

This is a repository copy of *Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/196690/>

Version: Published Version

Article:

Motlhale, Melitah, Muchengeti, Mazvita, Bradshaw, Debbie et al. (10 more authors) (2023) Kaposi sarcoma-associated herpesvirus, HIV-1 and Kaposi sarcoma risk in black South Africans diagnosed with cancer during antiretroviral treatment rollout. *International Journal of Cancer*. ISSN 1097-0215

<https://doi.org/10.1002/ijc.34454>






Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

Melitah Motlhale^{1,2}  | Mazvita Muchengeti^{1,2,3}  | Debbie Bradshaw⁴ |
 Wenlong Carl Chen^{1,5}  | Mwiza Gideon Singini^{1,2}  | Chantal Babb de Villiers⁶ |
 Cathryn M. Lewis^{7,8} | Noemi Bender⁹ | Christopher G. Mathew^{5,6,8} |
 Robert Newton^{10,11} | Tim Waterboer⁹ | Elvira Singh^{1,2†}  | Freddy Sitas^{4,12,13}

¹National Cancer Registry, National Health Laboratory Service, Johannesburg, South Africa

²Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

³South African DSI-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa

⁴Burden of Disease Research Unit, South African Medical Research Council, Cape Town, South Africa

⁵Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

⁶Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

⁷Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK

⁸Department of Medical and Molecular Genetics, Faculty of Life Sciences and Medicine, King's College London, London, UK

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.

† Deceased.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *International Journal of Cancer* published by John Wiley & Sons Ltd on behalf of UICC.

⁹Division of Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

¹⁰MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda

¹¹University of York, York, UK

¹²Centre for Primary Health Care and Equity, School of Population Health, University of New South Wales Sydney, Sydney, Australia

¹³Menzies Centre for Health Policy and Economics, School of Public Health, University of Sydney, Sydney, Australia

Correspondence

Melitah Motlhalé, National Cancer Registry, National Health Laboratory Service, Sandringham, Johannesburg, South Africa. Email: melitahm@nicd.ac.za

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 1988¹³ 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.

Characteristics	Cases (n = 1237)				Controls (n = 6649)			
	Males		Females		Males		Females	
	Total	KSHV seropositive N (%)	Total	KSHV seropositive N (%)	Total	KSHV seropositive N (%)	Total	KSHV seropositive N (%)
	658	648 (98.48)	579	560 (96.72)	2288	747 (32.65)	4361	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,

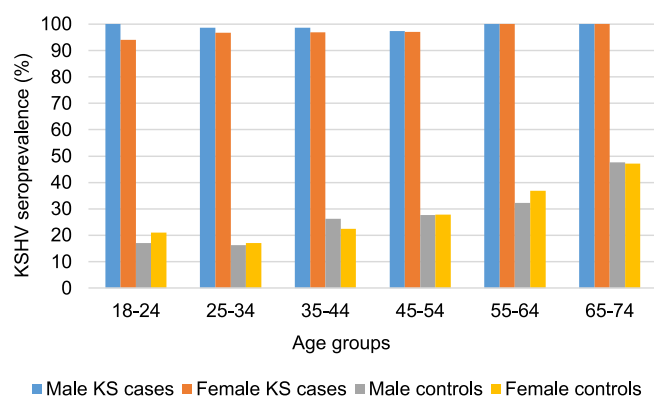


FIGURE 1 Age-associated seroprevalence of KSHV antibodies in KS cases and controls. [Color figure can be viewed at wileyonlinelibrary.com]

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

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
$P_{\text{heterogeneity}}$		<.001
HIV status		
Negative	1.00	1.00
Positive (all ages)	3.66 (3.31-4.05)	4.21 (3.74-4.73)
$P_{\text{heterogeneity}}$.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)
$P_{\text{heterogeneity}}$.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.

Variables	Adjusted OR (95%CI)		
	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)
$P_{\text{heterogeneity}}$.297	
Positive (LANA only)	61.50 (44.86-84.35)		
Positive (K8.1 only)	64.12 (47.47-86.63)		
$P_{\text{heterogeneity}}$.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 methods²⁶ 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.

