**Association of Kaposi's Sarcoma-associated Herpesvirus viral load and clinical features of Kaposi's sarcoma in HIV-infected patients in South Africa**

**by**

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# DECLARATION

I, Rebecca Tiberekana hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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**Abstract**

***Background.*** Kaposi's sarcoma is an AIDS-defining illness caused by Kaposi's Sarcoma-associated Herpesvirus (KSHV). The level of HIV immune suppression and extent of KSHV DNA determine the development of Kaposi's sarcoma. Common manifestations of Kaposi's sarcoma include skin and mucosal involvement, lymphoedema, and visceral disease.

***Objectives.*** The purpose of this study was to explore the association between KSHV DNA viral load and clinical extent of Kaposi's sarcoma.

***Methods.*** This was a cross sectional study involving 100 patients with Kaposi sarcoma who attended Groote Schuur Hospital for treatment. The cohort had previously participated in a study investigating polymorphisms in the KSHV G-protein coupled receptor gene. KSHV viral load was determined by PCR of DNA extracted from peripheral blood mononuclear cells. Demographic and clinical data were collected, and logistic regression was applied to study association of KSHV viral load with the predominant presenting feature in patients with symptomatic KS.

***Results*** The cohort was predominantly male (63.16%) with a median age of 37.76 years. Median CD4 count was 235 cells/microlitre (64.0-418.0) and most patients (95.7%) had T1 disease. There was no association between KSHV DNA levels and clinical extent of Kaposi's sarcoma. Relative risk ratio (RRR) of 1.006 (95% CI 0.974-1.006) p=0.174. Serum KSHV levels were not associated with outcome of treatment, however, patients who had poor outcomes had elevated KSHV levels.

***Conclusion.*** There was no relationship between KSHV viral load and clinical extent of Kaposi's sarcoma in this study. KSHV DNA viral load may be useful in predicting which patients will require aggressive treatment for Kaposi's sarcoma

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## Introduction

Kaposi's sarcoma (KS) was the first malignancy to be classified as an Acquired Immune Deficiency Syndrome (AIDS)-defining illness in 1981 (1) and is the commonest malignancy in sub-Saharan Africa (2,3).

KS is a multicentric tumour arising from vascular and lymphatic epithelial cells(4) . Clinically, it is characterised by development of purplish macular lesions which affect multiple sites. These include the skin, lymphatics, the oral cavity, and several viscera, among others. Although different epidemiologic types of KS exist, for this study we focused on Human Immunodeficiency Virus (HIV)-associated KS. Other epidemiologic types include classic KS, iatrogenic KS, and endemic KS (5).

It is associated with HIV infection and co-infection with Human Herpesvirus-8 (HHV8) also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV). It has been suggested that levels of KSHV viraemia may be an indicator for the development of KS. Whitby (6) detected KSHV in 52% of patients with KS compared to none out of 134 blood donors and 26 HIV negative controls. They used CD4 count to correlate the level of immunity and found that detection of KSHV increased with increase in immune suppression. They followed up 143 patients who did not have KS at baseline for a median of 30 months. The results were that 6 out of 11 patients who were KSHV positive developed KS, compared to 12 out of 132 who were KSHV negative. This study suggested that KSHV has a causal role in the development of KS.

Development of KS results from an interplay of several factors like HIV viral load and KSHV DNA-emia (7). HIV 1 affects its development by production of the HIV Tat (transactivating) protein which indirectly increases angiogenesis. The Tat protein also increases viral loads and causes expression of viral oncogenes like v-GPCR and v-bcl2 (8). HIV promotes cytokine production which in turn initiate KS tumour development and progression.

The diagnosis of Kaposi's sarcoma is made by clinical evaluation and confirmed with histology which usually shows proliferation of abnormal vascular spaces and spindle cells. Other investigations to define the extent of disease include chest radiograph, stool occult blood, CD4 count and HIV viral load (9). Staging of Kaposi's sarcoma in the pre-HAART era was based on tumour extent, immunological status as defined by the CD4 count, and presence of systemic illness(10) . This study involved 294 patients in eight ACTG therapeutic trials. The patients were staged according to the extent of their tumour (T), severity of immune suppression (I) and presence of other HIV- related systemic illness, S. Severity of immune suppression was defined

as CD4 of less than 200/microlitre. Patients were classified as good risk (subscript 0), or poor risk (subscript 1). Univariate and multivariate analyses were used to determine associations between the staging variables, and further analyses were done to evaluate the predictive value of this staging system. The findings were that survival was shorter for poor risk category of the TIS variables. It was statistically significant for tumour extent and level of immune suppression both with $p<0.001$ and not statistically significant for systemic illness ($p=0.04$). Multivariate analysis showed that level of immune suppression gave the most predictive information followed by tumour extent. Nasti (11) did a study to validate the ACTG staging in the era of HAART and found that CD4 count did not provide prognostic information. As such, they recommended staging using only 2 variables, tumour extent T and presence of systemic illness, S.

