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Research article

Risk factors for high anti-HHV-8 antibody titers (≥1:51,200) in black, HIV-1 negative South African cancer patients: a case control study

Janet M Wojcicki*[1], Rob Newton[2], Margaret I Urban[3], Lara Stein[3], Martin Hale[4], Moosa Patel[5,6], Paul Ruff[5,9], Ranjan Sur[5,7], Dimitra Bourboulia[10] and Freddy Sitas[3,8]

Address: [1]Center for AIDS Prevention Studies, University of California, San Francisco, United States, [2]Cancer Epidemiology Unit, the Radcliffe Infirmary, Oxford University, United Kingdom, [3]The South African Cancer Epidemiology Research Group, South Africa, [4]Department of Anatomical Pathology, the National Health Laboratory Service and the University of the Witwatersrand, South Africa, [5]Division of Medical Oncology, Department of Medicine, University of Witwatersrand, Faculty of Health Sciences,, South Africa, [6]The Haematology/Oncology Division, Department of Medicine at Chris Hani Baragwanath Hospital, South Africa, [7]McMaster University Hamilton Regional Cancer Centre, Department of Radiation Oncology, Hamilton, Ontario, Canada, [8]The New South Wales Cancer Council, Woolloomooloo, Australia, [9]Johannesburg Hospital, Johannesburg, South Africa and [10]Cancer Research UK, Viral Oncology Group, Wolfeon Institute for Biomedical Research, University College London, UK

Email: Janet M Wojcicki* - jwojcicki@psg.ucsf.edu; Rob Newton - rob.newton@cancer.org.uk; Margaret I Urban - margaret.urban@nhls.ac.za; Lara Stein - lara.stein@nhls.ac.za; Martin Hale - martin.hale@nhls.ac.za; Moosa Patel - patelmm@medicine.wits.ac.za; Paul Ruff - ruff@medicine.wits.ac.za; Ranjan Sur - rksur@yahoo.com; Dimitra Bourboulia - d.bourmpoulia@ucl.ac.uk; Freddy Sitas - freddys@nswcc.org.au

* Corresponding author

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Abstract

Background: Infection with human herpesvirus 8 (HHV-8) is the necessary causal agent in the development of Kaposi's sarcoma (KS). Infection with HIV-1, male gender and older age all increase risk for KS. However, the geographic distribution of HHV-8 and KS both prior to the HIV/AIDS epidemic and with HIV/AIDS suggest the presence of an additional co-factor in the development of KS.

Methods: Between January 1994 and October 1997, we interviewed 2576 black in-patients with cancer in Johannesburg and Soweto, South Africa. Blood was tested for antibodies against HIV-1 and HHV-8 and the study was restricted to 2191 HIV-1 negative patients. Antibodies against the latent nuclear antigen of HHV-8 encoded by orf73 were detected with an indirect immunofluorescence assay. We examined the relationship between high anti-HHV-8 antibody titers (≥1:51,200) and sociodemographic and behavioral factors using unconditional logistic regression models. Variables that were significant at p = 0.10 were included in multivariate analysis.

Results: Of the 2191 HIV-1 negative patients who did not have Kaposi's sarcoma, 854 (39.0%) were positive for antibodies against HHV-8 according to the immunofluorescent assay. Among those seropositive for HHV-8, 530 (62.1%) had low titers (1:200), 227 (26.6%) had medium titers (1:51,200) and 97 (11.4%) had highest titers (1:204,800). Among the 2191 HIV-1 negative patients, the prevalence of high anti-HHV-8 antibody titers (≥1:51,200) was independently associated with increasing age (ptrend = 0.04), having a marital status of separated or divorced (p = 0.003), using

wood, coal or charcoal as fuel for cooking 20 years ago instead of electricity (p = 0.02) and consuming traditional maize beer more than one time a week (p = 0.02; p-trend for increasing consumption = 0.05) although this may be due to chance given the large number of predictors considered in this analysis.

Conclusions: Among HIV-negative subjects, patients with high anti-HHV-8 antibody titers are characterized by older age. Other associations that may be factors in the development of high anti-HHV-8 titers include exposure to poverty or a low socioeconomic status environment and consumption of traditional maize beer. The relationship between these variables and high anti-HHV-8 titers requires further, prospective study.

Background

Human herpesvirus 8 (HHV-8, also known as Kaposi's sarcoma-associated herpesvirus) is understood to be the necessary, causal agent in the development of Kaposi's Sarcoma (KS) [1–3]. HHV-8 has been detected in the lesions of nearly all patients with Kaposi's sarcoma [4,5] and it predicts the development of Kaposi's sarcoma when found in the blood [3,6].

