Oral HIV-associated Kaposi sarcoma: A clinical study from the Ga-Rankuwa area, South Africa

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ORAL HIV-ASSOCIATED KAPOSI SARCOMA: A CLINICAL STUDY FROM THE GA-RANKUWA AREA, SOUTH AFRICA.

By

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RESEARCH REPORT

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Supervisor: Professor L. Feller

Co-supervisor: Professor L. Pantanowitz

DECLARATION

I declare that the research report hereby submitted to the University of Limpopo, for the degree of Master of Dentistry in Periodontology and Oral Medicine has not been previously submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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DEDICATION

To my family:

Thank you for the encouragement, unwavering belief and support.

ACKNOWLEDGEMENTS

ABSTRACT

Background: Kaposi sarcoma (KS) is the most common neoplasm diagnosed in HIV-seropositive subjects. HIV-associated KS (HIV-KS) may affect any body system and the disease may be slowly progressing or fulminant. Oral involvement is frequent and extensive oral HIV-KS is associated with poor prognosis.

Methods: All cases of oral HIV-KS treated in the Department of Periodontology and Oral Medicine over a period of seven years were included in this retrospective study. A record was made regarding the clinical and laboratory features, and differences in these features between males and females were statistically tested. The differences between the percentages of the different clinical appearances of oral HIV-KS lesions; and between the features of oral HIV-KS in patients who contracted HIV infection before the diagnosis of oral KS and those who were diagnosed with HIV infection at the time of oral KS presentation were also tested.

Results: Of the 37 patients included in the study, 54% were females and 46% were males and two patients (5%) were children. In 21 patients (57%) the initial presentation of HIV-KS was in the mouth. Seventeen patients (46%) were diagnosed with HIV infection and oral KS at the same time. At the time of oral HIV-KS diagnosis, 29 patients (78%) had multiple lesions affecting one or several sites.

There were no statistically significant differences between males and females regarding the clinical and laboratory features of oral HIV-KS except for the size of the lesions. The percentage of lesions <10mm was significantly lower in females than males (*chi-squared test*: p=0.007), whereas the percentage of lesions ≥10mm≤50mm was significantly higher in females than in males (*chi-squared* test: p=0.0004). There were significantly more patients with multiple oral HIV-KS lesions than patients with single oral HIV-KS lesions (*binomial distribution test:* p=0.0003). At the time of oral HIV-KS diagnosis, the difference between

the average CD4+ T cell counts of the patients who were concurrently diagnosed with HIV infection and oral KS (130cells/mm^3), and those who contracted HIV infection before developing oral HIV-KS (90 cells/mm^3) was not statistically different.

Nine patients (24%) developed facial lymphoedema in association with multifocal exophytic oral HIV-KS lesions. The average CD4+ T cell counts of these patients at the time of oral HIV-KS diagnosis was 28 cells/mm^3, and was statistically significantly lower (*t-test*: p= 0.01) than the average CD4+ T cell count (133 cells/mm^3) of those who did not develop facial lymphoedema. All the patients with facial lymphoedema died, on average within two weeks from the occurrence of facial lymphoedema. One patient (2.7%) developed immune reconstitution inflammatory syndrome (IRIS) – associated oral HIV-KS, and his oral HIV-KS resolved following administration of highly active antiretroviral therapy (HAART) and systemic cytotoxic chemotherapy, and surgical excision.

Out of the 28 patients who were not lost to follow-up, 21 (75%) died, on average within 13.6 weeks from the time of oral HIV-KS diagnosis and seven (25%) survived. At the time of oral HIV-KS diagnosis the difference in the average CD4+ T cell count of the patients who died (64 cells/mm^3) and those who survived (166 cells cells/mm^3) was statistically significant (*t-test*: p=0.016). The prognosis of the patients who received cytotoxic chemotherapy was better than the prognosis of those who received only HAART, or those who were HAART-naïve.

Conclusions: Oral HIV-KS affects females more frequently than males (M:F = 1:1.2), and it is not uncommon in children. A lower CD4+ T cell count at the time of oral HIV-KS diagnosis is associated with a poor prognosis. Patients who develop facial lymphoedema during the course of HIV-KS disease, die soon thereafter. Oral HIV-KS can be successfully treated with systemic cytotoxic chemotherapy.

1. INTRODUCTION

Kaposi sarcoma (KS) is a multicentric angioproliferative disorder of endothelial origin (Wang et al., 2004). It predominantly affects mucocutaneous sites, but may also affect visceral organs; and it is characterized microscopically by angiogenesis, the presence of spindle-shaped tumour cells, inflammatory cell infiltrate dominated by mononuclear cells, extravasated erythrocytes and oedema (Montaner et al., 2004).

There are four clinical-epidemiological variants of KS: classic KS, endemic KS, iatrogenic KS and HIV-associated KS (HIV-KS). These variants develop in distinct populations of subjects, and in all of them, the mouth may be affected. HHV8 is a critical factor, although not on its own sufficient for the development of all the clinical-epidemiological variants of KS (Schwartz, 2004; Feller et al., 2006; Feller and Lemmer, 2008).

KS is the most frequently observed neoplasm in HIV-seropositive subjects (Letang et al., 2010). HIV-KS may develop at any stage of HIV infection including the stage of early HIV-seropositivity, but it is more prevalent at a lower CD4+ T cell count; and concurrently with the diminution in the number of CD4+ T cells, oral KS lesions tend to enlarge and become exophytic (Feller at al., 2007a; Feller and Lemmer 2010). Advanced oral lesions may become ulcerated, secondarily infected and may interfere with mastication (Feller and Lemmer., 2010). Cutaneous and as well as visceral involvement is common in HIV-KS (Pantanowitz et al., 2010).

Oral HIV-KS lesions may be single or multifocal, initially present as macules that progress to papulo-nodular lesions and ultimately become confluent forming large exophytic masses

(Feller et al., 2007a; Feller et al., 2007b; Feller and Lemmer, 2010). Oral HIV-KS lesions harbour a higher load of HHV8 than cutaneous HIV-KS lesions, and advanced nodular/exophytic oral HIV-KS lesions have a higher HHV8 load than early macularpapular lesions (Pak et al., 2007; Feller et al., 2010b); and oral fluid contains a higher HHV8 load than does any other body secretion (Canon et al., 2003; Marcelain et al., 2004; Feller et al., 2010b).