The first line of treatment for HIV-associated Kaposi sarcoma is highly active antiretroviral therapy (HAART) (12). Localised Kaposi sarcoma may be further treated with local therapies like radiotherapy, cryotherapy or intralesional chemotherapy. In Sub-Saharan Africa, most patients with HIV associated Kaposi sarcoma present with advanced disease. Systemic treatment is required to improve quality of life in addition to HAART (13). Patients with advanced KS may present with lymphoedema, pulmonary KS, gastrointestinal disease, or extensive cutaneous disease. Combining chemotherapy and highly active antiretroviral therapy (HAART) has been shown to improve clinical response and quality of life (14,15).

The standard of care for systemic chemotherapy according to the NCCN Harmonized guidelines for Sub-Saharan Africa is liposomal doxorubicin 20mg/$m^2$ intravenously every three weeks or paclitaxel at a dose of 100mg/ $m^2$ every two weeks or 135mg/ $m^2$ every three weeks. Other acceptable first line regimens are intravenous bleomycin 10-15IU combined with intravenous vincristine 1.4mg/ $m^2$ every two to three weeks for six cycles (NCCN Harmonized guidelines 2019). Krown (16) demonstrated superiority of paclitaxel for treatment of advanced Kaposi's sarcoma over bleomycin/vincristine combination and etoposide in low- and middle-income countries.

At Groote Schuur Hospital, patients are treated with intravenous bleomycin at 10IU/ and vincristine 1.4mg $m^2/m^2$ in addition to HAART as first line therapy. This chemotherapy is administered every two weeks for 6-20 cycles, depending on tolerability and clinical response. In 2017 Paclitaxel was approved for use for KS in the public sector in South Africa. However, at Groote Schuur Hospital, bleomycin and vincristine are used as first line because this regimen

is cheaper than paclitaxel. Duration of chemotherapy is based on symptom control and is limited by adverse effects (17).

A study done in Johannesburg and Soweto, South Africa, showed that seroprevalence of KSHV is high and is associated with Kaposi sarcoma at high titres (18). They interviewed 3591 black African patients with cancer and tested 3344 of them for HIV 1 and KSHV antibodies. They found that 51 patients had Kaposi's sarcoma whereas 3293 patients had other malignancies. Only 32% of the non-KS group had KSHV antibodies compared to the 51 patients with Kaposi Sarcoma who had a seroprevalence of KSHV of 83%. This difference was statistically significant (p=0.001).

In Uganda, Wakeham had similar results where she found KSHV antibody levels to be significantly higher among cases of Kaposi's sarcoma than controls (p= 0.001)(19).

In Harare, Zimbabwe (20) a blinded cross-sectional analysis of peripheral blood KSHV DNA levels among patients with AIDS-related Kaposi's sarcoma was done. Their sample consisted of 31 patients with various clinical stages of Kaposi's sarcoma. They found that there was no association between plasma KSHV DNA levels and KS disease stage, however, levels of KSHV DNA in peripheral blood mononuclear cells were strongly associated with clinical stage of Kaposi's sarcoma (p=0.005).

Nsubuga (21) studied the relationship of KSHV and KS burden in Kampala, Uganda and found that although elevated plasma KSHV viral load was associated with the rate of development of new lesions of Kaposi's sarcoma, it was not associated with KS burden (clinical extent). They described high KS burden as more than twenty KS cutaneous lesions. Their sample size was 74 patients.

Another study (22), found an increased detection rate of KSHV DNA in peripheral blood mononuclear cells in 41 patients with stages III and IV Kaposi's sarcoma. They staged their patients according to the Krigel classification. However, their study was based on patients with classic Kaposi's sarcoma.

Elevated plasma KSHV viral load was associated with high HIV viral load and had increasing and decreasing trends in patients on treatment with HAART (23).

In terms of prognostic utility, it was demonstrated that elevated KSHV viral load was associated with increased hospital mortality in a cohort of 682 HIV-infected patients admitted at a local hospital in Cape Town, South Africa (24) .This study suggests that testing for KSHV

and titre levels could be important for prognostication of clinical syndromes and malignancies associated with KSHV. However, only 10 (1.5%) of their patients had symptomatic KS. They found that KSHV viral loads were lower for KS than for other diseases caused by KSHV. This is consistent with another study (25) which showed that KSHV may be undetectable in a quarter of assays in patients with KS but is higher in patients with primary effusion lymphoma or multicentric Castleman's disease.