Not all individuals infected with HHV-8 develop KS suggesting the presence of a co-factor in the development of the malignancy [7,8]. HIV infection, other immunosuppression, male gender and older age all increase risk [9,10]. To explain the geographical variation in KS incidence world-wide, researchers have proposed additional co-factors. In particular, researchers have suggested that infection with HHV-8 later in life, high socioeconomic status and/or exposure to substances in the water or soil may be potential co-factors increasing risk for KS in adulthood [11–13]. High anti-HHV-8 antibody titers have been correlated with high HHV-8 viral load and increased risk for development of KS [14–16], but risk factors other than age and length of infection for elevated titers have not been determined [17,18]. The aim of our study was to identify risk factors for high titers to HHV-8 (≥1:51,200) as a means to better understand risk factors for Kaposi's sarcoma among HIV-seronegative, black adults in South Africa. Using a database of information on over 2000 HIV-1 negative black, South African hospitalized cancer patients, we conducted a case control study of risk factors for high titers to HHV-8 (≥1:51,200) using patients with high titers as cases and HHV-8 infected patients with low titers as controls (median titer 1:200).

Methods

Study participants

The participants included in our analyses were part of a large epidemiologic study conducted by researchers from the South African National Cancer Registry and the Department of Medicine of the University of the Witwatersrand, in collaboration with investigators in the United Kingdom as described elsewhere [14]. The study was conducted between January 1994 and October 1997 at three Johannesburg hospitals (Chris Hani-Baragwanath, Hillbrow and Johannesburg). Trained nurses interviewed 2576 black inpatients with cancer using a questionnaire in the language of the patient (most commonly Zulu or Sotho).

Serologic Tests for HHV-8 and HIV-1

The serum samples were shipped by air on dry ice to the Institute of Cancer Research in London for HHV-8 testing. Details of the testing procedure are described elsewhere [14]. Briefly, a B-cell lymphoma (primary effusion lymphoma) cell line, BCP-1, infected with HHV-8 but not Epstein Barr virus (EBV) was used for an indirect immunofluorescence assay to detect IgG antibodies against HHV-8 antigen. All assays were examined by a single observer [2,19]. Slides were screened by ultra-violet microscopy for the latent nuclear antigen of HHV-8 encoded by orf73 [2,20–24]. Serum samples that were positive for antibodies against HHV-8 by the immunofluorescence assay were scored as low (median titers were 1:200), medium (1:51,200) or high (1:204,800) according to the intensity of the fluorescent signal. These scores correlated well with intensity of fluorescence as measured by fluorescence-activated cell sorter (FACS) analysis described in detail in Sitas et al. 1999.

Statistical Analysis

Within this group of 2576 black inpatients interviewed between 1994–1997, serum samples from 2329 (90 percent) were tested for antibodies to HHV-8. We restricted analyses to those patients who were HIV-1 negative and without KS. A total number of 2191 HIV-1 negative patients without KS who were tested for HHV-8 form the basis for the analysis presented in this paper. We decided to restrict the study to HIV-1 negative patients so as to remove the confounding between level of immunosuppression and high HHV-8 titers or risk of Kaposi's sarcoma [25]. Since the factors influencing high titers to HHV-8 are not known, we initially examined the relation between high antibody titers to HHV-8 (≥1:51,200) and all questions from our questionnaire including age, sex, education, place of birth, place of residence, parity, number of lifetime sexual partners, history of contraceptive use for

Table 3: Some factors characterizing wealth in relation to high anti-HHV-8 antibody titers*

Characteristic	high titer/total pos (%)¥	aOR * 95%CI
Walls of home (building materials used)		
Brick§	268/727 (36.9)	1.0
Other	56/127 (44.1)	1.49 (1.01–2.21)
Test of Homogeneity¶		χ^2 = 4.04, p = 0.04
Location for cooking food now		
Inside home§	308/808 (38.1)	1.0
Outside home	16/45 (35.6)	0.92 (0.49–1.73)
Test of Homogeneity¶		χ^2 = 0.06, p = 0.80
Type of fuel used for cooking food now		
Electricity §	229/597 (38.4)	1.0
Other	95/256 (37.1)	0.99 (0.73–1.34)
Test of Homogeneity¶		χ^2 = 0.01, p = 0.93
Type of fuel used for heating now		
Electricity§	228/595 (38.3)	1.0
Other	96/258 (37.2)	1.00 (0.73–1.35)
Test of Homogeneity¶		χ^2 = 0.00, p = 0.98
Location for cooking food 20 years ago		
Inside§	282/749 (37.7)	1.0
Outside	41/108 (39.8)	1.10 (0.72–1.69)
Test of Homogeneity¶		χ^2 = 0.20, p = 0.65
Type of fuel used for cooking food 20 years ago		
Other§	306/771 (39.7)	1.0
Electricity	17/81 (21.0)	0.44 (0.25–0.78)
Test of Homogeneity¶		χ^2 = 8.08, p = 0.005
Type of fuel used for heating 20 years ago		
Other§	307/773 (39.7)	1.0
Electricity	16/79 (20.3)	0.43 (0.25–0.73)
Test of Homogeneity¶		χ^2 = 8.5, p = 0.004
Eyes watering in house from smoke		
No§	142/432 (32.9)	1.0
Yes/	160/365 (43.8)	1.56 (1.16–2.08)
(More than 5 Years)		
Test of Homogeneity¶		χ^2 = 8.82, p = 0.003
Working Now		
No§	261/673 (38.7)	1.0
Yes	54/167 (32.3)	0.85 (0.58–1.26)
Test of Homogeneity¶		χ^2 = 0.72, p = 0.39
On pension/disability		
No§	153/449 (34.1)	1.0
Yes	163/392 (41.6)	1.01 (0.67–1.52)
Test of Homogeneity¶		χ^2 = 0.00, p = 0.97
Occupational status		
White-collar§	37/89 (41.6)	1.0
Non-active	31/99 (31.3)	0.71 (0.38–1.33)
Farming	14/41 (34.2)	0.63 (0.29–1.38)
Industry	72/181 (39.8)	0.85 (0.50–1.44)
Domestic	130/331 (39.3)	0.85 (0.52–1.41)
Unspecified	15/32 (46.9)	1.53 (0.65–3.59)
Test of Homogeneity¶		χ^2 (5df) = 4.49, p = 0.48

