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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 Kaposi sarcoma–associated herpesvirus (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 6 years of age. Early infection may be an important risk factor for KSHV pathogenesis and viral shedding in saliva, leading to transmission.

Key Words: Kaposi sarcoma–associated herpesvirus, seroconversion age, antibody values, Uganda

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 antibody titers 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 titers to KSHV are an important predictors of risk of Kaposi's sarcoma (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 immunoglobulin G (IgG) antibody values.

METHODS

Study Population

Samples collected from children enrolled in the Entebbe Mother and Baby Study were tested for KSHV IgG antibody responses retrospectively. Entebbe Mother and Baby Study was initiated as a randomized 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 semiurban 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, the Uganda National Council for Science and Technology 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 6 years of age (annual 6) were tested for KSHV IgG antibodies to identify KSHV seropositive children. KSHV seropositivity was defined by seropositivity 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

TABLE 1. Association Between Age of KSHV Seroconversion, Sex, Age and KSHV Antibody Levels Among Children From Uganda

	K8.1				ORF73			

AI: Antibody Levels at Age 6 yr From 158 Children

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted* GMR (95% CI)	P	Crude GMR (95% CI)	P	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	0.73 (0.64–0.83)	<0.0001

AII: Antibody Levels at Age 6 yr From 100 Children With All Consecutive Samples

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted* GMR (95% CI)	P	Crude GMR (95% CI)	P	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	0.68 (0.58–0.80)	<0.0001

BI: Antibody Levels at Age 9 yr From 158 Children

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted* GMR (95% CI)	P	Crude GMR (95% CI)	P	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	0.75 (0.66–0.85)	<0.0001

BII: Antibody Levels at Age 9 yr From 100 Children With All Consecutive Samples

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted* GMR (95% CI)	P	Crude GMR (95% CI)	P	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	1.03 (0.88–1.20)	0.737

CI: Antibody Levels at Ages 1, 2, 3, 4, 5, 6 and 9 yr From 158 Children

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted† GMR (95% CI)	P	Crude GMR (95% CI)	P	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	0.59 (0.53–0.64)	<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	1.65 (1.53–1.77)	<0.0001

CII: Antibody Levels at Ages 1, 2, 3, 4, 5, 6 and 9 yr From 100 Children With All Consecutive Samples

	K8.1				ORF73			
	Crude GMR (95% CI)	P	Adjusted† GMR (95% CI)	P	Crude 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	0.78 (0.72–0.85)	<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	1.22 (1.15–1.03)	<0.0001	1.29 (1.22–1.37)	<0.0001

*Adjusted for sex and seroconversion age.
†Adjusted for age, sex and seroconversion age.

AI and AII show the association between age of KSHV seroconversion, sex and KSHV antibody levels at age 6. BI and BII show the association between age of KSHV seroconversion, sex and KSHV antibody levels at age 9. CI and CII show 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. AI and CII show results from 100 children with all 6 consecutive samples plus 58 children missing 1–4 samples. AII and CII show results from 100 children with all 6 consecutive samples. BI shows results from 130 children with samples available at age 9. BII shows results from 79 children with available samples at age 9, and have all previous consecutive samples. GMR and 95% CI obtained by calculating the $\log_{10}$ exponent of the regression coefficient and the 95% CI, respectively. Regression coefficient, 95% CI and P value were obtained using linear regression after $\log_{10}$ transformation of K8.1- and ORF73-specific total IgG MFI. MFI was obtained using the bead assay. K8.1 and ORF73 IgG antibody responses were analyzed using separate linear regression models. 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.

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 enzyme-linked immunosorbent assay 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 2000 beads per well in assay/wash buffer (1% bovine serum albumin in 1 × phosphate buffered saline), to make a total volume of 100 μ per well. The mixture was incubated for an hour under gentle agitation and washed with wash buffer thereafter. Detection antibody of 100 μ of 0.5μL/mL [goat F(ab′)² antihuman IgG R-phycoerythrin (R-PE) conjugate] was added and incubated for 30 minutes under gentle agitation. After washing, 100 μ of assay

buffer was added per well, agitated for 2 minutes and the plate read using a Bioplex200 machine (Luminexcorp, Austin, TX) to obtain the median fluorescence intensities (MFIs). Each plate contained 3 negative and 3 positive control wells plus 2 blank wells. The cutoff MFI values for OFR73 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, TX). Antibody values (measured as MFIs) were log_{10} transformed. Linear regression was used to examine the relationship between KSHV IgG antibody responses at 6 and 9 years of age and seroconversion age while adjusting for sex. To investigate the association between seroconversion age and antibody values (KSHV IgG antibodies) at all time-points/ages (1, 2, 3, 4, 5, 6 and 9), we used mixed models with random effects. Random-effects modeling provided associations that were independent of the duration of infection and accounted for correlation of results from the same child at different time points. Geometric mean ratios (GMRs) and their 95% confidence intervals (CIs) were obtained by calculating the log_{10} exponent of the regression coefficients and their 95% CI, respectively. Antibodies to K8.1 and ORF73 were analyzed using separate regression models.

RESULTS

Age at KSHV Seroconversion

The number of children who were KSHV seropositive at 6 years of age was 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 was 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 1, AII and CII) were comparable to those from 158 participants (Table 1, AI and CI) at 6 years of age and at all ages combined. At 9 years of age, the available samples from the 100 participants were 79 which might have reduced the power of the study to detect statistically significant differences (Table 1, BII). Among the 158 KSHV seropositive 6-year-old children analyzed, 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%) and 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 1, CI). On the other hand median antibody values generally decreased with increasing age at primary infection (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C950).