The role of plasma KSHV DNA levels as a marker of response to treatment, was evaluated in Harare, Zimbabwe (26) in a study conducted with the hypothesis that clinical response of AIDS-related KS is associated with suppression of KSHV viral load. Out of 90 participants with mostly T1 disease, 50 received chemotherapy, at the discretion of the treating physician, in addition to antiretroviral therapy and patients were followed up to week 96. The other 40 patients received HAART with or without radiotherapy as determined by the treating physician. Median CD4 counts increased from 124 cells/microlitre to 281 cells/microlitre CD4 counts, and HIV-1 viral load reduced after treatment. Plasma KSHV DNA levels decreased from 660 to less than 25 copies per ml, and KSHV DNA levels in peripheral mononuclear blood cells decreased from 2790 to 37 copies per 106 cells (p=0.001) for each comparison. There were 16% deaths and 15% lost to follow up. Pre-treatment plasma HHV8 levels of less than 660 copies/ml were associated with greater survival (OR 2.83, 95%CI 1.07-7.53, p=0.04) and with a better clinical response (OR 6.38, 95% CI 1.68-24.19, p=0.006).

The specific objectives of this study were to explore the relationship between KSHV levels and clinical presentation of patients with Kaposi sarcoma and to evaluate if KSHV levels could have prognostic utility in the management of Kaposi sarcoma.

**Methods**

Medical records and laboratory results of 100 patients with symptomatic Kaposi sarcoma who attended the KS clinic at Groote Schuur Hospital Department of Radiation Oncology between 2014 and 2018 were reviewed.

The study population consisted of patients who had participated in a study investigating polymorphisms in the Kaposi's sarcoma associated herpes virus G-protein coupled receptor gene and ephrin receptor A2 gene in South African Kaposi's sarcoma tumours (UCT HERC REF:279/2008). The patients in this study had given consent to having their samples used for

further research in the future by consenting to entry of their clinical information into EPR. All patients who receive care in the Radiation Oncology department are routinely required to consent for their clinical information to be entered into an electronic patient register (EPR) for research purposes. Every radiotherapy folder has a sticker where consent for EPR is registered. The EPR is approved by UCT HREC with reference R016/2013. Furthermore, all these participants consented specifically for their blood to be drawn for HHV8 testing under the previous study (UCT HERC REF:279/2008). As such, a waiver of consent was requested from the UCT health sciences faculty research ethics committee. The protocol was submitted to the UCT faculty research ethics committee for approval and permission was granted from Groote Schuur Hospital administration to carry out this research.

Eligible participants were over 18 years of age, HIV positive with a diagnosis of Kaposi's sarcoma either clinically or histologically confirmed. All were seropositive for KSHV as determined by ELISA coated with K8.1 and recombinant ORF73 antigens on plasma isolated from blood samples. These samples had been prepared in the previous study referred to above (UCT HERC REF:279/2008). We abstracted the KSHV results from an existing dataset.

Patients with classic or endemic Kaposi's sarcoma and those with missing medical records were excluded. Of the 100 participants there were 2 HIV negative patients and records of 3 patients could not be traced and these were excluded from analysis. Demographic and clinical information was obtained from 95 participants. Clinical information collected from medical records included predominant site of disease, presence of HIV-related systemic illness, chemotherapy received, HAART use, and response to treatment. Laboratory results including haematology, chemistry, CD4 count, and HIV viral load were obtained from the National Health Laboratory Service system and radiology data was obtained from PACS.

Specimens for KSHV ELISA and DNA viral load determination had been collected at different points of treatment during the previous study. Samples were collected either pre-chemotherapy at the first oncology visit, mid-chemotherapy, which were defined as having received at least four cycles of chemotherapy at the time of sample collection; or post-chemotherapy. Post-chemotherapy was defined as the end point of treatment for the patient in the oncology department, either at point of complete response or stable disease. These definitions were for purposes of this study.

Definition of the patients' outcomes/response to first line treatment were as follows:

1. Complete response was resolution of macroscopically visible Kaposi's sarcoma lesions.

2. Partial response was a 50% reduction in number or size of Kaposi's sarcoma lesions.
3. Progressive disease was at least 25% increase in number or size of KS lesions in patient.

Relapse was recurrence of symptomatic Kaposi's sarcoma after completing systemic chemotherapy (16,27). We defined ART defaulters as patients who had stopped ART for more than 30 days (28).

***Variables and measurement.***

The dependent (outcome) variables were T1 sub stage of KS and response to treatment. The primary independent variable was KSHV viral load (KSHVvl), but in three different categories according to the time of sample collection: pre-chemotherapy (KSHVvl_pc), mid-chemotherapy (KSHVvl_mc) and post-chemotherapy (KSHVvl_post). Covariates and/or potential confounders considered include age (years), sex (male or female) and the most recent HIV viral load (HIVvl_rec). ARV compliance and coincident tuberculosis were not considered.