* Adjusted for age category < 35, 35–44, 45–54, 55–64, or ≥ 65 and sex. ¥ Data were not available for all patients for all variables. § These patients served as the reference group. ¶ All values were calculated with the χ^2 test with 1 df unless noted otherwise.

women, frequency and type of alcohol consumption, use and frequency of tobacco and other lifestyle variables such as fuel use for cooking and heating and building materials used in house construction. Odds ratios were calculated by unconditional logistic regression adjusting for age group (<35, 35 to 44, 45 to 54, 55 to 64, or ≥ 65)

Table 1: Sociodemographic Characteristics of Participants in relation to HHV-8 seropositivity and anti-HHV-8 titer level.

	Total	No. HHV-8 neg (%)	No. HHV-8 pos (%)	No. low titer (%)	No. med. titer (%)	No. high titer (%)
TOTAL	2191	1337 (61.0)	854 (39.0)	530 (62.1)	227 (26.6)	97 (11.4)
Sex						
Male	857 (39.1)	522 (60.9)	335 (39.1)	207 (61.8)	85 (25.4)	43 (12.8)
Female	1334 (60.9)	815 (61.1)	519 (38.9)	323 (62.2)	142 (27.4)	54 (10.4)
Age group						
<35	205 (9.4)	157 (76.6)	48 (23.4)	38(79.2)	9(18.8)	1 (2.1)
35–44	333 (15.2)	220 (66.1)	113 (33.9)	77 (68.1)	31(27.4)	5 (4.4)
45–54	533 (24.4)	343 (64.4)	190 (35.7)	121 (63.7)	51 (26.8)	18(9.5)
55–64	539 (24.6)	319 (59.2)	220 (40.8)	133 (60.5)	60(27.3)	27(12.3)
≥ 65	578 (26.4)	297 (51.4)	281(48.6)	159 (56.6)	76(27.1)	46(16.4)
Marital status						
Single	527 (24.1)	352 (66.8)	175 (33.2)	124 (70.9)	42 (24.0)	9 (5.1)
Married	1161 (53.1)	703 (60.6)	458 (39.5)	292 (63.8)	115 (25.1)	51 (11.1)
Widowed	344 (15.7)	182 (52.9)	162 (47.1)	87 (53.7)	52 (32.1)	23 (14.2)
Divorced	153 (7.0)	95 (62.1)	58 (37.9)	27 (46.6)	18 (31.0)	13 (22.4)
Separated						
No. of lifetime sexual Partners						
0–2	455 (22.6)	288(63.3)	167 (36.7)	103 (61.7)	47 (28.1)	17 (10.2)
3–4	1051 (52.2)	628(59.8)	423 (40.3)	267 (63.1)	111 (26.2)	45 (10.6)
≥ 5	508 (25.2)	306(60.2)	202 (39.8)	123 (60.9)	51 (25.3)	28 (13.9)
Education						
None	581 (26.7)	310 (53.4)	271 (46.6)	153(56.5)	82(30.3)	36(13.3)
1–5 years	878 (40.3)	539 (61.4)	339 (38.6)	219 (64.6)	83 (24.5)	37 (10.9)
≥ 6 years	719 (33.0)	479 (66.6)	240 (33.4)	156 (65.0)	60 (25.0)	24 (10.0)
Occupation						
White-collar	270 (13.5)	181 (67.0)	89 (33.0)	52 (58.4)	25 (28.1)	12 (13.5)
Non-active	237 (11.8)	138 (58.2)	99 (41.8)	68 (68.7)	21 (21.2)	10 (10.1)
Farming	97 (4.8)	56 (57.7)	41 (42.3)	27 (65.9)	7 (17.1)	7 (17.1)
Industry	559 (27.9)	378 (67.6)	181 (32.4)	109 (60.2)	47 (26.0)	25 (13.8)
Domestic	756 (37.7)	424 (56.1)	332 (43.9)	202 (60.8)	97 (29.2)	33 (9.9)
Unspecified	88 (4.4)	56 (63.6)	32 (36.4)	17 (53.1)	10 (31.3)	5 (15.6)
Place of birth						
Rural	1245 (59.0)	729 (58.6)	516 (41.5)	324 (62.8)	133 (25.8)	59 (11.4)
Urban	866 (41.0)	553 (63.9)	313 (36.1)	188 (60.1)	89 (28.4)	36 (11.5)
Place of residence						
Urban	1698 (79.6)	1038 (80.0)	259 (20.0)	413 (62.6)	170 (25.8)	77 (11.7)
Rural	435 (20.4)	660 (79.0)	176 (21.1)	103 (58.5)	54 (30.7)	19 (10.8)

and sex as indicated using STATA [26]. All p-values are 2-sided. Numbers of cases and controls in the tables do not always add up to the total because of missing values.

