Received: 13 July 2022 | Revised: 8 December 2022 | Accepted: 16 December 2022

DOI: 10.1002/ijc.34454

CANCER EPIDEMIOLOGY

IJC INTERNATIONAL JOURNAL of CANCER

Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout

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Abstract

Kaposi sarcoma-associated herpesvirus (KSHV) causes Kaposi sarcoma (KS). The risk of KS is amplified in HIV-immunosuppressed individuals and antiretroviral therapy (ART) reduces KS incidence. Reliable data on the relationship between these factors are lacking in Africa. We used questionnaires and serum from 7886 black South Africans (18-74 years) with incident cancer, recruited between 1995 and 2016. ART rollout started in 2004. We measured associations between KS, HIV-1 and KSHV before and after ART rollout. We measured seropositivity to HIV-1, KSHV latency-associated nuclear antigen (LANA) and glycoprotein (K8.1) and calculated case-control-adjusted odds ratios (OR_{adj}) and 95% confidence intervals (CI) in relation to KS and KSHV infection, before (1995-2004), early (2005-2009) and late (2010-2016) ART rollout periods. KSHV seropositivity among 1237 KS cases was 98%. Among 6649 controls, KSHV seropositivity was higher in males ($OR_{adj} = 1.4$ [95%CI 1.23-1.52]), in persons with HIV, ($OR_{adj} = 4.2$ [95%CI 3.74-4.73]) and lower in high school leavers ($OR_{adj} = 0.7$ [95%CI 0.59-0.83]). KSHV seropositivity declined over the three ART rollout periods (37%, 28% and 28%, $P_{trend} < .001$) coinciding with increases in high school leavers over the same periods (46%, 58% and 67%, $P_{trend} < .001$). HIV-1 seroprevalence increased from 10% in the pre-ART period to 22% in the late ART period ($P_{trend} < .001$). Compared to HIV-1 and KSHV seronegatives, KSHV seropositives yielded an OR for KS of 26 (95%CI 11-62) in HIV-1

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; ELISA, enzyme-linked immunoassay; HIV-1, human immunodeficiency virus-subtype 1; IARC, International Agency for Research on Cancer; ICD-O, International Classification of Diseases for Oncology; IFA, immunofluorescence assays; IQR, interquartile range; JCS, Johannesburg Cancer Study; K8.1, Kaposi sarcoma-associated herpesvirus glycoprotein; KS, Kaposi sarcoma; KSHV, Kaposi sarcoma-associated herpesvirus; LANA, latency-associated nuclear antigen; OR, odds ratio; OR_{adj}, adjusted odds ratio.

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Funding information
South African Medical Research Council; National Health Laboratory Service; UK Medical Research Council, Grant/Award Number: MRC- RFA-SHIP 01-2015

seronegative participants and an OR of 2501 (95%CI 1083-5776) in HIV-1 seropositive participants. HIV-1 increases the risk of KS in those infected with KSHV by 100-fold. Declines in KSHV seroprevalence coincide with ART rollout and with improvements in educational standards and general hygiene.

KEYWORDS
HIV, Kaposi sarcoma, Kaposi sarcoma associated herpesvirus, seropositivity, South Africa

What's new?

The risk of Kaposi sarcoma, which is caused by Kaposi sarcoma-associated herpesvirus (KSHV), is amplified in HIV-immunosuppressed individuals, and antiretroviral therapy (ART) reduces KS incidence. However, reliable data are lacking in Africa. Here, the authors found KS odds ratios for both HIV and KSHV-seropositive individuals ranging from 2000-fold in males to 5000-fold in females compared to seronegative participants. A decline was observed in KSHV antibody positivity levels over the ART period, but not in HIV prevalence. The clinical utility of a serological assay in high-risk KSHV regions is of limited value unless the specificity of the KSHV assay improves.