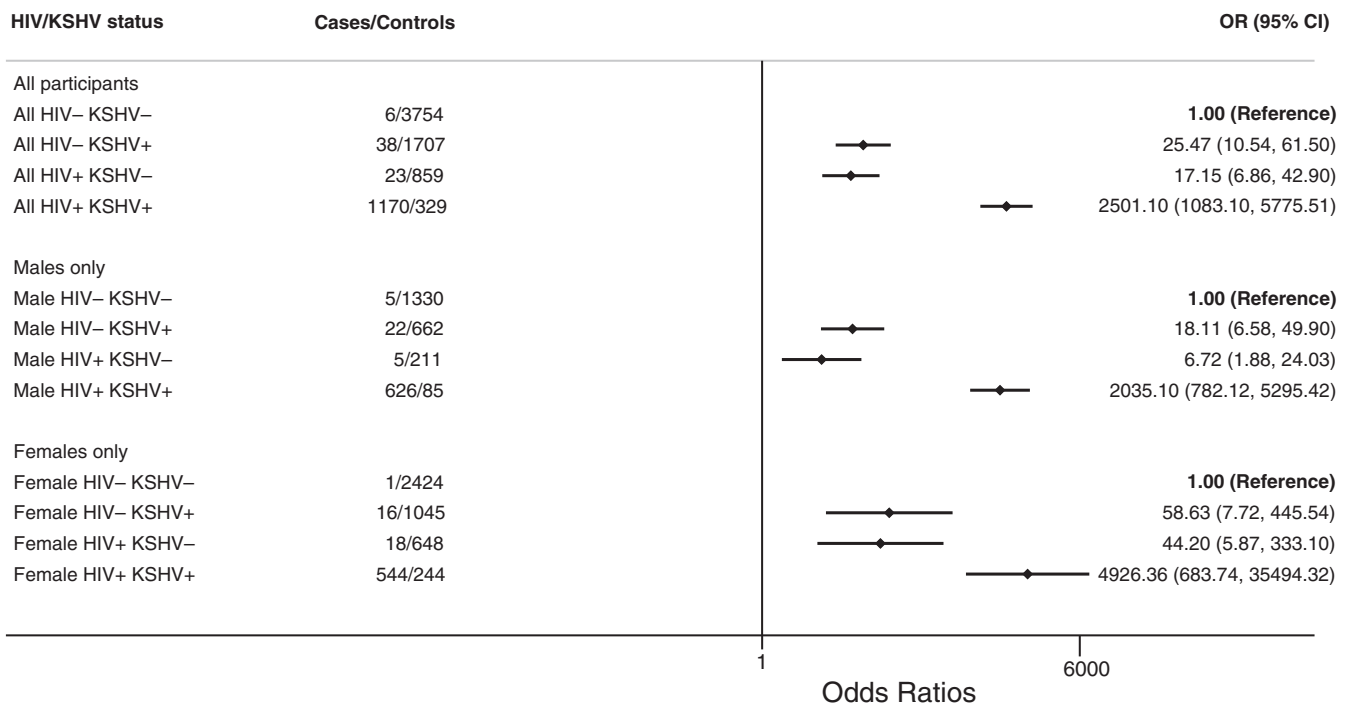


FIGURE 2 The relationship between HIV and KSHV seropositivity and KS risk. Odds ratios adjusted for age, education, and the number of sexual partners.

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 time⁶ 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 Africa³⁴ 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 findings⁶ 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. **Melilah Motlhale, Freddy Sitas and Elvira Singh:** conceptualized the study. **Melilah 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.