Factors that were significant in bivariate analyses at p= 0.10 were included in a multivariate logistic regression analysis, to identify which factors, if any, were independently associated with high HHV-8 titers. Goodness of fit was assessed by the Hosmer-Lemeshow test [27]. The variable "use of fuel for warming 20 years ago" was not considered in the multivariate model because it was collinear with the variable "use of fuel for cooking 20 years ago." The variable "frequency of sorghum beer consumption" was also not considered in the multivariate model because it was collinear with the variable "frequency of traditional maize beer consumption." We examined the confounding effect of other variables. In general, confounding was defined when inclusion of a variable in the multivariate model resulted in a change of more than 15 percent in odds ratios of factors already present in the model. Variables that had a considerable number of "missings" were modeled in our analysis with a separate "missing values" category.

Table 2: Socio-demographic factors in relation to the risk of high anti-HHV-8 titers (≥ 1:51, 200) *

Characteristic	high titer/total pos (%)¥	aOR * 95%CI
Sex		
Male§	128/335 (38.2)	1.0
Female	196/518 (37.8)	1.08 (0.80–1.45)
Test of Homogeneity¶		χ^2 = 0.25, p = 0.62
Age group		
<35§	10/48 (20.8)	1.0
35–44	36/113 (31.9)	1.76 (0.79–3.93)
45–54	69/190 (36.3)	2.16 (1.01–2.41)
55–64	87/220 (39.6)	2.48 (1.18–5.25)
≥ 65	122/281 (43.4)	2.96 (1.42–6.19)
Test for trend¶		χ^2 = 11.16, p = 0.001
Educational level		
None§	118/270 (43.7)	1.0
1–5	120/339 (35.4)	0.75 (0.54–1.05)
≥ 6 years	84/240 (35.0)	0.83 (0.57–1.21)
Test for trend¶		χ^2 = 1.08, p = 0.30
Place of birth		
Rural §	192/516 (37.2)	1.0
Urban	125/312 (40.1)	1.29 (0.95–1.74)
Test of Homogeneity¶		χ^2 = 2.73, p = 0.10
Place of birth		
Other§	212/558 (38.0)	1.0
Gauteng	112/290 (38.6)	1.16 (0.85–1.56)
Test of Homogeneity¶		χ^2 = 0.84, p = 0.36
Place of residence		
Urban§	247/659 (37.5)	1.0
Rural	73/176 (41.5)	1.26 (0.89–1.78)
Test of Homogeneity¶		χ^2 = 1.84, p = 0.18
Place of residence		
Gauteng§	243/648 (37.5)	1.0
Other	81/205 (39.5)	1.15 (0.83–1.60)
Test of Homogeneity¶		χ^2 = 0.82, p = 0.37

* Adjusted for age category < 35, 35–44, 45–54, 55–64, or ≥ 65 and sex. ¥ Data were not available for all patients for all variables. § These patients served as the reference group. ¶ All values were calculated with the χ^2 test with 1 df unless noted otherwise.

Results

High antibody titers (≥1:51,200) against HHV-8 in Relation to Demographic and Behavioral Factors

Of the 2191 HIV-1 negative samples who did not have Kaposi's sarcoma, 854 (39.0%) were positive for antibodies against HHV-8 according to the immunofluorescent assay. Among those seropositive for HHV-8, 530 (62.1%) have low titers (1:200), 227 (26.6%) have medium titers (1:51,200) and 97 (11.4%) have highest titers (1:204,800). Table 1 provides some socio-demographic information about our sample in relation to HHV-8 infection and level of anti-HHV-8 antibody titers.

The prevalence of high titers did not differ significantly by sex being 38.2% (128/335) in men and 37.8% (196/518) in women (p = 0.62). However, the prevalence of high titers increased linearly with age from 20.8% in those under 35 years (10/48), 31.9% in those 35–44 (36/113), 36.3% for ages 45–54 (69/190), 39.6% for ages 55–64 (87/220) to 43.4% (122/281) in those 65 years or older (p-trend =0.001). Anti-HHV-8 high antibody titers were not associated with educational status (p = 0.30), place of birth (p = 0.10), province of birth (p = 0.36), place of residence (p = 0.18) or province of residence (p = 0.37) (Table 2).