1 | INTRODUCTION

Kaposi sarcoma (KS) is caused by Kaposi sarcoma-associated herpesvirus (KSHV) and is consistently associated with HIV.[1] In 2020, GLOBOCAN reported 34 000 new cases and 15 000 deaths from KS, the majority arising in sub-Saharan Africa.[2]

Molecular methods have been used to detect the presence of KSHV DNA in cells, tissues and body fluids.[3] Serological antibody detection methods against latent and lytic KSHV proteins include immunofluorescence assays (IFA), Western blots and enzyme-linked immunosorbent assay (ELISA). Earlier assays[3,4] focused on positivity to either latent or lytic antibodies (LANA encoded by ORF73 or the lytically expressed K8.1glycoprotein encoded by ORF65) making accurate comparisons difficult, but recent assays can detect both. In South African adults the prevalence KSHV infection in adults ranges from 16 to 75% using IFA or ORF65 ELISA to detect latent antibodies.[5-10] In other parts of the world, KSHV seroprevalence ranges between two and 5%.[11] More recently a rapid multiplex immunoassay for detecting KSHV antibodies reported sensitivity of 95.1% and specificity of 91.4% in detecting LANA and K8.1.[12]

In black South Africans reported in the pathology-based National Cancer Registry the KS male to female ratio was 7:1 in 1988[13] declining to 1.6:1 in 2019.[14] In Sub Saharan Africa males have 2% increased odds of seropositivity to KSHV in comparison to females, the differences in KS development between males and females is suggesting that non-viral risk factors could be at play.[15] Evidence on the role of KSHV in KS development showed relative risks ranging from over 10- to 100-fold, mainly in the context of HIV-1 infection.[1] In Uganda, antibody titers to K8.1 and especially LANA increase significantly prior to a KS diagnosis.[16]

Non-viral risk factors for KSHV infection prior to HIV-1 are based on small inconclusive studies. Demographic and lifestyle risk factors for HIV-associated KS from Africa is inconsistent and includes ethnicity, livestock ownership, rare use of shoes, higher education, affluence-associated occupations, extended contact with water, number of sexual partners and a history of STDs.[17-20] In another recent study using data from the Johannesburg cancer study (JCS) we showed an association between KS and alcohol and smoking, in contrast to evidence from previous smaller studies.[21]

The JCS aimed at measuring the relative importance of known and emerging risk factors for cancer in a black African population, mainly residents of Johannesburg or Soweto. Recruitment of people diagnosed with cancer began in 1995 and ended in 2016. In 1999, based on 51 cases of KS and 3293 participants with cancers other than KS (controls), using a "first generation" labor-intensive LANA IFA to detect KSHV, we showed an OR of 1683 for KS in relation to HIV-1 positivity and high KSHV titers.[6] Other studies on KSHV and HIV were based on small numbers of KS cases and reported that the risk of KS increased with increasing titer of antibodies against KSHV, independently of HIV infection.[16,22] With 1237 participants recruited over a 21-year period with a new diagnosis of KS (cases) and 6649 participants diagnosed with infection unrelated cancer types (controls) and using high-throughput multiplex serology, we are now able to provide more reliable estimates on the relationship between KS and KSHV, HIV-1 and the association between KSHV and select demographic and lifestyle co-factors. Recruitment coincided with national HIV-antiretroviral therapy (ART) rollout in 2004. We therefore also measured the prevalence of KSHV and HIV-1 in non-KS controls before and after ART rollout and measured the association between HIV-1, KSHV and KS during this period.

2 | METHODS

2.1 | Study setting and design

JCS is described elsewhere.[23] Between 1995 and 2016, the JCS recruited approximately 25 000 consenting black South Africans newly diagnosed with any cancer aged between 18 and 74 years, referred to the medical oncology and radiation therapy departments of Charlotte Maxeke Johannesburg Academic Hospital from associated referral hospitals and clinics. Participants were interviewed in English, Zulu or Sotho using a structured questionnaire to collect sociodemographic and behavioral characteristics. Interviewers collected an optional venous blood sample in a serum separator tube (SST), aliquoted for routine HIV testing and future serological assays

TABLE 1 Socio-demographic characteristics of study participants (cases and controls) by KSHV status.

