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Kaposi's sarcoma associated herpesvirus in a rural Ugandan cohort: 1992-2008

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Summary

We found that KSHV seroprevalence and antibody titres in this long-standing rural Ugandan cohort are the highest yet reported and changed little over time, perhaps reflecting frequent viral reactivation and persistently elevated transmission and risk of developing Kaposi's sarcoma.

Abstract

Background: The prevalence and titres of antibodies against Kaposi's sarcoma associated herpesvirus (KSHV) in rural Africa are not completely understood, nor are their trends over time in populations in which HIV is also endemic. We examined prevalence, titres, temporal trends and determinants of anti KSHV antibodies in each of three time periods (1990-91, 1999-2000 and 2007-2008) within a long-standing, rural population-based cohort in southwestern Uganda.

Methods: For each period, we measured antibodies to the K8.1 and ORF73 KSHV antigens in ~ 3000 people of all ages (1:1 sex ratio).

Results: In all periods, KSHV prevalence increased rapidly through childhood to ~ 90% by age 15 years, plateauing at ~ 95% thereafter. Similarly, antibody titres, particularly against the lytic antigen K8.1, were amongst the highest seen and increased significantly with age, suggesting sustained viral replication in this population. Male sex was also independently associated with higher prevalence, whereas HIV co-infection was not. A modest reduction in prevalence among children was noted in the most recent period.

Discussion: KSHV seroprevalence and antibodies titres in this rural Ugandan population are the highest yet reported, perhaps reflecting frequent viral reactivation and persistently elevated transmission.

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8) is the causative agent of Kaposi's sarcoma (KS) [1, 2]. Unlike other human herpesviruses, KSHV is not ubiquitous in human populations, but varies in prevalence geographically and in HIV risk groups [3]. Incidence of KS in both HIV-infected and uninfected populations largely reflect these differences in KSHV prevalence.

KS was a relatively common malignancy in parts of sub-Saharan Africa prior to the AIDS epidemic and its incidence increased dramatically as HIV spread, becoming the commonest malignancy in countries such as Uganda [4]. The epidemiology of KSHV and KS in Uganda has been studied by ourselves [5-7] and others [8-10] but much remains to be elucidated. Specifically, few studies have addressed the effects of the changing HIV epidemic on KSHV over time and most studies have been based in urban hospitals and clinics, rather than in rural populations.

The General Population Cohort (GPC) was established in rural southwestern Uganda as a HIV natural history study[11]. It is a population-based cohort study in which individuals of all ages and both sexes in a defined geographical community have been followed (with yearly visits and blood sampling) for over 20 years. We sought to investigate the prevalence and determinants of KSHV infection in this cohort and, any potential changes over time.

Methods

The General Population Cohort (GPC) in Kyamulibwa, South West Uganda was established by the UK Medical Research Council and the Uganda Virus Research Institute (UVRI) in 1989 to study the dynamics of HIV in a typical rural Ugandan population [12]. More recently, research activity has broadened to include the epidemiology and genetics of other communicable and of non-communicable diseases (NCDs), including cancer, cardio-vascular disease and diabetes [11].

In brief, the GPC is a community-based open cohort study of residents of neighbouring villages within a sub-county, lying about 40km from the shores of Lake Victoria. The population is scattered across the country-side in villages defined by administrative boundaries with a few concentrated in small trading centres. A population of approximately 10,000 people in a cluster of 15 adjacent villages was studied from 1989 to 1999. In 2000 the GPC was expanded to cover a further 10 villages. The cohort is dynamic with new births, deaths and migration reported at each round of follow-up and the current population under survey includes approximately 22,000 people. Data are collected though an annual census, questionnaire and serological survey. Details of sexual behaviour, medical, sociodemographic and geographic factors are recorded. Blood specimens are obtained at each annual survey. Serum is tested for HIV-1 and the remainder is stored at -80°C in Entebbe (Uganda Virus Research Institute). Since the start of the study, the seroprevalence of HIV in the study population has remained relatively stable at about 8% [11].