It is estimated that in 22% of HIV-seropositive subjects with KS, the initial presentation of HIV-KS is in the mouth; and in up to 71% of subjects with HIV-KS, sooner or later the mouth will be affected (Fauci and Lane, 2005; Epstein et al., 2005).

The exact nature of KS is a matter of controversy, and it is still unclear whether the disease is a reactive phenomenon or a true malignancy (Pantanowitz and Dezube, 2004; Wood and Feller 2008). It is suggested that HIV-KS starts as a reactive polyclonal angioproliferative response to HHV8 infection, but with time some of the polyclonal KS cells evolve into oligoclones, and some advanced oral HIV-KS lesions may have arisen from a monoclonal expansion of HHV8 infected endothelial/spindle cells accounting for the aggressive nature of the disease in these cases (Rabkin et al., 1997; Gill et al., 1998; Feller et al., 2007b; Wood and Feller, 2008; Feller and Lemmer, 2008; Pantanowitz et al., 2010).

2. THE CLINICAL-EPIDEMIOLOGICAL VARIANTS OF KAPOSI SARCOMA

There are four clinical-epidemiological variants of KS: classic KS, endemic KS, iatrogenic KS and HIV-associated KS (HIV-KS). These variants develop in distinct populations of subjects, and in all of them, the mouth may be affected. HHV8 is a critical factor, although

not on its own sufficient for the development of all the clinical-epidemiological variants of KS. Other co-factors such as profound immune impairment, HIV-tat protein, inflammatory cytokines, angiogenic mediators, or genetic predisposition are necessary for the development of KS (Friedman-Kien et al., 1990).

Classic KS mostly affects men of Mediterranean, eastern European or Jewish descent between the ages of 40 and 70 years. Sites affected by this variant of KS involves the skin of the lower extremities and mucosae. Classic KS runs a chronic, indolent course with a good prognosis (Iscovich et al., 2000).

Iatrogenic KS develops in patients who have received an organ transplant (bone marrow transplantation, kidney transplant) and/or are on chronic immunosuppressive drug therapy (corticosteroids, cyclosporine, azathioprine) for other reasons (Tappero et al., 1993). This variant of KS may have a chronic course similar to classic KS although in 50% of subjects it has an aggressive course, affecting multiple organs, lymph nodes and different anatomical mucosae. Iatrogenic KS has a median survival of months to years (Antman and Chang, 2000). However regression of KS lesions has been noted in some cases when the immunosuppressive therapy was discontinued, confirming the role which immunosuppression plays in the pathogenesis of KS (Tappero et al., 1993; Schwartz, 2004).

African endemic KS is a variant of KS affecting black African HIV-seronegative subjects, living in geographical locations where HHV8 is endemic. Two forms of this variant are observed affecting two distinct age groups:

- The fulminant lymphadenopathic form that affects subjects between the ages of 5 and 15 years and is characterized by rapidly enlarging tumours of lymph nodes and

occasionally internal organs, resulting in death within two to three years of diagnosis (Tappero et al., 1993; Neville et al., 2002).

- The second form known as the nodular form affects females between the ages of 20 and 40 years, is characterized by wide spread involvement, resulting in death within five to eight years of diagnosis. This form of KS is unrelated to immune deficiency and runs a more aggressive course than the classic or iatrogenic variants of KS but is less aggressive than HIV-KS (Tappero et al., 1993).

HIV-KS is common in African countries where HIV and HHV8 infections are endemic and anti-retroviral medication is not always available. KS is the most frequently observed neoplasm in HIV-seropositive subjects and accounts for severe morbidity and mortality (Letang et al., 2010). HIV-KS may develop at any stage of HIV infection including the stage of early HIV-seropositivity, but it is more prevalent at a lower CD4+ T cell count; and concurrently with the diminution in the number of CD4+ T cells, oral KS lesions tend to enlarge and become exophytic (Feller at al., 2007a; Feller and Lemmer 2010).

3. PATHOGENESIS OF HIV-ASSOCIATED KAPOSI SARCOMA

3.1 The role of human herpesvirus 8 (HHV8) in the pathogenesis of KS

HHV8 is a gamma virus belonging to the herpesvirus family. It is causally linked to development of KS, multicentric Castleman disease and primary effusion lymphoma (PEL) (Beyari et al., 2003). HHV8 infects KS tumour spindle cells, tumour macrophages and normal appearing endothelial cells in all KS variants. HHV8 infection is acquired either by sexual transmission, by mother to child transmission during pregnancy or delivery, or by close non-sexual contact. The prevalence of HHV8 infection in the general population varies according to geographic locations. HHV8 seroprevalence is lower in North America,

Northern Europe and Asia (<20%) but higher in Africa (20-40%) and accordingly Africa has the highest frequency of KS in the world (Ablashi et al., 1999).

There are three significant facts that implicate HHV8 in the pathogenesis of KS. Firstly, the DNA sequences of all epidemiological variants of KS contain HHV8 DNA (Schwartz, 2004); secondly anti-HHV8 antibodies are present in subjects at high-risk of developing KS, and lastly the development of KS is preceded by the seroconversion of HHV8 (Feller et al., 2007b).

HHV8 is essential for the development of KS, and the pathogenesis of KS is associated with several HHV8 genes. With utilization of specific signal transduction pathways, these genes have the capacity to transform HHV8 infected cells into KS tumour cells (Sullivan et al., 2010).

HHV8 genome contains several genes which are homologous to human genes. Activation and resultant expression of these genes allows HHV8 to promote cell proliferation and survival, and to stimulate angiogenesis and disease progression (Sullivan et al., 2010).

Like all herpesviruses, HHV8 carry lytic phase and latent phase genes. During the lytic phase, HHV8 expresses the viral G-protein coupled receptor (vGPCR), a viral homologue of interleukin (IL)-6 and viral homologue genes of macrophage inflammatory protein (MIP) – 1, 2 and 3, which drive the replication of HHV8 (Sullivan et al., 2010).

vGPCR is a member of the CXC chemokine G-protein coupled receptor family with significant homology to IL-8. vGPCR has ligand independent activity and has the ability to induce the production of chemokines (IL-8, MIP-1), cytokines (IL-1β, TNF-α, IL-6) and growth factors (VEGF, bFGF), that function in an autocrine and paracrine fashion (Masood et al., 2002).