The results for variables characterizing wealth in South Africa are displayed in Table 3. A few of the factors associated with poverty were linked with an increased risk of high anti-HHV-8 antibody titers. The risk of high anti-HHV-8 antibody titers was increased in those who lived in structures that were constructed out of tin, mud, wood or other building materials as opposed to brick (OR = 1.49, 95%CI 1.01–2.21; p = 0.04). The risk of high anti-HHV-8 antibody titers was lower in those who had used electricity 20 years ago both for fuel to cook food and also as a means to heat their homes (for warmth) (OR = 0.44,

Table 4: Factors related to smoking and alcohol use in relation to high anti-HHV-8 antibody titers*

Characteristic	High titer/total pos (%)¥	aOR * 95%CI
Smoke cigarettes/pipes		
No§	179/486 (36.8)	1.0
Past	96/233 (41.2)	1.12 (0.77–1.62)
Yes	46/128 (35.9)	1.00 (0.64–1.57)
Test of Homogeneity¶		χ^2 (2df) = 0.43, p = 0.80
Use of snuff		
No§	255/691 (36.9)	1.0
Past	22/57 (38.6)	0.97 (0.54–1.71)
Current	47/101 (46.5)	1.48 (0.95–2.30)
Test of Homogeneity¶		χ^2 (2df) = 2.56, p = 0.11
Consumption of maize beer		
Never§	277/761 (36.4)	1.0
Less than 1× week	18/50 (36.0)	0.92 (0.51–1.69)
More than 1× week	13/17 (76.5)	5.10 (1.64–15.87)
Most days	13/20 (65.0)	3.07 (1.19–7.90)
Test for Trend¶		χ^2 = 9.49, p = 0.002
Consumption of sorghum beer		
Never§	250/692 (36.1)	1.0
Less than 1× week	47/116 (40.5)	1.12 (0.74–1.70)
More than 1× week	15/25 (60.0)	2.46 (1.08–5.64)
Most days	7/13 (53.9)	1.93 (0.64–5.86)
Test for Trend¶		χ^2 = 4.29, p = 0.04
Consumption of other homemade beer		
Never§	249/636 (39.2)	1.0
Less than 1× week	43/151 (28.5)	0.52 (0.34–0.78)
More than 1× week	18/38 (47.4)	1.18 (0.60–2.31)
Most days	7/18 (38.9)	0.91 (0.34–2.41)
Test for Trend¶		χ^2 = 1.46, p = 0.23
Consumption of homemade spirits		
Never§	284/776 (36.6)	1.0
Less than 1× week	15/27 (55.6)	2.26 (1.03–5.00)
More than 1× week	4/8 (50.0)	1.60 (0.39–6.53)
Most days	13/25 (52.0)	2.05 (0.91–4.60)
Test for Trend¶		χ^2 = 5.11, p = 0.02
Consumption of commercial beer		
Never§	187/508 (36.8)	1.0
Less than 1× week	21/48 (43.8)	1.32 (0.72–2.43)
More than 1× week	38/81 (46.9)	1.35 (0.83–2.21)
Most days	74/211 (35.1)	0.85 (0.58–1.250
Test for Trend¶		χ^2 = 0.24=, p = 0.63
Consumption of commercial spirits		
Never§	257/680 (37.8)	1.0
Less than 1× week	29/82 (35.4)	0.85 (0.52–1.39)
More than 1× week	12/39 (30.8)	0.68 (0.33–1.41)
Most days	22/45 (48.9)	1.59 (0.85–2.99)
Test for Trend¶		χ^2 = 0.37, p = 0.54
Consumption of wine		
Never§	278/733 (37.9)	1.0
Less than 1× week	23/68 (33.8)	0.83 (0.49–1.41)
More than 1× week	3/6 (50.0)	1.58 (0.31–8.01)
Most days	13/27 (48.2)	1.63 (0.75–3.56)
Test for Trend¶		χ^2 = 0.90, p = 0.34

Table 4: Factors related to smoking and alcohol use in relation to high anti-HHV-8 antibody titers* *(Continued)*

Consumption of any alcohol		
No§	162/432 (37.5)	1.0
Yes	159/416 (38.2)	0.94 (0.69–1.29)
Test of Homogeneity¶		χ^2= 0.13, p = 0.72
Consumption of any maize or sorghum traditional beers		
No§	245/682 (35.9)	1.0
Yes	75/166 (45.2)	1.37 (0.95–1.97)
Test of Homogeneity¶		χ^2 = 2.85, p = 0.09

* Adjusted for age category < 35, 35–44, 45–54, 55–64, or ≥ 65 and sex. ¥ Data were not available for all patients for all variables. § These patients served as the reference group. ¶ All values were calculated with the χ^2 test with 1 df unless noted otherwise.

95%CI 0.25–0.78; p = 0.005 for cooking; OR = 0.43 95%CI 0.25–0.73; p = 0.004 for warmth). Similarly, those individuals who reported that their eyes had watered in their homes as a result of excessive smoke exposure for more than 5 years (potentially related to the use of coal or wood for fuel) also had an increased risk of having high anti-HHV-8 titers (OR = 1.56, 95%CI 1.16–2.08; p = 0.003). Other variables associated with socioeconomic status (occupational status, cooking food indoors versus outdoors, and employed/unemployed) were not associated with high HHV-8 titers.

Table 4 displays the results for variables characterizing smoking and alcohol use in this population. We did not find any association between being a current or past smoker, the use of snuff, consumption of commercial beers, commercial spirits, wine and increased risk for high titers. We did find an increased risk of high titers among those who consumed traditional maize and sorghum beers with increased risk for those consuming maize beer more than once a week (OR = 5.10, 95%CI 1.64–15.87) and for those consuming most days (OR = 3.07, 95%CI 1.19–7.90; p-trend = 0.002). Among those who drink traditional sorghum beer more than once a week risk was also elevated (OR = 2.46, 95%CI 1.08–5.64; p-trend = 0.04). Similarly, consumption of homemade spirits was also associated with an increased risk of having high titers with increased risk for those consuming homemade spirits less than one time a week (OR = 2.26, 95%CI 1.03–5.00; p-trend = 0.02).