We estimated KSHV seroprevalence over the last 20 years from three cross-sectional samples of participants, at one each of three time points. Census rounds 3 (1991-‘92), 11 (1999-2000) and 19 (2007-‘08) were chosen, based on the availability of samples for testing and the inclusion of

children in those rounds. At each time point, we randomly sampled individuals from four age classes: 0-14, 15-24, 25-44 and 45 and older; 1200, 600, 600, and 600 people respectively (males and females in equal ratio) were selected. We restricted our selection to people living in the 15 villages included the study from its inception. In these villages, the population size and the age and sex structure has remained stable during the study period.

We selected a total of 9112 samples, 3112 in round 3 and 3000 each in rounds 11 and 19. Sampling was independent in each of the rounds, and because there were no restrictions on resampling, overall we sampled 7601 individuals; 1101 were randomly sampled twice and 205 three times. For a small number of selected individuals, serum specimens were unavailable; in such cases, another participant was randomly selected from the remaining individuals of the same round, age class, and sex.

Antibodies to KSHV K8.1 and ORF73 (LANA) were determined by recombinant protein ELISA as previously described[13]. Samples were considered seropositive if they were positive for either antigen. A random selection of 521 samples were also tested using previously described peptide EIAs for K8.1 and ORF 73 [14] [15]. A total of 1100 samples from HIV- uninfected individuals in round 19 were selected for titration based on the K8.1 optical density distribution: 300, 250, 150, 150 and 150 samples from the highest to lowest quintiles. KSHV K8.1 and ORF 73 antibodies were titrated by recombinant assays as previously described [16].

Multivariate hierarchical logistic regression models were utilized when examining prevalence; for antibody levels (titres as number of doubling dilutions and log-transformed optical densities, OD), linear models were used. Statistical significance was determined using likelihood ratio tests; all p values were two-sided. Nonparametric correlations were assessed between OD

obtained with protein ELISA and peptide EIA, and between OD and titres. Assay agreement was evaluated with Cohen's kappa statistic. Analyses were carried out using StataSE v13 (StataCorp LP, College Station, TX).

Ethical approval for this study was granted by the Uganda Virus Research Institute Research Ethics Committee and by the Uganda National Council for Science and Technology.

Results

Characteristics of the study sample are shown in Table 1. Overall, median age was 18.7 years (IQR 10.7-39.5) and was slightly, but significantly lower in 2000 and 2008 than in 1992; there were also statistically significant, albeit minor differences between rounds in the number of participants per village (median 218; IQR 191-263) and per household (median 2; IQR 2-4).

In each round, crude KSHV prevalence rose rapidly in childhood reaching about 90% by aged 15 years in all rounds (Figure 1A). Crude HIV prevalence was very low among children; over time, it tended to decrease in young adults, while in older adults it was essentially stable or tended to increase (Figure 1B). Adjusting for age, sex, HIV serostatus and round, KSHV prevalence increased with age (p trend <0.001) and was significantly higher in males than in females (p <0.001), regardless of age. There was no significant interaction between sex and age group. Compared to 1992, prevalence was higher in 2000 and lower in 2008 in adjusted models(Table 2). The decline in crude KSHV prevalence in the last period was most pronounced in children (Figure 1A, Table S1); Table S2 presents results of adjusted analyses by narrowed age categories in children. HIV seropositivity was not independently associated with KSHV seroprevalence in adjusted models.

The prevalence of KSHV in this study was higher than in several previous reports. To exclude potential assay-related misclassification, we repeated KSHV serology on a subsample, utilising peptide based EIAs used by various research groups [14, 15]. We have previously observed that, for US populations, peptide EIAs are less sensitive than protein ELISAs (Whitby and Dollard, unpublished observations) especially for ORF 73, which is a complex protein with many non-

linear epitopes [17]. In this subset, the seropositivity for K8.1 was 89% by recombinant protein ELISA and 92% by peptide EIA, while the seropositivity for ORF73 was 89% by recombinant protein ELISA and 69% by peptide EIA (Figure S1). Inter-test agreement was 95% for K8.1and 72% for ORF73, (κ=0.7 and κ=0.3, respectively). Inter-assay correlation was good for ORF73 (κ= 0.51) and excellent for K8.1(κ 0.80). These confirmatory data with alternative assays provide reassurance that our data represent valid prevalence estimates for this population.