Interleukin (IL)–6 is an inflammatory cytokine that in health plays a role in inflammation. In KS, the HHV8 lytic gene produces a homologue of IL-6 (vIL-6). vIL-6 directly promotes proliferation of HHV8 infected cells and indirectly induces the expression of vascular endothelial growth factor (VEGF) and cellular IL-6 resulting in proliferation of uninfected endothelial cells. Viral MIP-1 mediates dysregulation of the host immune responses and initiates angiogenesis, a characteristic feature of Kaposi sarcoma (Masood et al., 2002).

The three latent genes expressed by HHV8 that play important roles in the pathogenesis of KS are: the latency associated nuclear antigen (LANA) which inhibits the functions of p53, subsequently blocking apoptosis; viral cyclin (vCyc) which inhibits cell cycle arrest; and a viral Fas-ligand interleukin (vFLIP) that protects virally infected cells from apoptosis (Feller et al., 2007b).

Persistent endothelial cell proliferation in response to HHV8 gene products within an environment of impaired immunity brought about by HIV, may result in dysregulated cell proliferation and survival, followed by cellular transformation and eventual progression to a monoclonal tumour (Ensoli et al., 2001; Krown, 2003; Bubman and Cesarman, 2003).

3.2 The role of HIV in the pathogenesis of HIV-associated Kaposi sarcoma

Not all persons infected with HHV8 develop KS, confirming that although HHV8 is an essential co-factor in the initiation of KS, it is not sufficient on its own to cause KS. It is evident that immune dysregulation is an essential co-factor in the development of KS. The association between KS and states of immunosuppression was first evident in solid organ recipients and/or in patients receiving chronic immunosuppressive drug therapy (Feller and Lemmer, 2008), and later in relation to HIV-induced immune impairment.

HIV co-infection is an important co-factor in the pathogenesis of HIV-KS. HIV is an RNA virus, infecting a subset of T cells phenotypically characterised by the presence of a CD4 molecule on the surface. During primary infection, HIV replicates rapidly in lymphoid tissue, resulting in a burst of viremia (Fauci and Lane, 2005).

Following primary infection, a robust immune response is mounted, however the virus skilfully escapes immune mediated clearance and paradoxically thrives on immune activation. A state of chronic infection is than established characterized by variable degrees of viral replication and a chronically activated immune system (Fauci and Lane, 2005).

Generalised immune deficiency is the hallmark of HIV, and occurs as a consequence of the aberrant immune activation. It is characterised by a qualitative and quantative decrease in CD4+ T cell function and numbers. CD4+ T cell depletion is a result of direct destruction of HIV infected cells and indirectly through immune exhaustion of aberrantly activated cells (Fauci and Lane, 2005; Lieberman et al., 2001).

A dysregulated cytokine network induced by the chronically activated immune system is a powerful mediator of HIV infection, since viral replication is controlled by the pro-inflammatory cytokines, TNF-α, IL-1β and IL-6 which act synergistically in an autocrine and paracrine fashion (Fauci and Lane, 2005).

HIV-KS originates in an environment induced by inflammatory T helper cytokines in association with an impaired immune system brought about by HIV. The inflammatory infiltrate in HIV-KS lesions comprises CD8+ T cells, monocytes, macrophages, and dendritic cells. These cells release inflammatory cytokines that act synergistically with HHV8 gene products, activating endothelial cells and consequently initiating the development of HIV-KS (Feller and Lemmer, 2008).

Increased levels of inflammatory cytokines have the potential to reactivate latent HHV8, resulting in an increase in HHV8 plasma load and spread of HHV8 in tissues, and an increase in HIV load. The HIV-associated high levels of inflammatory cytokines trigger endothelial cells to express activation factors which may initiate and promote the development of HIV-KS (Barillari and Ensoli, 2002).

HIV Tat-protein, a transcriptional activator of HIV gene expression is thought to play an important role in the pathogenesis of HIV-KS by directly promoting HHV8 replication. HIV Tat protein is released by HIV infected T cells into the extracellular matrix where it synergises with upregulated cytokines and growth factors to promote angiogenesis and the progression of HIV-KS. Mechanisms employed to accomplish this involve the induction of growth factors and interaction with integrins on the surface of endothelial and spindle cells (Connick et al., 2004).

4. COURSE OF HIV-ASSOCIATED KAPOSI SARCOMA

The course of HIV-KS in the absence of highly active antiretroviral therapy (HAART) is unpredictable. It can be a mild, slowly progressive disease, but usually is rapidly progressive and life threatening. The mild form of HIV-KS is usually confined to the skin, oral mucosa and lymph nodes (Krown et al., 1997). The aggressive form of HIV-KS in contrast is associated with disseminated visceral and cutaneous lesions, intra-oral exophytic lesions (Feller and Lemmer, 2008), with facial lymphoedema (Feller et al., 2010b; Feller et al., 2008a; Feller and Lemmer 2010) and with increased viral load of HHV8 in peripheral blood mononuclear cells (Nsubuga et al., 2008; Feller et al., 2010a).

The clinical presentation of HIV-KS is independent of the CD4+ T cell count. HIV-KS may present at any stage of HIV infection, however with a decrease in CD4+ T cell count and

resultant immune suppression, KS lesions tend to rapidly enlarge in size (Fauci and Lane, 2005).

It is estimated that in 22% of HIV-seropositive subjects with KS the initial presentation of HIV-KS is in the mouth; and in up to 71% of subjects with HIV-KS, sooner or later the mouth will be affected (Epstein et al., 2005; Fauci and Lane, 2005). Early oral KS lesions are usually macular in appearance but progress to papular-nodular lesions and eventually may enlarge and coalesce presenting as exophytic lesions. Advanced oral HIV-KS lesions have the potential to cause underlying alveolar bone resorption, with resultant tooth loss (Lager et al., 2003).