	Cases (n = 1237)				Controls (n = 6649)			
	Males		Females		Males		Females	
Characteristics	Total 658	KSHV seropositive N (%) 648 (98.48)	Total 579	KSHV seropositive N (%) 560 (96.72)	Total 2288	KSHV seropositive N (%) 747 (32.65)	Total 4361	KSHV seropositive N (%) 1289 (29.56)
Median age (IQR)	38 (33-44)	38 (33-44)	33 (29-40)	33 (29-40)	56 (49-64)	59 (51-66)	50 (41-57)	53 (45-60)
	N	N (%)	N	N (%)	N	N (%)	N	N (%)
HIV status								
Negative	27	22 (81.48)	17	16 (94.12)	1992	662 (33.23)	3469	1045 (30.12)
Positive	631 (95.90)	626 (99.21)	562 (97.06)	544 (96.80)	296 (12.94)	85 (28.72)	892 (20.45)	244 (27.35)
Age group (years)								
18-24	11	11 (100)	51	48 (94.12)	76	13 (17.11)	76	16 (21.05)
25-34	213	210 (98.59)	275	266 (96.73)	105	17 (16.19)	392	67 (17.09)
35-44	282	278 (98.58)	163	158 (96.93)	232	61 (26.29)	1023	230 (22.48)
45-54	117	114 (97.44)	67	65 (97.01)	582	161 (27.66)	1411	393 (27.85)
55-64	27	27 (100)	21	21 (100)	787	254 (32.27)	1016	374 (36.81)
65-74	8	8 (100)	2	2 (100)	506	241 (47.63)	443	209 (47.18)
Place of residence								
Urban	630	622 (98.73)	543	524 (96.50)	2073	669 (32.27)	3964	1155 (29.14)
Rural	26	25 (96.15)	34	34 (100)	209	76 (36.36)	387	129 (33.33)
Missing	2	1 (50.00)	2	2 (100)	6	2 (33.33)	10	5 (50.00)
Education								
None	30	29 (96.67)	24	23 (95.83)	263	120 (45.63)	425	164 (38.59)
Primary	150	148 (98.67)	90	89 (98.89)	833	305 (36.61)	1166	419 (35.93)
Secondary and tertiary	478	471 (98.54)	465	448 (96.34)	1187	321 (27.04)	2764	705 (25.51)
Missing					5	1 (20.00)	6	1 (16.67)
Number of sexual partners								
0-1	16	15 (93.75)	24	23 (95.83)	100	27 (27.00)	403	130 (32.26)
2-5	205	202 (98.54)	321	306 (95.33)	771	275 (35.67)	2476	727 (29.36)
6 or more	256	252 (98.44)	108	105 (97.22)	792	276 (34.85)	471	140 (29.72)
Unknown	181	179 (98.90)	126	126 (100)	625	169 (27.04)	1011	292 (28.88)
ART period								
Pre-ART 1995-2004	157	152 (96.82)	138	129 (93.48)	653	260 (39.82)	1180	416 (35.25)
Early ART 2005-2009	226	224 (99.12)	228	221 (96.93)	687	214 (31.15)	1407	374 (26.58)
Late ART 2010-2016	275	272 (98.91)	213	210 (98.59)	948	273 (28.80)	1774	499 (28.13)

(in this case KSHV). Sera were stored at −25°C. Over 90% of tumors were microscopy/histopathology verified.[24] Cancer types were coded to their primary site and morphology using International Classification of Diseases for Oncology Third edition (ICD-O3) and presented in ICD-10 format. For our study cases were participants with a new diagnosis of KS (C46) (N = 1237 [658 males and 579 females]). Controls were participants diagnosed with cancer types not known to be infection-related (N = 6649 [2288 males and 4361 females]). Figure S1 outlines the distribution of cases and controls. Table S1 shows the distribution of cancer types and KSHV seroprevalence in the controls.

2.2 | Serology for HIV-1 and KSHV

HIV-1 antibody testing was performed locally by the National Health Laboratory Services using the Vironostika (HIV Uniform II plus O) micro-ELISA assay.[24] Aliquots were shipped on dry ice without any case/control identifiers to colleagues at the German Cancer Research Center (Deutsches Krebsforschungszentrum-DKFZ) in Heidelberg, who tested for antibodies against KSHV LANA and K8.1 using a multiplex serological assay based on a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent beads on a Luminex platform.[25] The final net (bead and GST background subtracted) Median Fluorescence Intensity (MFI) values were analyzed at a dilution of 1:1000 and all values below +1 MFI were set to +1 MFI. KSHV seropositives were defined as positive for either LANA or K8.1. KSHV seronegatives were LANA and K8.1 negative (Table S2). Table S3 shows the distribution of the participants who had KSHV serology data vs those who did not.