We examined the distribution of antibody levels, as measured by OD, across the study population and saw that the pattern was strikingly different from any other population we had observed, with considerably more subjects having high OD levels for both ORF 73 and K8.1. Figure S2 compares OD distributions from this study to those observed in a prior large multinational population-based study conducted using the same ELISAs[18]. The pattern was most dramatic for K8.1. In multivariate models including age, sex, round and HIV infection, OD increased significantly with age for both K8.1 and ORF 73 (p trend<0.001, Table 3, Figure 2). OD were also significantly higher for both antigens in males compared to females (p<0.001) and in 2000 compared to 1992, and tended towards a decrease in 2008. K8.1 and ORF73 OD were slightly, but significantly lower in HIV seropositive individuals compared to HIV negative persons.

To further examine antibody levels, we selected another sub-sample of 1100 HIV-seronegative specimens from 2008 with a wide range of OD and performed ELISA titrations for ORF 73 and K8.1. As expected, OD was an imperfect surrogate measure for titre, underestimating the antibody level at the highest titres because of the limited dynamic range of ELISA (Figure S3). However, analysing titres yielded findings that differed little from the results obtained with OD

(Table S3 and S4, Figure S4) except that ORF73 titres did not differ between sexes after adjusting for age.

Discussion

Our study demonstrates that in a rural population in South West Uganda, KSHV prevalence is higher than in any other population reported to date and that this population also has strikingly high levels of KSHV antibodies. Since both the prevalence and the antibody levels seen in this population are so much higher than has been previously reported for Uganda[19-21], we corroborated our findings on subsets of samples using peptide EIAs and ELISA titration. These confirmatory assays reinforce the validity of our data.

The disparity between the results of the current study (prevalence >90%) and previously reported prevalence estimates (~40-60%) can possibly be explained in part by differences in antigens and assay formats (recombinant ELISA and peptide EIA vs. immunofluorescent assays) or cut-offs, but also are likely to reflect the study population and design. Most previous studies have been conducted in clinics or hospitals and in urban communities and have recruited participants in a relatively narrow age range and selected socioeconomic and health status[9, 22, 23], or have included only a small sample size[8]. Strengths of our study design include the large sample size (>9000 specimens in total) and the complete enumeration of this rural population, which has been followed for more than two decades. This allowed for the unbiased recruitment of participants of both sexes and all ages and health conditions, rather than selected groups that can be studied in ante-natal clinics or other health centres.

KSHV transmission is known to vary geographically even within a region, for example in Northern Italy's Po Valley, significant differences were observed in KSHV prevalence in elderly people living in the district in which the Po and Oglio rivers converged compared to an adjacent district without a major river [24]. Similarly, in the Gauteng province of South Africa, KSHV

prevalence varied considerably between ante-natal clinics reflecting local geographic variations [25]. Ecological and lifestyle factors may underlie these local variations. In a country such as Uganda, geographic differences [26, 27], are likely to be greatest between urban and rural settings. It should be noted that 75% of the population of Uganda lives in rural areas, according to the 2014 national census. Previously reported co-factors for KSHV transmission include malaria and other parasitic infections [7, 28, 29], use of surface water [6] and HIV infection [28]. We did not have complete data on exposures other than HIV for the entire study duration. Further detailed studies of KSHV prevalence according to local geography are warranted, to elucidate the effect of these exposures and of additional hitherto unidentified ecological and lifestyle factors.

Previous reports have observed KSHV infection occurring during childhood in sub-Saharan Africa, consistent with our data[21, 30-32]. However, our data show a more rapid increase in prevalence in early childhood compared to previous studies. We also observed significantly higher prevalence in males than in females, contrary to previous studies that have not observed a significant difference in prevalence by sex[8, 21]. This finding, which needs to be further investigated, might be in part mediated by higher antibody levels in males, which can minimize false negatives in serodiagnosis.