We analyzed consumption of alcoholic beverages dichotomizing study participants into non-drinkers and drinkers and found no association between being a drinker of alcoholic beverages and increased risk for high titers. However, dichotomizing participants into those who drink maize and/or sorghum beers with those who do not, the results were marginally significant with drinkers at marginally increased risk (OR 1.37, 95%CI 0.95–1.97; p = 0.09). In South Africa, sorghum beer is often supplemented with maize as a filler as it is cheaper; as maize will supplement sorghum beers, we thought it was necessary to look at the combined group of maize and sorghum to indicate overall exposure to maize.

In Table 5, we looked at associations between markers of sexual activity and reproductive risk factors and high anti-HHV-8 titers. The only factor that had any statistical significance for high anti-HHV-8 titers was marital status with those individuals who were separated/divorced having increased risk in comparison with unmarried individuals (OR = 2.44, 95%CI 1.30–4.58; p = 0.01). We did not find any associations with number of sexual partners (p-trend = 0.54). We also did not find any associations between the various reproductive risk factors including age at menarche, parity, use of oral and injectable contraceptives and risk for high HHV-8 titers.

Those risk factors from Tables 2–5 that were significantly associated with high anti-HHV-8 antibody titers at the 1% level are summarized in Table 6. After adjustment for age group, sex and each other, 4 variables remained independently associated with high anti-HHV-8 titers. People with high anti-HHV-8 titers were more likely to be older (p-trend = 0.04, OR for ≥ 65 years = 2.38, 95%CI 1.04–5.44; p = 0.04), separated or divorced (OR = 2.73, 95%CI 1.41–5.28; p = 0.003), consuming traditional maize beer more than one time a week (OR = 4.16, 95%CI 1.26–13.80; p = 0.02; p-trend for increasing consumption = 0.05) and were less likely to have used electricity for cooking 20 years ago (OR = 0.50, 95%CI 0.27–0.91; p = 0.02).

Discussion

The primary aim of this study was to identify risk factors for high HHV-8 titers in HIV-seronegative, black South African adults as means to identify potential co-factors in the development of Kaposi's sarcoma. Studies have found that the risk of KS is increased with increasing anti-HHV-8 titers [13,14]. Similarly, high anti-HHV-8 titers have been correlated with high HHV-8 viral load, as it is believed that the titer of antibodies against HHV-8 reflects viral load [16]. As has been described by Sitas et al., 1999,

Table 5: Factors related to sexual exposures and reproduction in relation to high anti-HHV-8 antibody titers

	high titer/total pos (%)¥	aOR * 95%CI
Number of lifetime husbands/wives		
0–1§	247/633 (39.0)	1.0
≥ 2	20/45 (44.4)	1.26 (0.68–2.35)
Test for Trend¶		χ^2 = 0.55, p = 0.46
Marital status		
Single§	51/175 (29.1)	1.0
Married	166/458 (36.2)	1.11 (0.73–1.69)
Widowed	75/162 (46.3)	1.54 (0.92–2.59)
Separated/Divorced	31/57 (54.4)	2.44 (1.30–4.58)
Test of Homogeneity¶ (3 df)		χ^2 = 10.6, p = 0.01
Number of lifetime sexual partners		
0–2§	64/166 (38.6)	1.0
3–4	156/423 (36.9)	0.94 (0.65–1.38)
≥ 5	79/202 (39.1)	1.11 (0.71–1.74)
Test for Trend¶		χ^2 = 0.37, p = 0.54
Pregnant (in lifetime)		
Yes§	189/501 (37.7)	1.0
No	8/17 (47.1)	1.65 (0.61–4.49)
Test of Homogeneity¶		χ^2 = 0.95, p = 0.33
Times pregnant (in lifetime)		
1§	11/39 (28.2)	1.0
2–3	57/149 (38.3)	1.69 (0.77–3.69)
4–5	54/142 (38.0)	1.50 (0.68–3.29)
≥ 6	68/173 (39.3)	1.45 (0.67–3.15)
Test for Trend¶		χ^2 = 0.03, p = 0.87
Times miscarriage (in lifetime)		
0§	6/12 (50.0)	1.0
1	37/107 (34.6)	0.56 (0.17–1.89)
≥ 2	39/100 (39.0)	0.61 (0.18–2.06)
Test for Trend¶		χ^2 = 0.10, p = 0.73
Parity		
0–2§	53/140 (37.9)	1.0
3–4	57/174 (32.8)	0.73 (0.45–1.18)
≥ 5	74/176 (42.1)	0.99 (0.62–1.59)
Test for Trend¶		χ^2 = 0.00, p = 0.95
Number of children (born alive) (for men)		
0–1§	10/26 (38.5)	1.0
2–3	29/82 (35.4)	0.80 (0.31–2.06)
4–5	31/80 (38.8)	0.76 (0.29–2.01)
≥ 6	49/126 (38.9)	0.69 (0.26–1.79)
Test for Trend¶		χ^2 = 0.50, p = 0.48
Number of mothers and fathers (children)		
1§	237/608 (39.0)	1.0
≥ 2	67/197 (34.0)	0.82 (0.58–1.16)
Test for Trend¶		χ^2 = 1.30, p = 0.25
Age when periods began		
10–13§	17/39 (43.6)	1.0
14	34/81 (42.0)	0.93 (0.42–2.04)
15	53/166 (31.9)	0.55 (0.27–1.14)
≥ 16	91/227 (40.1)	0.77 (0.38–1.56)
Test for Trend¶		χ^2 = 0.46, p = 0.50
Age when periods ended		
≤ 45§	32/94 (34.0)	1.0
46–49	40/100 (40.0)	1.11(0.60–2.08)
≥ 50	85/198 (42.9)	1.24 (0.69–2.23)
Test for Trend¶		χ^2 = 0.74, p = 0.39