2.3 | Statistical analysis

Aside from descriptive statistics, we examined the association between antibodies against KSHV by sex, HIV status, sociodemographic factors and ART periods. Prevalence odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional unmatched logistic regression to identify risk factors associated with KSHV seropositivity among controls. We

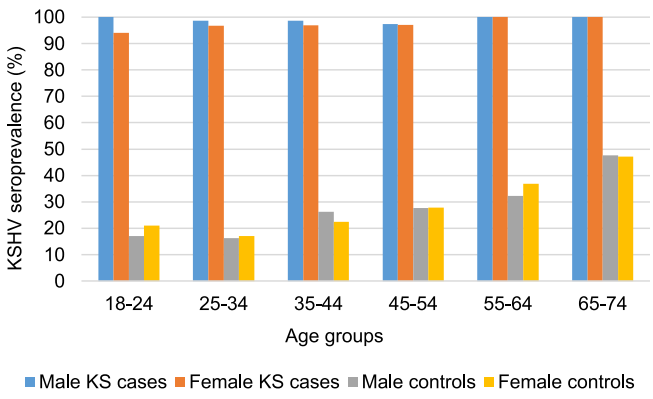

FIGURE 1 Age-associated seroprevalence of KSHV antibodies in KS cases and controls. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Univariate and multivariate logistic regression analysis of risk factors of KSHV infection in controls (for breakdown see Table S2).

Variables	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Sex		
Males	**1.50 (1.37-1.65)**	**1.37 (1.23-1.52)**
Females	1.00	1.00
$P_{heterogeneity}$		<.001
HIV status		
Negative	1.00	1.00
Positive (all ages)	**3.66 (3.31-4.05)**	**4.21 (3.74-4.73)**
$P_{heterogeneity}$		.004
Age group (years)		
18-24	1.00	1.00
25-34	**1.89 (1.40-2.55)**	1.35 (0.98-1.86)
35-44	1.07 (0.80-1.43)	0.92 (0.67-1.25)
45-54	0.73 (0.55-0.97)	0.82 (0.60-1.12)
55-64	0.82 (0.62-1.10)	1.07 (0.78-1.46)
65-74	1.32 (0.98-1.78)	**1.84 (1.33-2.55)**
P_{trend}		.014
Place of residence		
Urban	1.00	1.00
Rural	0.96 (0.82-1.13)	0.98 (0.82-1.17)
$P_{heterogeneity}$		.611
Education		
None	1.00	1.00
Primary	0.91 (0.77-1.07)	0.86 (0.72-1.03)
Secondary and tertiary	**0.80 (0.68-0.93)**	**0.70 (0.59-0.83)**
P_{trend}		<.001
Number of sexual partners		
0-1	1.00	1.00
2-5	1.19 (0.99-1.44)	1.04 (0.85-1.26)
6 or more	**1.62 (1.32-1.97)**	1.16 (0.93-1.45)
Unknown	1.16 (0.95-1.42)	1.00 (0.77-1.25)
P_{trend}		.762
ART period		
Pre-ART 1995-2004	1.00	1.00
Early ART 2005-2009	**0.78 (0.71-0.86)**	**0.73 (0.64-0.82)**
Late ART 2010-2016	**0.74 (0.68-0.81)**	**0.68 (0.60-0.78)**
P_{trend}		<.001

Note: Adjusted for sex, HIV status, age group, ART-period, place of residence, level of education and number of sexual partners. The Significance of Bold values indicates *P less than or equal to 0.05*

TABLE 3 Case-control risks of developing KS stratified by sex.