ORCID

Melilah Motlhale  <https://orcid.org/0000-0003-4703-7057>

Mazvita Muchengeti  <https://orcid.org/0000-0002-1955-923X>

Wenlong Carl Chen  <https://orcid.org/0000-0002-3248-4906>

Mwiza Gideon Singini  <https://orcid.org/0000-0002-2897-1728>

Elvira Singh  <https://orcid.org/0000-0003-1259-2122>

REFERENCES

- Bouvard V, Baan R, Straif K, et al. A review of human carcinogens—part B: biological agents. *Lancet Oncol.* 2009;10(4):321-322.
- Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249.
- Ablashi DV, Chatlynne LG, Whitman JE, Cesarman E. Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8. *Diseases Clin Microbiol Rev.* 2002;15(3):439-464.
- Moore PS, Gao SJ, Dominguez G, et al. Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J Virol.* 1996;70(1):549-558.
- Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. *Br J Cancer.* 2003;88:1-3.
- Sitas F, Carrara H, Beral V, et al. Antibodies against human herpesvirus 8 in black south African patients with cancer. *N Engl J Med.* 1999;340(24):1863-1871.
- Wilkinson D, Sheldon J, Gilks CF, Schulz TF. Prevalence of infection with human herpesvirus 8/Kaposi's sarcoma herpesvirus in rural South Africa. *South Afr Med J.* 1999;89(5):554-557.
- Blumenthal MJ, Schutz C, Meintjes G, et al. EPHA2 sequence variants are associated with susceptibility to Kaposi's sarcoma-associated herpesvirus infection and Kaposi's sarcoma prevalence in HIV-infected patients. *Cancer Epidemiol.* 2018;56:133-139.
- Blumenthal MJ, Schutz C, Barr D, et al. The contribution of Kaposi's sarcoma-associated herpesvirus to mortality in hospitalized human immunodeficiency virus-infected patients being investigated for tuberculosis in South Africa. *J Infect Dis.* 2019;220(5):841-851.
- Blumenthal MJ, Lambarey H, Chetram A, Riou C, Wilkinson RJ, Schäfer G. Kaposi's sarcoma-associated herpesvirus, but not Epstein-Barr virus, co-infection associates with coronavirus disease 2019 severity and outcome in South African patients. *Front Microbiol.* 2022;12:1-10.
- Chatlynne LG, Ablashi DV. Seroepidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV). *Semin Cancer Biol.* 1999;9(3):175-185.
- Logan C, Todorof K, Fiorillo SP, et al. Rapid multiplexed immunoassay for detection of antibodies to Kaposi's sarcoma-associated herpesvirus. *PLoS One.* 2016;11(9):e0163616.
- Sitas F, Newton R. Kaposi's sarcoma in South Africa. *JNCI Monogr.* 2000;28:1-4.
- South African National Cancer Registry. *Cancer in South Africa 2019. Full Report; 2019.*
- Begré L, Rohner E, Mbulaiteye SM, Egger M, Bohlius J. Is human herpesvirus 8 infection more common in men than in women? Systematic review and meta-analysis Lorin. *Int J Cancer.* 2016;139(4):776-783.
- Wakeham K, Johnston WT, Nalwoga A, et al. Trends in Kaposi's sarcoma-associated herpesvirus antibodies prior to the development of HIV-associated Kaposi's sarcoma: a nested case-control study. *Int J Cancer.* 2015;136(12):2822-2830.
- Semango GP, Charles RM, Swai CI, et al. Prevalence and associated risk factors for Kaposi's sarcoma among HIV-positive patients in a referral hospital in northern Tanzania: a retrospective hospital-based study. *BMC Cancer.* 2018;18(1):1-8.
- Rohner E, Kasaro M, Msadabwe-Chikuni SC, Stinson K, Mohamed Z, Tweya H. Treatment and outcome of AIDS-related Kaposi sarcoma in South Africa, Malawi and Zambia: an international comparison. *Pan Afr Med J.* 2017;28(261):1-8.
- Rogena EA, Simbiri KO, De Falco G, Leoncini L, Ayers L, Nyagol J. A review of the pattern of AIDS defining, HIV associated neoplasms and premalignant lesions diagnosed from 2000-2011 at Kenyatta national hospital. *Kenya Infect Agent Cancer.* 2015;10(1):1-7.
- Ziegler JL, Newton R, Katongole-Mbidde E, et al. Risk factors for Kaposi's sarcoma in HIV-positive subjects in Uganda. *Aids.* 1997;11(13):1619-1626.
- Motlhale M, Sitas F, Bradshaw D, et al. Lifestyle factors associated with sex differences in Kaposi sarcoma incidence among adult black south Africans: a case-control study. *Cancer Epidemiol.* 2022;78:102158.