Table 5: Factors related to sexual exposures and reproduction in relation to high anti-HHV-8 antibody titers *(Continued)*

Oral contraceptives		
No§	168/424 (39.6)	1.0
Yes	25/86 (29.1)	0.69 (0.40–1.17)
Test of Homogeneity¶		χ^2 = 1.88, p = 0.17
Injectable contraceptives		
No§	165/425 (38.8)	1.0
Yes	28/87 (32.2)	0.93 (0.55–1.59)
Test of Homogeneity¶		χ^2 = 0.07, p = 0.80

* Adjusted for age category < 35, 35–44, 45–54, 55–64, or ≥ 65 sex. ¥ Data were not available for all patients for all variables. § These patients served as the reference group. ¶ All values were calculated with the χ^2 test with 1 df unless noted otherwise.

the relationship between high anti-HHV-8 titers and KS may be similar to the relationship between EBV and African Burkitt's lymphoma [28] and nasopharyngeal cancer [29]. For these two cancers, high antibody titers correlate well with the risk of disease.

In addition to HIV infection, which dramatically increases risk for KS, which has been well described, the discrepancy between the geographical distribution of KS both prior to and with the AIDS epidemic suggests the presence of an additional co-factor in the development of this malignancy [7,30]. As Dedicoat and Newton note, HHV-8 is common in Botswana (76–87% seroprevalence) and the Gambia (29–84% seroprevalence) but KS was rare in these areas prior to the HIV epidemic. Among the community of approximately 45,000 Ethiopian Jews in Israel, HHV-8 seroprevalence is between 39–57%, however, there has been only one case of KS documented from 1982 to 1998 [8].

We examined approximately 50 potential risk factors in relation to high anti-HHV-8 titers. In our final analysis, only those factors that were significant at the 1% level were considered in the multivariate model. Risk factors that were significant for high anti-HHV-8 titers included older age and socioeconomic variables. The associations we found between age and increased risk for high HHV-8 titers concur with results from other studies [18,31]. In contrast with the findings of Plaincoulaine et al. 2002, however, we found no greater risk for high HHV-8 titers in males than females. Looking at a subset of our sample above age 65, we also found no greater risk for males than females with high titers being 43.9% (68/155) in men and 42.9% (54/126) in women (χ^2 (1df) = 0.28, p = 0.59). Other studies have found that the incidence of KS is higher in males than females in endemic areas such as sub-Saharan Africa and the Mediterranean region [10,32,33]. As we found no difference between males and female in risk for high anti-HHV-8 titers, to explain this discrepancy, there may be additional risk factors for the development of KS.

Increasing titers associated with increasing age may be related to the natural aging process or may be a marker for length of infection as suggested by other studies [17]. It is difficult to speculate about length of infection with this cross-sectional, prevalence data. Risk factors for high titers potentially include sexual risk factors. Although we did not find any associations between lifetime number of sexual partners and high anti-HHV-8 antibody titers, we did find an association between being separated/divorced and increased risk for high anti-HHV-8 antibody titers. Being separated or divorced could be a proxy for having more sexual partners, particularly for women, who may find themselves impoverished after divorce and in need of engaging in survival sex or sex work. Alternatively, being divorced or separated may suggest low socioeconomic status.

High HHV-8 titers were associated with variables measuring socioeconomic status. Using electricity 20 years ago was protective. Electricity was used by a fraction of the urban and rural black South African population 20 years ago and was a good marker of socioeconomic status at that time. In our study, only 11.7% (257/2191) had access to electricity 20 years ago. This contrasts with the almost universal access to electricity in urban areas of Gauteng province (the province where 76.6% (1681/2191) of the sample lives) as reported in the 1996 census [34]. Having been born in an urban area was marginally associated with increased risk (p = 0.08) as was using cheaper, non-brick building materials such as tin, mud or wood for home construction (p = 0.09). Crowding may be more of a problem in urban areas and in poorer households and could be a risk factor for high titers. Research has indicated that stressors can increase EBV antibody titers through the steady-state expression of latent herpes viruses [35,36]; it is possible that stressors correlated with low SES may account for an increase in anti-HHV8 titers. However, high anti-HHV-8 antibody titers may also be a marker of length of infection [17] particularly as those who were living in a higher SES environment 20 years ago are protected against having high HHV-8 titers. As this