We observed changes in KSHV prevalence over time: the overall trend was an increase in prevalence from 1992 to 2000 followed by a decrease in 2008, even below 1992 levels. It is likely that multiple factors contributed to these changes in prevalence. Changes in the risk of KSHV acquisition in children may reflect changes in KSHV immune control, reactivation and shedding in mothers, siblings and other individuals potentially transmitting the virus. Thus, changes in HIV

prevalence and the introduction of cART are potential contributing co-factors. All UGPC participants are provided with routine free medical care, including HIV/AIDS treatment, which have been accompanied by substantial improvements in life expectancy [33]. Although in our study HIV infection per se did not influence the risk KSHV seropositivity, HIV has been shown to be a risk factor for virus replication and shedding. Moreover, we have demonstrated that malaria and certain parasitic infections are a risk factor for KSHV acquisition in Uganda [7, 28, 29], thus recent increases in effective malaria control measures, periodic deworming campaigns and possibly other changes in the health or behaviour of this population, often centred on children, may play a role. This is consistent with our observation of a recent decrease in prevalence in childhood, during which most individuals acquire KSHV. To further elucidate the contribution of these and others co-factors, prospective studies of incident infection and transmission will be needed, with particular focus on children.

The high levels of antibodies in this population, especially anti-K8.1 antibodies, are more comparable to levels seen in patients with KSHV-related diseases than in healthy persons. One interpretation is that high K8.1 levels reflect persistent or frequent KSHV reactivation, which is an important risk factor for KS. Examining KS incidence was not an objective of the present work; we previously published a retrospective KS case control study nested in the same cohort, spanning approximately the same time period[16]. In that study, KS cases already had significantly higher titres of anti-KSHV antibodies years before presentation and their titres further rose throughout follow-up to diagnosis. Consistent with this notion, in the present study OD were higher in older participants and among males; both factors associated with an increased risk of KS. The sex disparity in antibody levels (particularly of anti-K81 antibodies, which is confirmed by titres) is present since childhood, suggesting that sex-specific factors make both

boys and men less able to control KSHV replication. Such phenomenon needs to be investigated further in different settings to examine generalizability and to generate hypotheses about possible mechanisms. More generally, the very high prevalence of KSHV in this population, combined with the high levels of antibodies is consistent with the high incidence of KS in this region both prior to and during the AIDS pandemic. Our findings may suggest that prevention and treatment of KS in this population may be considerably more challenging than in other populations.

Further studies are needed to understand these findings. Specifically, studies of the correlates of high OD are warranted, including KSHV viral load in peripheral blood mononuclear cells, and clinical and environmental factors associated with viral replication. In summary, our study provides new and exciting insights into the epidemiology of KSHV in rural Uganda in relation to changes over the past two decades and opens new avenues of study to help understand the epidemiology of KS.

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Footnotes

Declaration of interests.

No author reports any conflict of interest

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Figure Legends

Figure 1

Crude seroprevalence of KHSV (A) and HIV (B) by age category, sex and round.

Figure 2

Mean antiK8.1(A) and antiORF73 (B) ELISA ODs by age category, sex and round; (C): mean ODs in children by age in years and sex.

Tables

Table 1. Characteristics of the study sample. Number (%) or median (IQR) are provided, as applicable

	Round		
	1990-1	1999-2000	2007-8
Sample size	3112	3000	3000
Age	18.8 (11.14- 39.6)	18.5 (11.1- 39.2)	18.5 (9.5- 39.6)
Males (N., %)	1495 (48%)	1500 (50%)	1500 (50%)
HIV+ (N., %)	195 (6.3%)	184 (6.4%)	191 (6.5%)
N. villages[#]	15	15	15
N./village[#]	220 (198- 259)	197 (181- 239)	214 (199- 293)
Households sampled[#]	1340	1418	1425
N. sampled /household[#]	3 (2- 5)	3 (2- 4)	3 (2- 4)

the same 15 villages were sampled in each round, however, the population within each village and household may have changed

Table 2. Risk factors for prevalent KSHV infection[#]. N=9077†

	OR*	(95%	CI)	p
Age				
0-14	Ref.			<0.0001
15-24	3.73	2.70	5.16	
25-44	4.02	2.84	5.67	
45+	5.31	3.61	7.82	
Sex				
F	Ref.			
M	1.56	1.26	1.92	<0.0001
Year				
1992	Ref.			
2000	1.64	1.26	2.13	<0.0001
2008	0.61	0.48	0.77	<0.0001

HIV+	0.74	0.47	1.16	0.192

[#]Hierarchical model accounting for individual and village clustering

†35 biospecimens not available

*Odds Ratio, adjusted for the other factors

Table 3. Antibody levels (OD) as a function of age, sex, HIV serostatus and calendar time[#].