In children in sub-Saharan Africa, African endemic KS and HIV-KS is more prevalent than any other epidemiological variants of KS (Wamburu et al., 2006). The African endemic variants of KS affect children and females more frequently than the classic or iatrogenic variants of KS. HIV-KS in children runs a course similar to the lymphadenopathic form of African endemic KS. Lymphadenopathy, oral involvement and respiratory dysfunction are frequent presenting clinical features in children with HIV-KS (Wamburu et al., 2006).

5. ORAL HIV-ASSOCIATED KAPOSI SARCOMA

Oral HIV-KS lesions may be single or multifocal, initially present as macules which progress to papulo-nodular lesions and ultimately become confluent forming large exophytic masses (Feller et al., 2007a; Feller et al., 2007b; Feller and Lemmer, 2010). Oral HIV-KS lesions carry a higher load of HHV8 than cutaneous HIV-KS lesions, and advanced nodular/exophytic oral HIV-KS lesions have a higher HHV8 load than early macularpapular lesions (Pak et al., 2007; Feller et al., 2010b); and oral fluid contains a higher HHV8 load

than does any other body secretion (Canon et al., 2003; Marcelain et al., 2004; Feller et al., 2010b).

Clinically, oral KS lesions are bluish-purple or red; may be indolent, slowly progressing or may be rapidly progressing, fulminant and locally aggressive. Oral HIV-KS most frequently affects the palate, gingiva and the dorsum of the tongue (Lager et al., 2003; Feller et al., 2006; Feller et al., 2007a; Feller et al., 2007b; Feller et al., 2008a; Feller et al., 2008b; Feller et al., 2010b; Feller and Lemmer, 2010). Rarely, long standing oral HIV-KS may cause resorption of the underlying alveolar bone and basal bone of the jaw resulting in tooth mobility and tooth loss (Konstantinopoulos et al., 2006; Feller et al., 2010b; Feller and Lemmer, 2010).

Early oral HIV-KS can be asymptomatic, however exophytic advanced oral HIV-KS may be ulcerated or superficially necrotic interfering with speech, mastication and swallowing; and may be painful when the lesions are secondarily infected (Feller and Lemmer, 2010).

The mortality rate of patients with oral HIV-KS is greater than patients with only cutaneous HIV-KS. The former has a 24-month median survival rate compared to the latter that have a 72-month median survival rate (Rohrmus et al., 2000).

6. HIV-KS AS AN IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS) PHENOMENON

Highly active antiretroviral therapy (HAART) used to treat HIV infection, although not directly affecting HHV8 replication, indirectly brings about a decrease in the incidence and prevalence of HIV-KS and improvement in the clinical manifestation of KS. However,

HAART does not ensure that KS will not develop, and despite HAART, KS remains the most frequent associated neoplasm in people infected with HIV (Pantanowitz et al., 2010). Furthermore, in a subset of HIV-seropositive subjects, KS may flare up early after the introduction of HAART, as part of the immune inflammatory reconstitution syndrome (IRIS) (Feller and Lemmer, 2008; Feller et al., 2008b; Pantanowitz and Dezube, 2010).

HIV-KS as an IRIS phenomenon is characterized by paradoxical resurgence or worsening of the symptoms of a pre-existing KS shortly after the introduction of HAART, probably as a consequence of reactivation of the partially restored inflammatory response towards HHV8. IRIS-associated HIV-KS occurs in younger subjects who have low CD4+ T cell counts at the time of introduction of HAART; and in subjects who display as indicators of immune reconstitution, a decrease in HIV viral loads and an increase in the CD4+ T cell count. In IRIS-associated HIV-KS there is a rapid clinical progression of KS and the development of new KS lesions, usually within eight weeks after the initiation of HAART (Feller and Lemmer 2008, Feller et al., 2008b).

The pathogenesis of IRIS-associated HIV-KS is speculative. HAART brings about a reduction in the HIV plasma viral load, a shift in the cytokine profile from a Th-2 to a Th-1 type cytokine profile, activation of HHV8 lytic genes and an improvement in cytotoxic CD8+ T cell responses. The reactivated HHV8 lytic genes and the increase in inflammatory cytokines can stimulate angiogenesis, transformation of HHV8-infected endothelial cells, and increase proliferation of spindle cells through autocrine and paracrine mechanisms. Together these pathobiological changes may mediate the development of IRIS (Feller and Lemmer, 2008).

The HHV8 specific cytotoxic T cell responses may also play a role in the development of IRIS-associated HIV-KS. These responses may be dysregulated and as a result may promote the immunoinflammatory reaction toward the HHV8 antigens thus stimulating the exuberant response seen in IRIS-associated HIV-KS (Feller et al., 2008a).

7. HIV-ASSOCIATED KAPOSI SARCOMA AND LYMPHOEDEMA

Extensive oral HIV-KS associated with facial lymphoedema in the absence of HAART and of cytotoxic chemotherapy appears to be an indication of a poor prognosis. Lymphoedema is characterised by an abnormal increase in protein rich interstitial fluid in the presence of normal capillary filtration and consequently resulting in oedema of the affected tissue. Oedema in contrast occurs when capillary filtration rate exceeds lymph drainage (Ruocco et al., 2002).

Lymphoedema that develops in persons with HIV-KS may be the result of HHV8 infection of lymphatic endothelial cells and cells resident in the lymph nodes. Consequently, the proliferating endothelial cells may obstruct and/or compress the lymphatics, resulting in leakage of protein-rich fluid into the interstitial spaces (Ruocco et al., 2002; Hengge et al., 2002; Witte et al., 1990; Konstantinopoulos et al., 2006).

Lymphoedema may herald HIV-KS, develop concurrently with the occurrence of HIV-KS, or most frequently may follow the clinical appearance of HIV-KS (Smith and Skelton, 2006). Lymphoedema that heralds the appearance of HIV-KS may be a predisposing factor in the development of HIV-KS. HHV8 induced proliferation of endothelial cells may lead to occlusion of lymphatic vasculature lumens. This may promote the concurrent development of KS and lymphoedema (Witte et al., 1990; Ramdial et al., 2006).

Recently, three cases of HIV-seropositive HAART-naive patients with a fulminant course of oral KS disease who died within three weeks after the onset of facial lymphoedema have

been reported (Feller et al., 2008a; Feller et al., 2010b). However, as all these patients were terminally ill with severe immunosuppression and multiple metabolic abnormalities, their death might or might not have been owing to their advanced HIV-KS and associated facial lymphoedema (Feller et al., 2008a; Feller et al., 2010b).