	Adjusted OR (95%CI)		
Variables	**Both sexes**	**Males**	**Females**
KSHV status			
Negative	1.00	1.00	1.00
Positive	**132.21 (86.30-202.52)**	**188.60 (88.36-402.57)**	**115.52 (68.56-194.66)**
$P_{heterogeneity}$		.297	
Positive (LANA only)	**61.50 (44.86-84.35)**		
Positive (K8.1 only)	**64.12 (47.47-86.63)**		
$P_{heterogeneity}$	.851		
HIV status			
Negative	1.00	1.00	1.00
Positive	**86.96 (60.36-125.29)**	**83.39 (50.21-138.51)**	**86.04 (50.26-147.30)**
M/F Cochran Q *P*-value		.934	
Place of residence			
Urban	1.00	1.00	1.00
Rural	1.05 (0.62-1.80)	1.17 (0.50-2.78)	1.09 (0.54-2.19)
Education			
None	1.00	1.00	1.00
Primary	0.76 (0.45-1.30)	1.17 (0.52-2.61)	0.59 (0.29-1.19)
Secondary and tertiary	0.97 (0.58-1.63)	1.63 (0.73-3.64)	0.74 (0.38-1.45)
No. of sexual partners			
0-1	1.00	1.00	1.00
2-5	1.18 (0.62-2.26)	0.95 (0.26-3.51)	1.21 (0.56-2.61)
6 or more	1.23 (0.63-2.43)	1.12 (0.30-4.14)	1.27 (0.55-2.93)
Unknown	1.22 (0.60-2.46)	1.57 (0.40-6.15)	1.02 (0.44-2.37)
ART-period			
Pre-ART 1995-2004	1.00	1.00	1.00
Early ART 2005-2009	0.84 (0.60-1.19)	0.69 (0.39-1.22)	0.95 (0.61-1.47)
Late ART 2010-2016	**0.64 (0.45-0.93)**	0.65 (0.35-1.19)	0.65 (0.40-1.04)
P_{trend}	.005	.160	.073

Note: Adjusted for HIV status, KSHV status, age group, place of residence, education, number of sexual partners and ART period.

used standard case-control analysis methods[26] and similar regression models to measure adjusted case-control ORs of developing KS stratified by sex, for HIV and KSHV status. We stratified the KS ORs by HIV and KSHV status. Covariates were sex, age group (18-34, 35-44, 45-54, 55-64 and 65-74 years), HIV status, residence (urban/rural), education (none, primary [up to 5 years], secondary or higher) and number of sexual partners (none or one, two to five, six or more, missing). ART periods were classified as pre-ART (1995-2004), early ART (2005-2009) and late ART (2010-2016). National ART coverage in adults rose to 14.8% in 2009 (early ART period) and 48.6% in 2015 (late ART).[27] Statistical analyses were performed using STATA software V15.0 (Stata Corp, College Station, TX). We used the Cochran Q-test (*admetan* function) to test for heterogeneity in ORs between sexes.

3 | RESULTS

3.1 | KSHV prevalence and risk factors in controls

Of 7886 samples tested for KSHV, 2958 were seropositive for LANA and 2479 for K8.1 see Table S2. Table 1 shows the socio-demographic characteristics of study participants stratified by KSHV status. Figure 1 shows the age distribution of KSHV seropositivity among cases and controls. KSHV seropositivity peaking at 47% in 65-74 years in controls. The median age of the participants at recruitment who were KSHV seropositive was 59 years for males and 53 for females. The KSHV test yields a sensitivity of 99%, but a specificity of 53%, (Figure 1).

The majority, 658 of the 1237 individuals diagnosed with KS were male (Table 1). Cases were younger with a median age of

38 (IQR 33-44) in males and 33 (IQR 29-40) in females. Most of the KS cases were HIV positive (95.9% in males and 97.1% in females), HIV prevalence among the controls was 12.9% in males and 20.5% among females. Most of the KS cases were KSHV seropositive (98.5% males and 96.7% females), KSHV seroprevalence among controls was 32.7% in males and 29.6% in females.

