0.68 (0.62 – 0.75)	<0.0001
Age	1.09 (1.02 – 1.17)	0.008	1.20 (1.12 – 1.28)	<0.0001	1.29 (1.22 – 1.37)	<0.0001

Table 1 A: shows the association between age of KSHV seroconversion, sex and KSHV antibody levels at age 6. **Adjusted for sex and seroconversion age.

Table 1 B: shows the association between age of KSHV seroconversion, sex and KSHV antibody levels at age 9. **Adjusted for sex and seroconversion age.

Table 1 C: shows the association between age of KSHV seroconversion, sex, age and KSHV antibody levels among children at ages 1, 2, 3, 4, 5, 6 and 9 combined from Uganda. **Adjusted for age, sex and seroconversion age.

Tables AI and CII show results from 100 children with all 6 consecutive samples plus 58 children missing 1 to 4 samples. Tables AII and CII show results from 100 children with all 6 consecutive samples. Table BI show results from 130 children with samples available at age 9years. Table BII show results from 79 children with available samples at age 9, and have all previous consecutive samples.

* GMR (Geometric Mean Ratio). GMR and 95% CI (confidence interval) obtained by calculating the log10 exponent of the regression coefficient and the 95% CI respectively. Regression coefficient, 95% CI and P value were obtained using linear regression after log10 transformation of K8.1 and ORF73 specific total IgG MFI (Median Fluorescence Intensity). MFI obtained using the bead assay. K8.1 and ORF73 IgG antibody responses were analysed using separate linear regression models. KSHV: Kaposi sarcoma associated herpesvirus. Seroconversion age is the age at which the children became KSHV seropositive. Age is the number of years the children had lived by the time of sample collection.