8. THE NATURE OF HIV-ASSOCIATED KAPOSI SARCOMA

The clinical appearance of HIV-KS evolves over time from early macular lesions to late nodular/exophytic lesions (Pantanowitz and Dezube, 2010). Tumour progression is associated with HHV8 gene expression which modulates cellular proliferation, transformation, cell signalling, cytokine production, immune evasion and angiogenesis (Pantanowitz and Dezube, 2010).

The clonal nature of HIV-KS is debatable. It is uncertain whether HIV-KS is a true neoplasm arising from a clonal expansion of a single HHV8 infected cell or a benign proliferation in response to HHV8 infection (Wood and Feller, 2008).

HIV-KS development is closely associated with inflammation and immune suppression. An early KS lesion is probably brought about by a reactive proliferation of HHV8 infected endothelial cells within an environment of inflammatory cytokines and impaired immunity (Pantanowitz and Dezube, 2010).

HHV8 oncoproteins which are expressed in advanced KS lesions dysregulate intracellular transduction pathways, cell cycle progression and apoptosis bringing about dysregulation of normal cellular proliferation, maturation and survival thus promoting tumour development (Wood and Feller, 2008).

Two techniques have been used to determine the clonality of KS lesional cells. The first technique involved analysing the inactivation pattern of human x-linked androgen receptor

(HUMARA) in females with KS. Results of this technique were conflicting and should therefore be interpreted with caution. Rabkin et al., 1995 and Rabkin et al., 1997 using this technique demonstrated that the HIV-KS cells were of monoclonal origin. On the other hand Delabesse at al., 1997 found that the KS lesional cells were polyclonal and Gill et al., 1998 found that the KS lesional cells had both monoclonal and polyclonal characteristics (Rabkin et al., 1995; Rabkin et al., 1997; Delabesse et al., 1997; Gill et al., 1998).

The second technique used to determine the clonality of HIV-KS lesional cells involves studying HHV8 terminal repeat sequences in advanced KS lesions. Judd et al., 2000 found that advanced KS lesional cells displayed all patterns of clonality. This finding supports the concept that early HIV-KS lesions may result from a reactive polyclonal angio-proliferative response to HHV8 infection, and with time, some of the polyclonal KS cells evolve into oligoclones, and some advanced oral HIV-KS lesions arise from a monoclonal expansion of HHV8 infected endothelial or spindle cells (Wood and Feller, 2008).

9. TREATMENT OF ORAL HIV-ASSOCIATED KAPOSI SARCOMA

Treatment of HIV-KS requires HAART, which brings about a decrease in HIV plasma load and immune restoration. HAART should ideally be introduced before advanced HIV-associated immunosuppression occurs. It reduces the risk of developing new KS lesions and may bring about regression of established lesions. However in HIV and HHV8 endemic areas of sub-Saharan Africa where access to HAART is limited, HIV-KS remains a growing health problem (Uldrick and Whitby, 2011).

The positive role of HAART alone in the management of early stage HIV-KS is well established, with clinical success rates more than 30%. However HAART is insufficient as a

sole mode of treatment for patients with aggressive disease that require tumour-specific treatment (Pantanowitz et al., 2010).

9.1 Additional treatment for the management of HIV-KS

Additional treatment is indicated in patients with visceral disease, rapidly progressive disease, cutaneous disease associated with oedema and ulceration, and advanced oral disease associated with lymphoedema (Di Lorenzo et al., 2007). Effective treatment modalities adjunct to HAART include radiotherapy, cryotherapy, photodynamic therapy, laser surgery, surgical excision and intralesional chemotherapy. KS lesions are radiosensitive, however it is not recommended in the treatment of oral HIV-KS, as it may induce life threatening oral mucositis, osteoradionecrosis and an increased frequency of opportunistic infections (Epstein, 1997).

Systemic chemotherapy is indicated in cases where there is concurrent skin and visceral KS involvement, rapidly progressive disease or life-threatening disease (Di Lorenzo et al., 2007), and it is advisable that this mode of therapy be introduced at an early macular-papular stage of oral HIV-KS as it may delay or prevent the development of widespread or exophytic oral HIV-KS, and the development of facial lymphoedema which both are indicators of a poor prognosis (Feller et al., 2010a; Feller and Lemmer, 2010). Chemotherapeutic drugs used with success in the treatment HIV-KS include vinblastine, paclitaxel, bleomycin, doxorubicin and daunorubicin (Epstein, 1997; Dezube et al., 2004). The type of treatment of HIV-KS depends on the risks, benefits and goals of the therapy implemented.

Lymphoedema that develops in association with HIV-KS should be treated parallel to HIV-KS. Treatment involves the use of diuretic agents and in cases where the lower extremities

are involved, by elevation of the lower limbs and use of compressive dressings. Emollients should be applied to the lymphoedematous area for skin preservation and to prevent secondary bacterial or fungal infections (Allen et al., 1995).

MATERIALS AND METHODS

Approval of the study was obtained from the Medical Research and Ethics Committee of the University of Limpopo, Medunsa campus, Pretoria, South Africa (MREC 0/212/2010:PG). All the files of patients with histologically and clinically confirmed oral HIV-associated Kaposi sarcoma (HIV-KS) treated in the Department of Periodontology and Oral Medicine, School of Oral Health Sciences, University of Limpopo, Medunsa campus, from January 2004 until November 2010 were retrieved.

In this retrospective study, the diagnosis of KS was confirmed by microscopic examination of incisional biopsy specimens by an oral pathologist. All biopsy specimens were fixed in 10% buffered formalin and were embedded in paraffin. Serial sections were stained with haematoxylin and eosin (H&E) for histological study. When there was any doubt as to the histopathological diagnosis, investigations for HHV8 or for vascular markers (CD34, for factor VIII antigen) were performed.

The HIV-serostatus of the patients was determined by enzyme linked immunosorbent assay (ELISA) and Western blot. All patients whose files were analysed had been appropriately counselled prior to HIV testing and again when blood results were disclosed to them, and all patients consented in writing to the use of their records for research purposes. The patients found to be HIV-seropositive had been referred to the regional HIV-clinic for treatment.