We investigated the association between age at KSHV seroconversion and antibody values to K8.1 and ORF73 antigens at 6 and 9 years of age using sex-adjusted linear regression modeling. At 6 years of age, 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 GMR (aGMR) of 0.81, 95% CI (0.73–0.91), $P = 0.001$ (Table 1, AI). 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 1, AI).

At 9 years of age, 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 aGMR of 0.67, 95% CI (0.57–0.79), $P > 0.0001$ (Table 1, BI). 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 1, BI).

To determine the effect of age of infection on antibody titers 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 and 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, aGMR 0.60, 95% CI (0.55–0.65), $P < 0.0001$ (Table 1, CI). 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 1, CI).

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, 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.

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MACRO- AND MICROVASCULAR PARAMETERS AFTER TOXIC SHOCK SYNDROME

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Abstract: Whether individuals who had toxic shock syndrome in childhood have differences in macro- and retinal microvascular parameters indicative of increased cardiovascular risk is unknown. We found no evidence of adverse macrovascular changes in 22 toxic shock syndrome participants compared with 60 control participants. Microvascular comparisons showed a reduction in retinal total fractal dimension, which has been associated with cardiovascular risk factors in children.

Key Words: toxic shock syndrome, intima-media thickness, arterial stiffness, microcirculation

Inflammation is central to the development of atherosclerosis, and pathogenic lesions in arteries are reported from early childhood onward.[1] Children with acute and chronic inflammatory diseases, including Kawasaki disease, inflammatory bowel disease, psoriasis and juvenile arthritis may have persistent adverse changes in their vascular structure and function, although the implications for later cardiovascular disease risk are largely unknown.[2–4] Toxic shock syndrome (TSS) is a rare, acute, life-threatening toxin-mediated illness causing fulminant vascular inflammation and dysfunction, often resulting in multiorgan failure. It is unknown whether children with TSS have changes in either the macro- or microvasculature years after the acute illness and are at increased long-term cardiovascular risk.

We aimed to investigate whether participants with past TSS have quantitative subclinical changes in macro- and retinal microvascular parameters indicative of a possible increased in cardiovascular risk. The retinal and coronary vasculature share many similarities and respond to common metabolic risk factors.[5] We hypothesized that, compared with control participants, TSS participants have a more adverse macrovascular profile [eg, increased carotid and aortic intima-media thickness (IMT), increased pulse wave velocity (PWV) and decreased carotid artery distensibility and compliance] and retinal microvascular profile (eg, wider venules, narrower arterioles, reduced fractal dimension and increased tortuosity).

PATIENTS AND METHODS

We performed a case-control study including participants aged 6–30 years who had TSS at least 2 years previously and control participants of similar age and sex, recruited from The Royal Children's Hospital Melbourne and Monash Medical Centre, Melbourne, Australia. Cases fulfilled the Centers for Disease Control and Prevention case definition for either probable or definite TSS.[6] Exclusion criteria were pregnancy, diabetes, known atherosclerotic cardiovascular disease, treatment for hypertension and/or hyperlipidemia and chronic auto-immune inflammatory conditions. The study was approved by the human research ethics committee of both hospitals, and written informed consent was obtained from the parents or adult participants.

All data were collected at a single visit after a minimum of 6 hours of fasting. Demographic data and anthropometric measurements (BC 418, Tanita, Tokyo, Japan) were obtained. Pubertal status was based on self-reported Tanner stage. The mean of 3 blood pressure measurements (SphygmoCor XCEL, AtCor Medical, NSW, Australia) was recorded. Blood was collected for measurement of high-sensitivity C-reactive protein (Abbott Architect, IL), glucose, triglycerides, total cholesterol, high density lipoprotein and low density lipoprotein cholesterol (Vitros 5600, Ortho-Clinical Diagnostics, NJ) during the study visit.

Carotid and Aortic Intima-Media Thickness

Ultrasound images of the carotid artery and the abdominal aorta were acquired using Vivid i (General Electronics Healthcare, Little Chalfont, UK) with simultaneous electrocardiogram (ECG) gating as previously described.[7,8] Cine loops of at least 5 cardiac cycles focused on the intima-media complex of the posterior wall of the right common carotid artery 1 cm proximal to the carotid bulb were recorded for offline analysis. Imaging was focused on the distal 10–15 mm of the abdominal aorta.

The IMT of the far wall 1 cm from the carotid bulb was measured at end diastole using a semiautomated software, Carotid Analyzer for Research (Medical imaging applications LLC, Coralville, IA). The "mean IMT" refers to the average IMT in the selected area of measurement while the "maximum IMT" to the thickest IMT measurement within that segment.[8] The mean of these "mean IMT" or "maximum IMT" measurements from 5 end-diastolic frames was used in analyses.[8]

The 5 best-quality frames of the abdominal aorta were selected from the recorded cine loops for analysis using the same procedure. All IMT measurements were performed by a single grader blinded to subject status. The intrarater reliability was assessed on 10 masked subjects. Intraclass correlation for carotid IMT and aortic IMT was 0.92 [95% confidence interval (CI): 0.69–0.98] and 0.89 (95% CI: 0.59–0.97), respectively.