***Statistical analysis.***

We used Stata version 15 (StataCorp, LLC, Texas, USA) for the analysis. Mean, standard deviation (SD) and, median were used to summarize numerical continuous variables. Frequencies and percentages were used to summarize categorical variables.Numerical continuous variables were analyzed as such. Categorical variables were also analyzed as such. For the associations between KSHVvl and T1_substage; and KSHVvl and outcomes of treatment, we used multivariate logistic regression (*mlogit* in Stata) because the dependent variable (outcomes), T1_substage and treatment outcomes are both multicategorical. The results are presented as relative risk ratios (RRR) with corresponding 95% confidence intervals and p values. Because the KSHVvl values were obtained at different times for different participants (data collection time points were heterogeneous), the data were analyzed with KSHVvl as an independent variable for each collection point separately.

## Results

***Patient characteristics***

The patients were predominantly male (63.16%). The median age of the patients was 37.76 years (range 24.31-62.31). Most patients had T1 disease (95.79%) (Table 1).

Patients with more severe clinical disease (T1 91 patients and I1 46 patients) were found to have a low baseline (pre chemotherapy) KSHV serum level compared to the T0 and I0

patients.(refer to supplementary Table 1). In the group where specimens were collected after at least 4 cycles of chemotherapy (mid-chemotherapy n= 41) the KSHVvl was significantly higher (11 times higher for T1, 26 times higher for I1 and 1.3 times higher for patients with S1) compared to the pre-chemotherapy group, (n=38). In the group where specimens were collected after completion/cessation of chemotherapy (n= 18) the KSHVvl for the I1 patients was about half the value of the patients with I0 . In the pre-chemotherapy collection group, the mean KSHVvl was higher among those with T0 and I0 than the T1 and I1. T0 substage was in a very small group of patients. Refer to table 1.

Mean HIV viral load was higher among patients in stages T1 and I1 than those in stages T0 and I0, respectively. However, the HIV viral load was higher among patients with stage S0 than those in stage S1.

Regarding HAART, there were more defaulters among patients in KS stage T1 (80.22%) than those in stage T0, and 84.75% of the I1 patients defaulted. There were fewer defaulters in stage S1 than stage S0.

Regarding investigations, there were more chest radiographs showing pulmonary Kaposi's sarcoma among patients in KS stages T1, 21patients (23.08% ) and I1 with 13 patients(28.26%) than those in T0 and I0, respectively. There seems to be more pulmonary KS among patients in S0 than S1 stage. (Refer to supplementary table 1 in appendices).

In addition, in general, the patients were anaemic, the median haemoglobin was 10.7g/dl IQR(4-15.7).

Table 1. ***Clinical and sociodemographic characteristics of study participants by stage of Kaposi's sarcoma***

| Characteristics | Variables | n (%) | Median | IQR |
|---|---|---|---|---|
| | | | | |
| **Patient demographics** | Sex | | | |
| | Male | 60 (63.16) | | |
| | Female | 35 (36.84) | | |
| | | | | |
| | Age (years) | | 37.76 | 24.31-62.31 |
| | | | | |
| **Clinical characteristics** | | | | |
| **KS Stage** | KS T Stage | | | |
| | T0 | 4 (4.21) | | |
| | T1 | 91 (95.79) | | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| | KS I Stage | | | |
| | I0 | 49 (51.58) | | |
| | I1 | 46 (48.42) | | |
| | | | | |
| | KS S Stage | | | |
| | S0 | 86 (90.53) | | |
| | S1 | 9 (9.47) | | |
| | | | | |
| **T1 sub-stage** | Lymphoedema | 55 (57.89) | | |
| | Extensive cutaneous disease | 21 (22.11) | | |
| | Pulmonary KS | 10 (10.53) | | |
| | GIT/ Other visceral disease | 7 (7.37) | | |
| | | | | |
| **Response to treatment** | Complete response | 3 (3.16) | | |
| | Partial response | 36 (37.89) | | |
| | Progressive disease | 2 (2.11) | | |
| | Relapse | 10 (10.53) | | |
| | Died | 20 (21.05) | | |
| | Loss to follow-up | 24 (25.26) | | |
| | | | | |
| **On ART for 1 year** | Yes | 91 (95.79) | | |
| | No | 1 (1.05) | | |
| | ART data unavailable | 3 (3.16) | | |
| | | | | |
| **Defaulted on ART** | Yes | 73(80.22) | | |
| | No | 2 (2.20) | | |
| | Default data unavailable | 16 (17.58) | | |
| | | | | |
| **Laboratory results** | CD4 count at diagnosis | 88(92.63) | 193.50 | 55.50-349.00 |
| | Most recent CD4 count | 78(82.10) | 235.00 | 64.00-418.00 |
| | Haemoglobin level (g/dL) | | 10.70 | 4.00-15.70 |
| | White Cell Count ($x10^9$) | | 6.73 | 2.03-41.21 |
| | Platelets ($x10^9$) | | 320.50 | 56.00-790.00 |
| | | | | |
| **Imaging (Chest X-ray)** | Absence of pulmonary KS | 59 (62.11) | | |
| | Pulmonary KS | 22 (23.16) | | |
| | Not done | 14 (14.74) | | |