For each patient included in the study the following details were recorded:

- The age, race and gender of the patients
- The oral site affected by the HIV-KS
- The clinical appearance of the oral HIV-KS
- The period of HIV-seropositivity before the KS diagnosis

- The CD4+ T cell count at HIV diagnosis and at oral HIV-KS diagnosis

- Whether the patient was on highly active antiretroviral therapy (HAART) at the time of oral HIV-KS diagnosis, or received HAART thereafter

- Any KS involvement of the skin

- The presence of facial lymphoedema

- The presence of an immune inflammatory reconstitution syndrome (IRIS)

- The treatment modality of oral HIV-KS

- The course and response to treatment of oral HIV-KS disease

- The survival time of the patients from the time of oral HIV-KS diagnosis to the end of the observation period

- And for those who died during the observation period, the time that elapsed from oral HIV-KS diagnosis to death.

A record was made of any relevant medical information from the patient's hospital files, and the presence of any HIV-associated oral diseases other than oral HIV-KS was documented. With regard to HIV-associated oral lesions other than KS, oral candidiosis, oral hairy leukoplakia and necrotizing gingivitis were diagnosed clinically.

The clinical appearance of oral HIV-KS was categorised into macular, papular, nodular, and exophytic types of lesions. The lesions were classified into three size groups: either smaller than 10mm; between 10mm and 50mm; or larger than 50mm. Lesions of oral HIV-KS were categorised as solitary or multifocal. Affected oral sites, and the number of lesions per site were documented. Lesions affecting the upper and lower retromolar area, and the soft palate were categorised as oropharyngeal lesions.

Statistical analysis

All data were entered into the Microsoft Excel program and analysed using a data analysis package which was written for it. *Chi-squared* test, *t-test* and *binomial-distribution* test were computed to conduct statistical hypothesis tests and to explore associations. P values < 0.05 were regarded as statistically significant.

The two-sample *chi-squared* test was used to test differences of the clinical and laboratory parameters of oral HIV-KS (at the time of oral KS diagnosis) between males and females. The two-sample *t-test* was used to test for differences between the average CD4+ T cell counts of:

1. Males and females at oral HIV-KS diagnosis

2. Males and females who were simultaneously diagnosed with HIV and oral HIV-KS

3. Males and females who contracted HIV infection before developing oral HIV-KS, at the time of HIV diagnosis

4. Males and females who contracted HIV infection before developing oral HIV-KS, at the time of oral HIV-KS diagnosis

5. Male and females who were receiving for some time HAART, at HIV-KS diagnosis

6. Male and females who were HAART-naïve at oral HIV-KS diagnosis

7. Male and females who had facial lymphoedema during the course of HIV-KS

8. Male and females who did not develop lymphoedema during the course of HIV-KS

9. Patients who were simultaneously diagnosed with HIV and oral KS and of patients who contracted HIV infection before developing oral HIV-KS, at the time of oral HIV-KS diagnosis

10. Patients who contracted HIV infection before developing oral HIV-KS, at the time of HIV diagnosis and at the time of oral HIV-KS diagnosis

11. HAART-naïve patients at oral HIV-KS diagnosis and patients who were on HAART for some time, at oral HIV-KS diagnosis

12. Patients who developed lymphoedema during the course of HIV-KS and patients who did not develop lymphoedema during the course of HIV-KS.

13. Patients who presented with cutaneous lesions at oral HIV-KS diagnosis and patients who did not present with cutaneous lesions at oral HIV-KS diagnosis

14. Patients who were alive at the end of the observation period and patients who died during the observation period

The *binomial distribution test* was used to test for differences between the percentages of the different phenotypes of oral HIV-KS; and between the percentage of patients who contracted HIV infection before the diagnosis of oral KS and those who were diagnosed with HIV infection at oral KS presentation.

RESULTS

Table 1: Clinical and laboratory features of the patients at the time of oral HIV-KS diagnosis

	Males	Females	Total
Number of patients (%)	**17 (46%)**	**20 (54%)**	**37(100%)**
Age (years)			
Mean	**34**	**33**	**33.4**
Range	*11-55*	*19-46*	*11-55*
Standard deviation	*12.53*	*7.69*	*10.08*
Tobacco usage (%)	**7 (41%)**	**2 (10%)**	**9 (24%)**
Number of patients in whom the initial presentation of HIV-KS was in the mouth	**8 (47%)**	**13 (65%)**	**21 (57%)**
Number of patients in whom the initial presentation of HIV-KS was concurrently in the mouth and skin	**3 (18%)**	**3 (15%)**	**6 (16%)**
Number of patients who developed cutaneous HIV-KS before oral HIV-KS diagnosis	**6 (35%)**	**4 (20%)**	**10 (27%)**
Other oral lesions present			
Pseudomembranous candidiasis	**7 (41%)**	**5 (25%)**	**12 (34%)**
Hairy leukoplakia		**1 (5%)**	**1 (2%)**
Necrotizing gingivitis	**1 (6%)**		**1 (2%)**
Total number of patients	**8 (47%)**	**6 (30%)**	**14 (38%)**
Average CD4+ T cell count at KS diagnosis (33 patients) [cells/mm³]	**141**	**85**	**107**
Range	*12 - 409*	*13 – 261*	*12 – 409*
Standard deviation	*117.40*	*77.99*	*106.99*
Number of patients diagnosed with HIV infection and oral KS at the same time	**10 (59%)**	**7 (35%)**	**17 (46%)**
Number of patients who contracted HIV infection before the diagnosis of oral KS	**7 (41%)**	**13 (65%)**	**20 (54%)**
Number of patients with single oral HIV-KS lesions	**5 (29%)**	**3 (15%)**	**8 (22%)**
Number of patients with multiple oral HIV-KS lesions	**12 (71%)**	**17 (85%)**	**29 (78%)**
Lesion phenotype			
Number of macular lesions	**9 (20%)**	**8 (17%)**	**17 (18%)**
Number of papular lesions	**10 (22%)**	**11 (23%)**	**21 (23%)**
Number of nodular lesions	**16 (36%)**	**17 (35%)**	**33 (35%)**
Number of exophytic lesions	**10 (22%)**	**12 (25%)**	**22 (24%)**
Total number of lesions	**45 (100%)**	**48(100%)**	**93 (100%)**
Lesion size			
Number of lesions <10mm	**15 (33%)**	**5 (10%)**	**20 (22%)**
Number of lesions ≥10mm≤50mm	**25 (56%)**	**40 (83%)**	**65 (70%)**
Number of lesions >50mm	**5 (11%)**	**3 (7%)**	**8 (8%)**
Total number of lesions	**45 (100%)**	**48(100%)**	**93 (100%)**