22. Newton R, Ziegler J, Bourboulia D, et al. Infection with Kaposi's sarcoma-associated herpesvirus (KSHV) and human immunodeficiency virus (HIV) in relation to the risk and clinical presentation of Kaposi's sarcoma in Uganda. *Br J Cancer*. 2003;89(3):502-504.
23. Chen WC, Singh E, Muchengeti M, et al. Johannesburg cancer study (JCS): contribution to knowledge and opportunities arising from 20 years of data collection in an African setting. *Cancer Epidemiol*. 2020;65:101701.
24. Stein L, Urban MI, O'Connell D, et al. The spectrum of human immunodeficiency virus-associated cancers in a south African black population: results from a case-control study, 1995-2004. *Int J Cancer*. 2008;122(10):2260-2265.
25. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione S-transferase fusion proteins. *Clin Chem*. 2005;51(10):1845-1853.
26. Day NE, Breslow N. In: Davis W, ed. *Statistical Methods in Cancer Research*. Vol 1. International Agency for Research on Cancer, Lyon, France: WHO; 1980.
27. Johnson LF, Dorrington RE, Moolla H. Progress towards the 2020 targets for HIV diagnosis and antiretroviral treatment in South Africa. *South Afr J HIV Med*. 2017;18(1):e694.
28. Butler LM, Dorsey G, Hladik W, et al. Kaposi sarcoma-associated herpesvirus (KSHV) Seroprevalence in population-based samples of African children: evidence for at least 2 patterns of KSHV transmission. *J Infect Dis*. 2009;200(3):430-438.
29. Malope-Kgokong BI, MacPhail P, Mbisa G, et al. Kaposi's sarcoma associated-herpes virus (KSHV) Seroprevalence in pregnant women in South Africa. *Infect Agent Cancer*. 2010;5(1):1-8.
30. Wakeham K, Webb EL, Sebina I, et al. Risk factors for seropositivity to Kaposi sarcoma-associated herpesvirus among children in Uganda. *J Acquir Immune Defic Syndr*. 2013;63(2):228-233.
31. Dollard SC, Butler LM, Jones AMG, et al. Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: insights on the origin of the "Kaposi's sarcoma belt". *Int J Cancer*. 2010;127(10):2395-2401.
32. Brayfield BP, Kankasa C, West JT, et al. Distribution of Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 in maternal saliva and breast milk in Zambia: implications for transmission. *J Infect Dis*. 2004;189(12):2260-2270.
33. Mbulaiteye SM, Pfeiffer RM, Engels EA, et al. Detection of Kaposi sarcoma-associated herpesvirus DNA in saliva and buffy-coat samples from children with sickle cell disease in Uganda. *J Infect Dis*. 2004;190(8):1382-1386.
34. Majaya E, Girdler-Brown BV, Muchengeti M, Singh E. The impact of the south African antiretroviral treatment programme on the age-standardised incidence rate of Kaposi sarcoma, 1999-2016: an interrupted time series analysis. *Int J Infect Dis*. 2021;102:20-27.
35. Motlhale M, Sitas F, Bradshaw D, et al. Epidemiology of Kaposi's sarcoma in sub-Saharan Africa. *Cancer Epidemiol*. 2022;78:102167.
36. Leao JC, Kumar N, Mclean KA, et al. Effect of human immunodeficiency virus-1 protease inhibitors on the clearance of human herpesvirus 8 from blood of human. *J Med Virol*. 2000;62:416-420.

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](https://doi.org/10.1002/ijc.34454)