*Abbreviations and acronyms. ksv8vl – Kaposi sarcom Herpesvirus-8 viral load; kshv8vl-pc – kshv8vl pre-chemotherapy; kshv8vl-mc – kshv8vl mid-chemotherapy; kshv8vl-post – kshv8vl post-chemotherapy; hivvl_rec – most recent viral load; Hb – haemoglobin; WCC – white cell count; plt – platelets; ART – antiretroviral therapy. * - ($x10^9$)*

### *Clinical extent/T1 substage of Kaposi sarcoma and serum KSHV8 levels*

The clinical extent, of Kaposi's sarcoma was further subdivided into five sub-groups in the T1 substage. In line with the current study, these are

1. KS IRIS (there were no patients in this sub-group),
2. Lymphoedema in 51 patients,
3. Extensive cutaneous disease in 22 patients,
4. Pulmonary KS in 10 patients and
5. Gastrointestinal tract (GIT) or other visceral site involved in 7 patients.

Although there were some patients with disease in more than one site, the predominant site is what was considered as a patient's T1 substage.

Table 2 shows the median values and standard deviations of KSHV-8 viral load by sub-group of the T1 substage.

**Table 2. Summary statistics of the KSHV and HIV viral load values by T1 substage**

| T1 sub-stage | KSHV/HIV vl | n (%) | Median | IQR |
|---|---|---|---|---|
| | | | | |
| **Lymphoedema** | KSHV vl pc | 14 (27.5) | 189.0 | 43.60-772.80 |
| | KSHV vl mc | 24 (47.1) | 48.0 | 3.00-401.50 |
| | KSHV vl post-c | 13 (25.5) | 70.5 | 61.00-1878.80 |
| | **Total** | **51 (100.0)** | - | |
| | | | | |
| | Recent HIV vl | 35 (-) | 35.0 | 20.00-243.00 |
| | | | | |
| **Extensive cutaneous disease** | KSHV vl pc | 11 (50.0) | 705.2 | 184.80-1910.30 |
| | KSHVvl mc | 8 (36.4) | 3.0 | 3.00-148.15 |
| | KSHV post-c | 3 (13.6) | 3.0 | 3.00-3.00 |
| | **Total** | **22 (100.0)** | - | |
| | | | | |
| | Recent HIV vl | 15 (-) | 48.0 | 20.00-139894.00 |
| | | | | |
| **Pulmonary Kaposi's Sarcoma** | KSHV vl pc | 3 (30.0) | 59.0 | 41.60-1066.90 |
| | KSHV vl mc | 6 (60.0) | 211.6 | 59.00-53565.40 |
| | KSHV vl post-c | 1 (10.0) | 112.1 | |
| | **Total** | **10 (100.0)** | - | |
| | | | | |
| | Recent HIV vl | 8 (-) | 4689.5 | 482.00-12708.50 |
| | | | | |
| **GIT/another visceral site** | KSHV- vl pc | 3 (42.9) | 107.0 | 3.00-200.30 |
| | KSHV-vl mc | 3 (42.9) | 4515.4 | 132.40-119760.50 |
| | KSHV- vl pc | 1 (14.3) | 24.7 | |
| | **Total** | **7 (100.0)** | - | |
| | | | | |
| | Recent HIV vl | 3 (-) | 5553.0 | 20.00-131330 |

Patients who presented with lymphedema and extensive cutaneous disease had high levels of pre-chemotherapy KSHV DNA. This was especially so for extensive cutaneous disease. The viral load levels reduced mid chemotherapy.

However, patients with visceral disease (lung and GIT) had low pre chemotherapy KSHV serum levels and they had remarkably high serum KSHV levels mid-chemotherapy. This is in comparison to the patients with extensive cutaneous disease and lymphedema. In all sub-groups

of the T1 substage, the mean HIV viral load was very high although it was highest among those with extensive cutaneous KS.