Clinical and laboratory features at the time of oral HIV-KS diagnosis

The study population comprised 37 patients with oral HIV-KS, all of whom were black persons. The mean age at the time of oral HIV-KS diagnosis was 33.4 years (Table 1), and the age distribution is shown in Figure 1. Two patients (5%) were children aged 10 and 11 years. Twenty females (54%) and 17 males (46%) were affected (M:F = 1:1.2). Nine patients (24%) were smokers. In 21 patients (57%) the initial presentation of HIV-KS was in the mouth; in six patients (16%) the initial presentation of HIV-KS was concurrently in the mouth and on the skin; and 10 patients (27%) developed cutaneous HIV-KS before the appearance of oral HIV-KS. Twelve patients (32%) had concomitant oral candidiosis, one (2%) had hairy leukoplakia and one (2%) had necrotizing gingivitis. The CD4+ T cell counts were obtained only for 33 patients, the mean CD4+ T cell count being 107cells/mm^3 (Table 1).
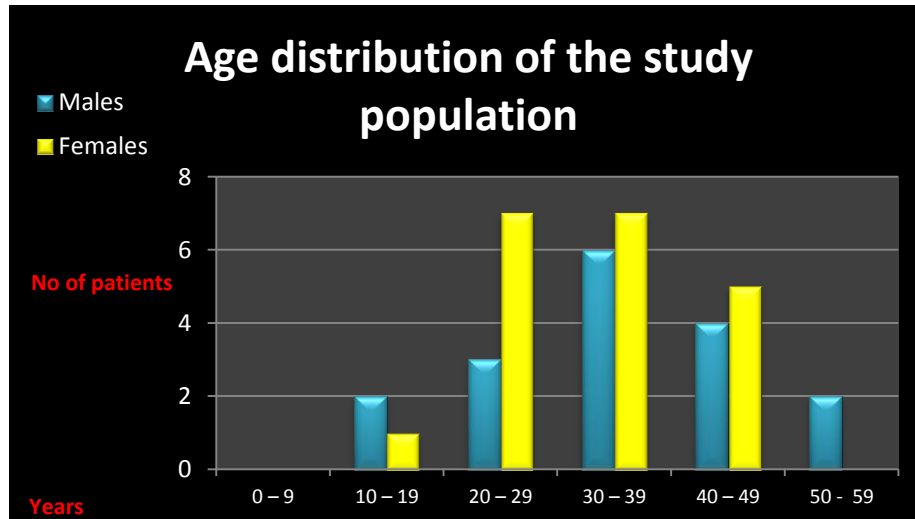

Figure 1

Seventeen patients (46%) were diagnosed with HIV infection and oral HIV-KS at the same time. The other 20 patients (54%) had already been diagnosed with HIV infection some time before the diagnosis of their oral HIV-KS (Table 1).

At the time of oral HIV-KS diagnosis, eight of the 37 patients (22%) had solitary oral lesions and 29 (78%) had multiple lesions affecting one or several oral sites. All together the 37 patients had 93 separate oral HIV-KS lesions, of which 17 (18%) were macular, 21 (23%) were papular, 33 (35%) were nodular and 22 (24%) were exophytic. Twenty lesions (22%) were < 10mm in size, 65 lesions (70%) were ≥10mm≤50mm and eight lesions (8%) were > 50mm (Table 1).

Twenty-eight oral HIV-KS lesions (30%) affected the gingiva, 24 (26%) affected the hard palate, 22 (24%) affected the oropharynx (upper and lower retromolar areas, and the soft palate), 14 (15%) affected the alveolar mucosa and five (5%) affected the dorsum of the tongue (Table 2). The oral lesions ranged in colour from pink to red to bluish-purple to deep brown.

Table 2: Oral sites affected by oral HIV-KS in relation to gender

	Males	Females	Total (%)
Gingiva	**13 (29%)**	**15 (31%)**	**28 (30%)**
Upper gingiva	7 (16%)	10 (21%)	17 (18%)
Lower gingiva	6 (13%)	5 (10%)	11 (10.8%)
Hard palate	**11 (24%)**	**13 (27%)**	**24 (26%)**
Oropharynx	**10 (22%)**	**12 (25%)**	**22 (24%)**
Alveolar mucosa	**8 (18%)**	**6 (13%)**	**14 (15%)**
Upper alveolar mucosa	4 (9%)	3 (6%)	7 (7.5%)
Lower alveolar mucosa	4 (9%)	3 (6%)	7 (7.5%)
Dorsum of tongue	**4 (9%)**	**1 (2%)**	**5 (5%)**
Total number of lesions	**45(100%)**	**48 (100%)**	**93 (100%)**

When the clinical and laboratory features of the patients at the time of oral HIV-KS diagnosis (Table 1) were compared between males and females using the *chi-squared test*, there were no statistically significant differences, except in the size of the lesions. The percentage of lesions <10mm was significantly lower in females than in males (p=0.007) whereas, the

percentage of lesions ≥10mm≤50mm was significantly higher in females than in males (p=0.004); and there were significantly more patients with multiple oral HIV-KS lesions than patients with single oral HIV-KS lesions (p=0.0003). Statistically, the percentage of patients diagnosed with HIV infection at the time of oral KS presentation was not significantly different to the percentage of patients who contracted HIV infection before the diagnosis of oral HIV-KS (*binomial distribution test*: p=0.3109).

HIV infection and HAART

The CD4+ T cell counts were available for only 33 of the 37 patients. The average CD4+ T cell count at the time of oral HIV-KS diagnosis was 107 cells/mm^3. Seventeen patients (46%) were diagnosed with HIV infection and oral KS at the same time, and CD4+ T cell counts were available for only 14 of these 17 patients. The mean CD4+ T cell count at the time of oral HIV-KS diagnosis was 130 cells/mm^3 (Table 3).