Table 6: Risk factors for high anti-HHV-8 antibody titers

	OR (high titers)‡	(95%CI) *p*-value
Age group		
<35§	1.0	
35–44	1.64 (0.71–3.77)	0.25
45–54	1.79(0.80–3.98)	0.16
55–64	2.03 (091–4.57)	0.09
≥ 65	2.38 (1.04–5.44)	0.04
Test for trend†		χ^2 = 4.45, p = 0.04
Place of birth		
Rural§	1.0	
Urban	1.33 (0.97–1.84)	0.08
Test of Homogeneity†		χ^2 = 3.10, p = 0.08
Material in Walls of Housing		
Brick§	1.0	
Other	1.43 (0.94–2.17)	0.09
Test of Homogeneity†		χ^2 = 2.86, p = 0.09
Type of fuel used for cooking 20 years Ago		
Other§	1.0	
Electricity	0.50 (0.27–0.91)	0.02
Test of Homogeneity†		χ^2 = 5.19, p = 0.02
Eyes water in house (from smoke)		
No§	1.0	
Yes/(More than 5 Years)	1.27 (0.92–1.75)	0.14
Test of Homogeneity†		χ^2 = 2.16, p = 0.14
Marital status		
Single§	1.0	
Married	1.20 (0.77–1.85)	0.42
Widowed	1.52 (0.89–2.59)	0.13
Separated/Divorced	2.73 (1.41–5.28)	0.003
Test of Homogeneity† (3df)		χ^2 = 10.31, p = 0.02
Consumption of maize beer		
Never§	1.0	
Less than 1× week	0.74 (0.38–1.42)	0.36
More than 1× week	4.16 (1.26–13.80)	0.02
Most Days	2.18 (0.79–6.04)	0.13
Test for Trend†		χ^2 = 3.72, p = 0.05
Consumption of homemade spirits		
Never§	1.0	
Less than 1× week	2.16 (0.93–5.03)	0.07
More than 1× week	1.00 (.20–5.05)	0.99
Most Days	1.68 (0.72–3.92)	0.23
Test for Trend†		χ^2 = 2.25, p = 0.13

† All values were calculated with the χ^2 test with 1df unless noted otherwise. ‡ In the multivariate model, all bivariately statistically significant variables at p = 0.1 are included and odds ratios are also adjusted for sex. Each variable was adjusted for all other variables in the table. The Hosmer-Lemeshow goodness-of-fit statistic = 5.66 with 8 df (p = 0.69). § These patients served as the reference group

study is based on prevalent HHV-8 infection, we can only hypothesize about the timing and length of infection in relation to risk for high anti-HHV-8 antibody titers.

Interestingly, an association with the consumption of maize beer was a significant risk factor for high titers. Given the association between consumption of traditionally brewed alcoholic beverages and high serum ferritin iron concentrations in black South Africans [37,38], consumption of traditional beers may facilitate the development of Kaposi's sarcoma. It has been reported that traditional beers have high concentrations of ionized, bioavailable iron (between 82.0 mg/l) as a result of being brewed and fermented in iron-clad pots [39,40]. Ziegler et al. has reported that there may be an association between exposure to the iron-rich soils of East and Central Africa

and the development of KS [12]. In a case-control study of KS in HIV positive Ugandan patients, Ziegler et al. found that those who drank home-made beer less than once a week had an OR of 2.65 95%CI (1.9–3.8) although there was no trend in risk with increased consumption [11]. Our association with maize beer, however, could be a chance finding since approximately 50 significance tests were performed. Unlike the protective association found between smoking and decreased KS risk by Goedert et al. [41], we found no association between smoking and high anti-HHV-8 antibody titers.

As this case control study was based on prevalent HHV-8 infection, it is important to emphasize the limitations of using this type of data. We were unable to establish a temporal relationship between high anti-HHV-8 antibody titers and the multitude of predictors evaluated in this study. Additionally, as some of our conclusions differ from studies that have evaluated risk factors for KS, there may be different risk factors for KS and anti-HHV-8 antibody titers that should be evaluated in future studies.

Conclusion

The associations we found between age, socioeconomic status and consumption of traditionally brewed alcoholic beverages and high anti-HHV-8 titers need to be examined in a prospective study. If there is an association between the development of KS and consumption of traditionally brewed beers due to the high levels of bio-available iron in these beers, public health campaigns could be directed at the replacement of iron containers with earthenware pots [42]. Additionally, specifics of the low socioeconomic environment that may increase risk for high anti-HHV-8 antibody titers such as crowding need to be examined in prospective study.

List of abreviations

HHV-8: Human herpesvirus 8.

KS: Kaposi's sarcoma

List of competing interests

None declared.

Author's contributions

FS conceived of the study, participated in its design, coordination and supervised the statistical analysis. JW was involved in the design of the study and performed the statistical analysis. RN was involved in the design of the study and supervised the statistical analysis. MU and LS were involved in the design of the study. MP, PR and RS supplied patients for this study. MH provided pathology reports and participated in the study design. DB performed the immunofluorescence assays. All authors read and approved the final manuscript.

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