KSHV seropositivity in controls (Table 2) was significantly associated with being male [OR_{adj} = 1.37 (95%CI 1.23-1.52)], HIV positivity [OR_{adj} = 4.21 (95%CI 3.74-4.73)] increased age group (P_{trend} = .014) and inversely associated with secondary or higher levels of education [OR_{adj} = 0.70 (95%CI 0.59-0.83), P < .001]. Compared to pre-ART, we observe lower KSHV seroprevalences in relation to the early and late ART rollout periods [respectively: OR_{adj} = 0.73 and OR_{adj} = 0.68, P_{trend} < .001]. By contrast, HIV prevalence among controls increased over the three ART periods from 7.8% to 15.5% in males and from 12.0% to 26.0% among females (P_{trend} < .001). The proportion of high school leavers increased over time (Tables S3 and S4). We found no association between KSHV seropositivity and residence or number of sexual partners.

3.2 | Factors associated with KS

Table 3 shows the association between KS and KSHV, HIV and selected risk factors including ART rollout period. KS is strongly associated with KSHV antibody positivity (OR_{adj} = 132.21 [95%CI 86.30-202.52]), males were similarly likely to develop KS as females (respectively OR_{adj} = 188.60, vs OR_{adj} = 115.52, M/F $P_{heterogeneity}$ = .297). The odds of developing KS among seropositive LANA and K8.1 cases vs controls were lower than results using the combined assay, but similar to each other: LANA OR_{adj} = 61.5 and K8.1 OR_{adj} = 64.1, $P_{heterogeneity}$ = .851. KS is also significantly associated with HIV positivity [OR_{adj} = 86.96; 95%CI 60.36-125.49] with no differences in risk between the sexes (Female OR_{adj} = 86.04, Male OR_{adj} = 83.39, M/F $P_{heterogeneity}$ = .934). There was no association between KS and residence, education, or number of sexual partners. There was a reduction in KS risk in relation to ART period in both sexes (P_{trend} = .005). Similar trends in KS risk were observed for males and females, however the reductions in sample size reduced statistical power (P_{trend} males = .16, females = 0.073).

3.3 | Association between KSHV, HIV seropositivity and KS

Using those who were HIV and KSHV seronegative as reference (Figure 2), the odds of KS among those who were HIV seronegative and KSHV seropositive was 25-fold higher [OR_{adj} = 25.47 (95%CI 10.54-61.50)]. The risk of developing KS in those who were KSHV and HIV seropositive increased by about 100-fold [OR_{adj} = 2501.10 (95%CI 1083.10-5775.51), notably OR_{adj} = 2035.10 in males and OR_{adj} = 4926.36 in females]. Twenty-three cases with KS were KSHV seronegative, possibly due to host or laboratory factors.