**Table 3. Summary statistics of the KSHV and HIV viral load values by treatment outcome**

| Response | KSHV/HIV vl | n (%) | Median | (IQR) |
|---|---|---|---|---|
| **Complete response** | KSHV vl pc | 1 (33.33) | 823.70 | |
| | KSHVmc | 2 (66.67) | 293.55 | 280.50 – 306.60 |
| | KSHV vl postc | 0 (0.00) | - | - |
| | **Total** | **3 (100.00)** | - | - |
| | | | | |
| | Recent HIV vl | 1 (-) | 20.00 | |
| | | | | |
| **Partial response** | KSHV pc | 13 (35.14) | 772.80 | 41.60 – 1910.30 |
| | KSHVmc | 14 (37.84) | 3.00 | 3.00 – 447.30 |
| | KSHV vl postc | 10 (27.03) | 62.30 | 24.70 – 10.50 |
| | **Total** | **37 (100.00)** | - | |
| | | | | |
| | Recent HIV vl | 23 (-) | 35.00 | 20.00 – 9689.00 |
| | | | | |
| **Progressive disease** | KSHVpc | 0 (0.00) | - | - |
| | KSHVmc | 1 (50.00) | 3.00 | |
| | KSHVpostc | 1 (50.00) | 4112.10 | |
| | **Total** | **2 (100.00)** | - | - |
| | | | | |
| | Recent HIV vl | 1 (-) | 22.00 | |
| | | | | |
| **Relapse** | KSHVvlpc | 2 | 219.00 | 59.00 – 434.30 |
| | KSHVmc | 0 (0.00) | - | - |
| | KSHVpostc | 2 (25.00) | 3.00 | 3.00 – 3.00) |
| | **Total** | **8 (100.00)** | - | - |
| | | | | |
| | Recent HIV vl | 8 (-) | 71.50 | 27.50 – 18197 |
| | | | | |
| **Died** | KSHVpc | 4 (20.00) | 480.70 | 146.20 – 1272.75 |
| | KSHVmc | 12 (60.00) | 95.70 | 20.25 – 672.80 |
| | KSHVpostc | 4 (20.00) | 984.95 | 47.05 – 2772.00 |
| | **Total** | **20 (100.00)** | - | - |
| | | | | |
| | Recent HIV vl | 13 (-) | 2811.00 | 20.00 – 139894.00 |
| | | | | |
| **Loss to follow-up** | KSHVpc | 9 (40.91) | 123.70 | 57.00 – 355.70 |
| | KSHVmc | 12 (54.55) | 250.50 | 29.60 – 29040.40 |
| | KSHVpostc | 1 (4.55) | 2948.60 | |
| | **Total** | **22 (100.00)** | - | - |
| | | | | |
| | Recent HIV vl | 16 (-) | 27.50 | 20.00 – 272.500 |

***The association of KSHVvl with the T1 substage.*** Table 4 shows results of the multinomial logistic regression for the association of KSHVvl with the T1 substage.

There is no relationship between serum KSHV DNA levels and clinical extent of Kaposi's sarcoma. The relative risk ratio for extensive cutaneous disease is 1.006 95% (CI 0.997-

1.014) p= 0.174. The relative risk ratio for visceral disease is 0.990 (CI 0.974-1.006) p=0.206

***Prognostic utility of KSHV serum levels.***

Response to therapy or outcomes of treatment has six subgroups. These, by frequency, include 1: complete response which was in 3 patients (3.3%), 2: partial response in 37 patients (40.2%), 3: progressive disease in 2 patients (2.2%), 4: relapse in 8 patients (8.7%), 5: died which was the outcomes of 20 patients (21.7%) and 6: loss to follow-up (LTFU) in 22 patients (23.9%).

**Table 4. Multinomial logistic regression for the association of KSHVvl with the T1 substage and treatment outcomes**

| Dependent variable | Category of dependent variable | Primary independent variable | RRR | 95% Confidence Interval | P value |
|---|---|---|---|---|---|
| | | | | | |
| **T1 sub-stage** | Lymphoedema (base) | KSHVvl_pc | - | - | - |
| | Extensive cutaneous disease | | 1.006 | 0.997 – 1.014 | 0.174 |
| | Pulmonary KS/GIT/Other | | 0.990 | 0.974 – 1.006 | 0.206 |
| | | | | | |
| **Response** | Complete | KSHVvl_pc | 1.525 | 3.05e-58 – 7.62e+57 | 0.995 |
| | Partial | | 1.003 | 0.999 – 1.006 | 0.520 |
| | Progressive disease | | - | - | - |
| | Relapse | | 0.997 | 0.989 – 1.005 | 0.514 |
| | Died | | 1.002 | 0.999 – 1.005 | 0.203 |
| | LTFU (base) | | - | - | - |

*Abbreviations and acronyms. GIT – gastrointestinal tract; kshv8vl – Kaposi Sarcom Herpesvirus-8 viral load; kshv8vl-pc – kshv8vl pre-chemotherapy; kshv8vl-mc – kshv8vl mid-chemotherapy; kshv8vl-post – kshv8vl post-chemotherapy; LTFU – lost to follow-up; RRR – relative risk ratio. * - comparison with a univariable model.*

Study patients who either died or were lost to follow-up (LTFU) had the highest KSHV viral load particularly those in the mid-chemotherapy and post-chemotherapy group. Those who died also had much higher HIV viral load than other groups followed by those who were LTFU. The latter particularly had much higher KSHVvl in the mid-chemotherapy group compared to any other group.

***The association of HHV8 levels with treatment outcome.*** There was no association between level of serum HHV8 DNA and treatment outcomes. RRR 1.002 (95% CI 0.999-1.005) p=0.203.

**Discussion**

Most of the patients had extensive Kaposi's sarcoma which is in keeping with some other resource-constrained settings.(13) The patients had very elevated HIV viral loads in general. Many patients with T1 disease had defaulted their antiretroviral therapy.