N=9077†

	K8.1 log10(OD)				ORF73 log10(OD)			
	Coeff*	(95%	CI)	p	Coeff*	(95%	CI)	p
Age$								
0-14				<0.001				<0.001
15-24	0.39	0.33	0.44		0.43	0.37	0.49	
25-44	0.43	0.37	0.49		0.53	0.47	0.60	
45+	0.55	0.49	0.62		0.79	0.73	0.86	
sex								
F	Ref				Ref			
M	0.18	0.13	0.23	<0.001	0.09	0.04	0.13	<0.001
Year								
1992	Ref				Ref			
2000	0.17	0.13	0.22	<0.001	0.18	0.13	0.23	<0.001
2008	-0.04	-0.09	0.00	0.067	-0.04	-0.08	0.01	0.151

HIV	-0.14	-0.24	-0.05	0.004	-0.15	-0.25	-0.06	0.002

[#]Hierarchical model accounting for individual and village clustering

*Coefficient adjusted for the other factors

†35 biospecimen not available

Figure 1.

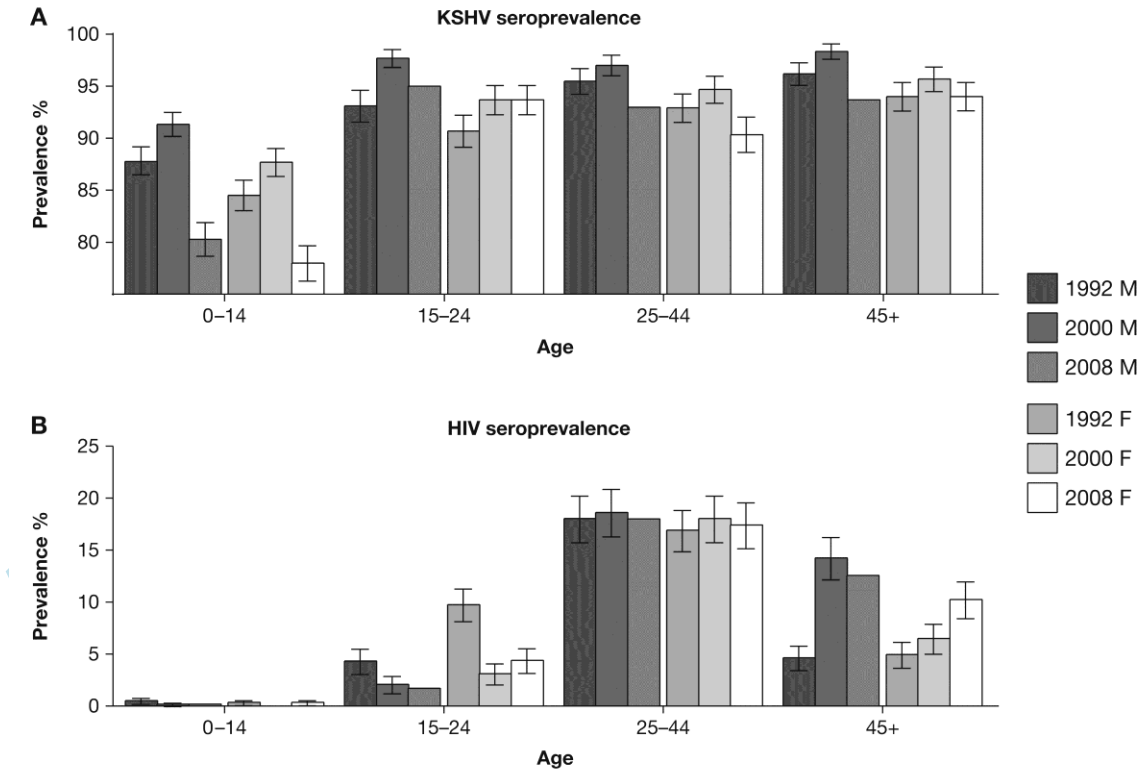

Figure 2.