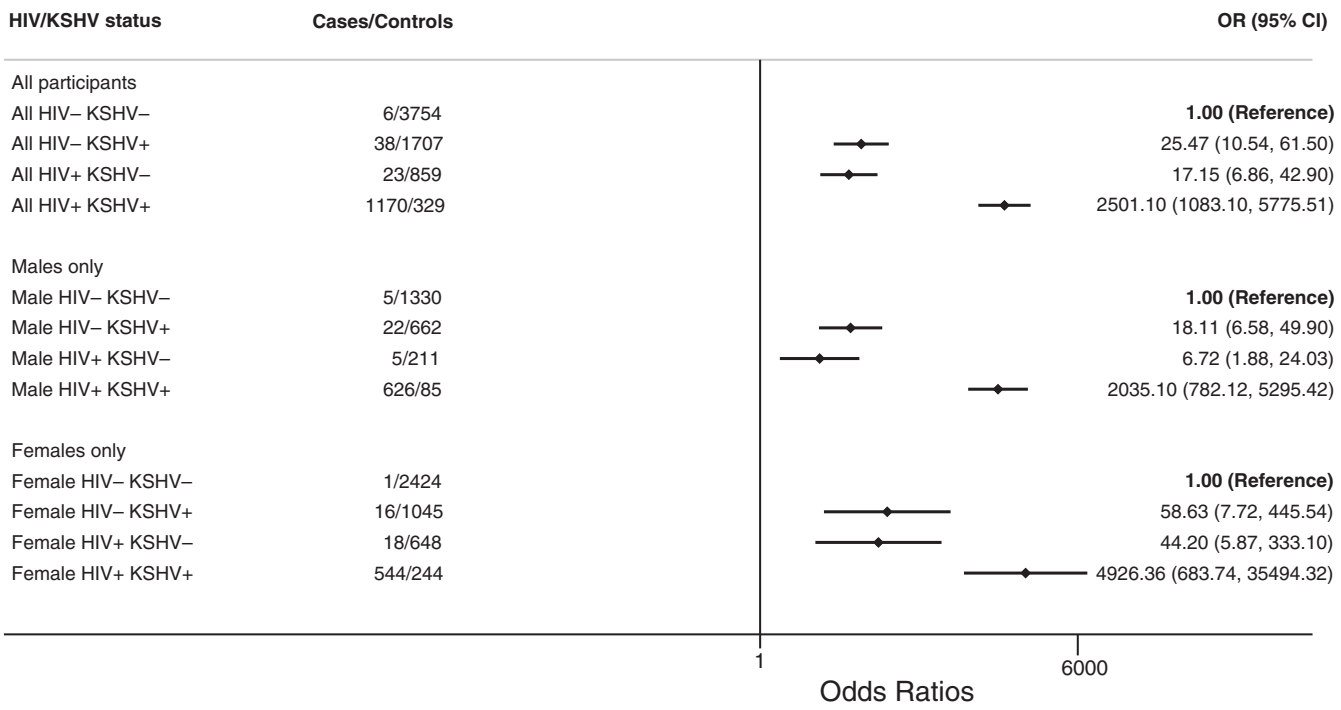

FIGURE 2 The relationship between HIV and KSHV seropositivity and KS risk. Odds ratios adjusted for age, education, and the number of sexual partners.

4 | DISCUSSION

In the previous study using JCS data, the immunofluorescence assay used had a sensitivity of 60%-80% in detecting KSHV antibodies.[6] That assay was labor-intensive, requiring microscopic visual inspection of each test.[6] In this case, we used a high throughput multiplex serological assay to detect antibodies against the KSHV LANA and K8.1, which exhibited increased detection of weak antibody responses.[25] Assuming all KS cases to be KSHV positive, this assay shows a sensitivity of 97.7%. Making comparisons of seroprevalence between different studies can be problematic because of the different serologic assays that evolved over time.[28] However our findings on the seroprevalence of antibodies against KSHV and age, lower education level and associations with HIV-1 are consistent with other studies from Africa.[6,29,30]

The increase of KSHV seroprevalence with increasing age (31% overall, from 20% in 18-34 year olds to 47% in those aged 65-74) suggests that KSHV may have been prevalent in this population for some time[6] or improvements in seroprevalence in relation to age (generation/cohort effect).[31-33] These findings are consistent with the findings from previous studies from South Africa reporting high seropositivities of KSHV.[5-10] Using LANA and K8.1 assays separately yielded lower but similar risks, so we could not replicate longitudinal data from Uganda showing an increase in Lytic antibody levels prior to KS development.[16]

Given the causal relationship between KSHV, HIV and KS, the seroprevalence of both viruses among participants with KS was over 95%. There was a decline in KS incidence after ART rollout which can be attributed to reduction in immune suppression among KS cases as seen in South Africa[34] and elsewhere in Africa.[35] This contrasts with HIV seroprevalence, which increased from 10% in the pre-ART period to 22% in the late-ART period, in keeping with improved post-HIV survival due to ART.

The study confirms previous findings[6] of the strong association between HIV and KS (about 80-fold), and KSHV and KS (ORs of 132-fold) with no statistically significant difference in risk between the sexes. When compared to HIV and KSHV negatives, HIV seropositivity had about a 100-fold effect on KS risk, increasing risks from 25-fold in KSHV+ and HIV− patients to 2500-fold in doubly infected (HIV+, KSHV+) patients. The risk for developing KS was higher in doubly infected females (5000-fold) compared to males (2000-fold). While KSHV is (a little) more common in male controls, the ORs observed for two major viral factors responsible for KS development cannot explain the observed 1.5- to 2-fold male predominance in KS incidence.