This is concerning because patients with more advanced clinical disease are not taking their HAART which is the first line of treatment for Kaposi's sarcoma. This means that some patients present for systemic chemotherapy despite their HIV not being controlled. HAART programs have expanded in South Africa to universal test and treat approach, but despite that, patients still present with advanced disease.Its been demonstrated that there are better virological outcomes when patients initiate HAART early and are consistent on it (29).

There was no association between clinical extent of Kaposi's sarcoma and serum HHV8 levels. Although our findings are not statistically significant, they are consistent with the findings by Campbell et al, 2000 who studied the relationship between KSHV in peripheral blood and clinical stage of Kaposi's sarcoma in Harare, Zimbabwe. This study compared serum KSHV and KSHV measured from peripheral blood mononuclear cells with clinical extent of disease. Differences between visceral versus "peripheral disease" (extensive cutaneous disease and lymphedema) were not mentioned in this study. The findings in our study show that patients with "peripheral disease" were more likely to have high pre chemotherapy KSHVvl than patients with visceral KS.

Our results are consistent with the findings of Nsubuga et al 2008, who found that although levels of serum KSHV were associated with rate of growth of new KS lesions, they were not associated with KS burden or clinical extent of disease. He defined high KS burden as over 20 cutaneous lesions on a patient.

We found that patients KSHV viral loads rise after some treatment and fall again. The higher KSHV viral load after a few cycles of chemotherapy could be attributed to an immune response or unmasking immune reconstitution.(23)

We found no association between serum KSHV levels and treatment outcomes for patients whose HHV8 levels were measured pre-chemotherapy. Elevated serum KSHV being

associated with poor outcomes is consistent with Borok (26)who did a study to find out how useful plasma KSHV DNA was as a marker of response to treatment. She found that serum KSHV levels of less than 660 copies/ml were associated with greater survival and better clinical response. In our study the patients with poor outcomes (death and loss to follow up) were found to have the highest levels of KSHV although these were measured after at least four cycles of chemotherapy, unlike in the study by Borok where only pre-treatment values were considered. Our findings are similar to another study in South Africa where patients with high values of KSHV viral load had an increased mortality rate and may benefit from more intensive management. (24)

***Limitations of the study***

Blood samples for HHV8 testing were collected at different times of treatment and as a result, the sample size for the pre chemotherapy group was small and is a limitation in terms of power of the study to make conclusions.

The patients started antiretroviral therapy at different times relative to the start of chemotherapy so accurate evaluation of the treatment response was not possible.

This was a retrospective study, and some information was missing from clinical records.

***Conclusions and Recommendations***

We find that there is no association between serum HHV8 viral load and clinical extent of Kaposi's sarcoma among our patients.

Serum HHV8 levels are not useful in predicting outcomes of treatment for Kaposi's sarcoma. However, it may have some utility as a prognostic indicator. We recommend a prospective study to further study the utility of KSHV DNA viral load as a prognostic indicator or a marker of treatment response among HIV-infected patients diagnosed with Kaposi's sarcoma. Further research into the variations of KSHV levels among different clinical presentations with Kaposi's sarcoma would be interesting. We recommend a multidisciplinary team approach to comprehensive care for these patients.

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*APPENDICES*

## KS DATA COLLECTION TOOL

### *Demographics*

ID number:

Age

Sex

### HIV characteristics

CD4 count

| | |
|---|---|
| Nadir | |
| At KS diagnosis | |
| Most recent | |

ART use

| | |
|---|---|
| naive | |
| defaulted | |
| On ART | |

ART duration prior to KS treatment

| | |
|---|---|
| Less than 1 year | |
| More than a year | |

HIV viral load

| | |
|---|---|
| Nadir | |
| At KS diagnosis | |
| Most recent | |

### KS characteristics

KSHV status

| | |
|---|---|
| positive | |
| negative | |

KSHV Viral load

| | |
|---|---|
| Pre chemotherapy | |
| Mid chemotherapy | |
| Post chemotherapy | |

Other tests done

| | |
|---|---|
| Full blood count: White cell count | |
| Haemoglobin | |
| Platelets | |
| Albumin | |
| Chest radiograph | |
| Abdominal ultrasound scan | |
| Faecal occult blood | |
| Gastroscopy | |
| | |

KS staging

T stage

| | |
|---|---|
| T0 | |
| T1 | |

I stage

| | |
|---|---|
| 10 | |
| I1 | |

S stage

| | |
|---|---|
| S0 | |
| S1 | |

T 1 sub staging

| | |
|---|---|
| KS IRIS | |
| Lymphedema | |
| Extensive cutaneous disease | |
| Pulmonary KS | |
| GIT/other visceral site | |