Our observed decline in KSHV seropositivity from about 35% pre-ART rollout to 28% in the latest period could suggest some level of KSHV viral clearance over time at a population level, either through direct action of protease inhibitors, or indirectly through immune system reconstitution.[36] However, as the bulk of KSHV transmission in Africa appears to be from person to person, improved hygiene conditions could also be responsible for the decline in KSHV as illustrated by our observed increased number of high school leavers observed over time. We did not collect income data but the proportion using electricity for cooking in the JCS increased from 70% in the first half of the study to 84% in the second half.[23]

4.1 | Limitations

Samples were tested for antibodies at the time of cancer diagnosis and the tumor may influence antibody titers. However, we found little evidence of this. The prevalence of KSHV is similar across all the cancer types constituting the controls (Table S2) and resemble those from independent studies. Study results are based on participants recruited from the catchment area of the largest tertiary hospital in Johannesburg/Soweto. Therefore, our findings might not be generalizable to other populations. Recall of past exposures such as numbers of sexual partners could be subject to recall or desirability bias. However, given all participants are patients with cancer we expect similar recall across cases and controls. Participants with KSHV serology were younger and more educated, however in all analyses we adjusted for both factors.

4.2 | Strengths of the study

Our study was conducted on a large sample size compared to previous studies.[5,6,30] The laboratory tests were performed without knowledge of the case/control status of the participants. Case-control referral biases are also minimized because both groups come from the same catchment areas and are referred through the same processes and departments. Over 90% of the tumors were histologically verified.[24] Controls for this analysis were carefully selected, comprising cancer types not known in the literature to be associated with infections.[23]

5 | CONCLUSION

Our findings highlight the high seroprevalence of antibodies against KSHV and the striking association between KSHV, HIV and KS found in African settings. ART rollout coincides with a decline in KSHV seroprevalence, in keeping with a decline in KS incidence after ART rollout.[35] By contrast we did not observe any declines in HIV prevalence over the same period. Our observed higher KS risks in KSHV and HIV seropositive females cannot explain the higher population male to female KS incidence ratios.

Treating underlying immune deficiency is the current way by which KS can be managed, however better targeted therapies are called for and better still a vaccine. Herpesviruses (of which KSHV is one of nine) are a serious complication in patients with HIV so ideally, aside from adherence to ARVs, a safe and effective herpes-virucidal agent could be explored. Having said that, identifying immune responses in the general population and co-factors predictive of KS disease are important areas to mitigate disease onset. Among HIV

positive patients, even though the risks of developing KS in those who test KSHV positive are large, this KSHV test yields a sensitivity of 99%, but a low specificity of 53% so a more specific KSHV test needs to be developed for it to be clinically useful in detecting KS in this high KSHV prevalence setting.

AUTHOR CONTRIBUTIONS

The work reported in the paper has been performed by the authors, unless clearly specified in the text. **Melitah Motlhale**, **Freddy Sitas** and **Elvira Singh**: conceptualized the study. **Melitah Motlhale**: performed data analysis, writing original draft, review and editing. **Freddy Sitas** and **Mazvita Muchengeti**: validation, resources, supervision writing—review and editing. Debbie Bradshaw, Wenlong Carl Chen, Mwiza Gideon Singini, Chantal Babb de Villiers, Cathryn M. Lewis, Tim Waterboer, Christopher G. Mathew, Freddy Sitas, Elvira Singh and Robert Newton are the members of Evolving Risk Factors for Cancers in African Populations (ERICA-SA) collaborative group. All authors read and provided feedback (writing: review and editing) to improve the final version of the manuscript.

FUNDING INFORMATION

The study was funded by the South African Medical Research Council, the National Health Laboratory Service and the UK Medical Research Council (with funds from the UK Government's Newton Fund) (MRC-RFA-SHIP 01-2015).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The primary study (JCS) and our study were approved by the University of Witwatersrand Human Research Ethics Committee (Medical) (Clearance certificate number: M191130). Informed consent was not feasible since secondary data was used for our study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Motlhale M, Muchengeti M, Bradshaw D, et al. Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout. *Int J Cancer*. 2023;1-9. doi:10.1002/ijc.34454