Survival

| | |
|---|---|
| alive | |
| dead | |

| | |
|---|---|
| Last ART clinic date if alive | |
| Date of death | |

**KS Treatment**

| | |
|---|---|
| Use of ART only | |
| ART and systemic chemotherapy | |

Type of chemotherapy

| | |
|---|---|
| Bleomycin/Vincristine | |
| Other | |

Response

| | |
|---|---|
| Complete response | |
| Partial response | |
| Progressive disease | |
| Relapse | |

KS relapse

| | |
|---|---|
| Date of relapse | |
| ART at relapse | |
| HIV viral failure at relapse | |

## Appendix 2. Supplementary Table 1.

## Clinical and demographic characteristics of patients with Kaposi's sarcoma

| Variables | Stage of Kaposi's Sarcoma | | | | | |
|---|---|---|---|---|---|---|
| | T | | I | | S | |
| | T0 (n = 4) | T1 (n = 91) | I0 (n = 49) | I1 (n = 46) | S0 (n = 86) | S1 (n = 9) |
| | | | | | | |
| Sex (n, %) | | | | | | |
| Male | 3 (75.0) | 57 (62.5) | 33 (67.4) | 27 (58.7) | 53 (61.6) | 7 (77.8) |
| Female | 1 (25.0) | 34 (37.4) | 16 (32.7) | 19 (41.3) | 33 (38.4) | 2 (22.2) |
| Total | 4 (100.0) | 91 (100.0) | 49 (100.0) | 46 (100.0) | 86 (100.0) | 9 (100.0) |
| | | | | | | |
| Age (years), (mean, SD) | 43.73 (8.8) | 38.8 (8.2) | 40.4 (6.9) | 37.4 (9.5) | 39.2 (8.2) | 36.1(9.6) |
| | | | | | | |
| kshvvl_pc (median) | 823.7 | 227.7 | 460.1 | 107.0 | 255.0 | 554.3 |
| | | | | | | |
| kshvvl_mc (median) | 3.00 (-) | 68.1 | 132.4 | 59.0 | 59.0 | 280.5 |
| | | | | | | |
| kshvvl_post(median) | - | 65.8 | 80.0 | 43.3 | 62.3 | 2091.3 |
| | | | | | | |
| hivvl_rec (median, SD) | 40.0 (28.3) | 39.5 | 35 | 64.5 | 35 | 8324.0 |
| | | | | | | |
| Hb (g/dL), (mean, SD) | 12.7 (1.9) | 10.5 (2.5) | 10.9 (2.6) | 10.3 (2.4) | 10.7 (2.6) | 10.2 (1.8) |
| | | | | | | |
| WCC (/L), (median)* | 7.96 | 6.73 | 6.98 | 6.16 | 6.53 | 7.55 |
| | | | | | | |
| Plt (/L), (mean, SD)* | 288.5(85.9) | 330.8 (148.0) | 311.1 (126.5) | 349.3 (164.2) | 329.6 (141.1) | 323.3 (194.1) |
| | | | | | | |
| Chest X-ray (n, %) | | | | | | |
| Normal | 3 (75.0) | 56 (61.5) | 33 (67.4) | 25 (56.3) | 55 (64.0) | 4 (44.4) |
| Pulmonary KS | 1 (25.0) | 21 (23.1) | 9 (18.4) | 13 (28.3) | 19 (22.1) | 3 (33.3) |
| Not done | 0 (0.0) | 14 (15.4) | 7 (14.3) | 7 (15.2) | 12 (14.0) | 2 (22.2) |
| Total | 4 (100.0) | 91 (100.0) | 49 (100.0) | 46 (100.0) | 86 (100.0) | 9 (100.0) |
| | | | | | | |
| On ART for 1 year | | | | | | |
| Yes (n, %) | 0 (0.0) | 8 (8.8) | 27 (55.1) | 32 (69.6) | 8 (9.3) | 0 (0.0) |
| No (n, %) | 3 (75.0) | 25 (27.5) | 16 (32.7) | 12 (26.1) | 25 (29.1) | 3 (33.3) |
| Missing (n, %) | 1 (25.0) | 58 (63.7) | 6 (12.2) | 2 (4.4) | 53 (61.6) | 6 (66.7) |
| Total (n, %) | 4 (100.0) | 91 (100.0) | 49 (100.0) | 46 (100.0) | 86 (100.0) | 9 (100.0) |
| | | | | | | |
| Defaulted on ART | | | | | | |
| Yes (n, %) | 0 (0.0) | 73 (80.2) | 38 (77.6) | 39 (84.8) | 13 (15.1) | 3 (33.3) |
| No (n, %) | 0 (0.0) | 2 (2.2) | 2 (4.1) | 0 (0.0) | 1 (1.2) | 1 (11.1) |
| Missing (n, %) | 4 (100.0) | 16 (17.6) | 9 (18.4) | 7 (15.2) | 72 (83.7) | 5 (55.6) |
| Total (n, %) | 4 (100.0) | 91 (100.0) | 49 (100.0) | 46 (100.0) | 86 (100.0) | 9 (100.0) |

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