The remaining 20 patients (54%) were already HIV-seropositive at the time of oral HIV-KS diagnosis, and on average, oral HIV-KS was diagnosed 52 weeks after the diagnosis of HIV infection.

Records of CD4+ T cell counts at the time of HIV diagnosis were available for 14 of these 20 patients, and at the time of oral HIV-KS diagnosis for 19 of these 20 patients (Table 3). The difference between the average CD4+ T cell count at the time of HIV diagnosis (164 cells/mm^3), and at the time of oral HIV-KS diagnosis (90 cells/mm^3) was not statistically significant (*t-test*: p=0.11).

At the time of oral HIV-KS diagnosis the difference between the average CD4+ T cell counts of the patients who were diagnosed concurrently with HIV and oral KS (130 cells/mm^3), and

those who contracted HIV infection before developing oral HIV-KS (90 cells/mm^3) was not statistically significant (*t-test*: p=0.296).

Seven patients (19%) have been on HAART for at least four weeks before their oral HIV-KS diagnosis, and ten (27%) started HAART around the time of, or soon after their oral HIV-KS diagnosis. Altogether, 17 out of the 37 patients (46%) with oral HIV-KS were on HAART during the observation period. The average CD4+ T cell counts of the seven patients receiving HAART before the time of oral HIV-KS diagnosis was 90 cells/mm^3 at the time of oral HIV-KS diagnosis (Table 3). Eleven patients (30%) did not receive HAART during the observation period.

Table 3: CD4+ T cell counts (cells/mm^3) of the participants

CD4+ T cell counts of the patients	Males	Females	Average
At the time of oral HIV-KS diagnosis (33 patients)	**141 (14)**	**85 (19)**	**107 (33)**
Standard deviation	*117.40*	*77.99*	*106.99*
Who were simultaneously diagnosed with HIV and oral KS (14 patients)	**163 (7)**	**97 (7)**	**130 (14)**
Standard deviation	*155.64*	*85.95*	*125.63*
Who contracted HIV infection before developing oral HIV-KS, at the time of HIV diagnosis(14patients	**210 (6)**	**129 (8)**	**164 (14)**
Standard deviation	*167.27*	*160.21*	*162.14*
Who contracted HIV infection before developing oral HIV-KS, at the time of oral HIV-KS diagnosis (19 patients)	**119 (7)**	**74 (12)**	**90 (19)**
Standard deviation	*112.92*	*75.55*	*90.75*
Receiving for some time HAART, at HIV-KS diagnosis (7 patients)	**160 (1)**	**78(6)**	**90 (7)**
Standard deviation	*0*	*71.49*	*72.19*
Who were HAART-naïve at oral HIV-KS diagnosis (26 patients)	**140 (13)**	**87 (13)**	**114 (26)**
Standard deviation	*137.98*	*83.75*	*114.97*
Who had facial lymphoedema during their course of oral HIV-KS (8 patients)	**24 (4)**	**31 (4)**	**28 (8)**
Standard deviation	*14.66*	*15.75*	*14.61*
Who did not have lymphoedema during the course of oral HIV-KS (25 patients)	**188 (10)**	**96 (15)**	**133 (25)**
Standard deviation	*129.50*	*82.60*	*111.33*

Thirty patients (81%) were HAART-naïve at the time of oral HIV-KS diagnosis. CD4+ T cell records were available for 26 of these 30 HAART-naïve patients at the time of oral HIV-

KS diagnosis. Their average CD4+ T cell count was 114 cells/mm^3 (Table 3), and statistically the difference between these patients average CD4+ T cell count and the average CD4+ T cell count of those who were on HAART at the time of oral HIV-KS diagnosis (90 cells/mm^3) was not significant (*t-test*: p = 0.606).

Facial lymphoedema

Nine patients (24%), five males (13%) and four females (11%) had facial lymphoedema. Three patients presented with facial lymphoedema at the time of oral HIV-KS diagnosis and six patients developed lymphoedema on average 2.3 weeks after the diagnosis of oral HIV-KS. All the patients with facial lymphoedema had multifocal exophytic oral HIV-KS lesions and their average CD4+ T cell count at the time of oral HIV-KS diagnosis was 28 cells/mm^3 (records of the CD4+ T cell counts were available for eight of the nine patients) compared to 133 cells/mm^3 of those patients who did not develop facial lymphoedema (Table 3). This difference in the average CD4+ T cell count was statistically significant (*t-test*: p= 0.01). All nine patients died very soon after the diagnosis of oral HIV-KS, on average within 2.0 weeks, regardless if they were on HAART. Statistically, the difference between the average CD4+ T cell count of females with lymphoedema (31 cells/mm^3) and males with lymphoedema (24 cells/mm^3) was not significant (*t-test*: p=0.54).

Cutaneous HIV-KS in relation to oral HIV-KS

Nineteen of the 37 patients (51%) with oral HIV-KS developed cutaneous HIV-KS. Ten patients (27%) developed cutaneous HIV-KS before the diagnosis of oral HIV-KS. In these patients the oral HIV-KS occurred on average 4.5 weeks after the diagnosis of cutaneous HIV-KS. Six patients (16%) concurrently developed cutaneous and oral HIV-KS (Table 1). Three patients (8%) developed cutaneous HIV-KS after the diagnosis of oral HIV-KS, on average 4.3 weeks later. Eighteen patients (49%) did not develop cutaneous HIV-KS during

the study period.   Statistically, the difference between the average CD4+ T cell count of the patients who had cutaneous HIV-KS at the time of oral HIV-KS diagnosis (113 cells/mm^3) and the average CD4+ T cell count (103 cells/mm^3) of those who did not have cutaneous HIV-KS at the time of oral HIV-KS diagnosis was not significant (*t-test*: p=0.77).

Immune reconstitution inflammatory syndrome (IRIS)-associated oral HIV-KS

One patient (3%) had IRIS-associated HIV-KS. The CD4+ T cell count of this female patient at the time of diagnosis of her HIV infection was 9 cells/mm^3, and after starting HAART her CD4+ T cell count resurged to 50 cells/mm^3 and she developed IRIS-associated HIV-KS, four weeks after her diagnosis with oral HIV-KS. The patient was treated with systemic cytotoxic chemotherapy, and after her oral HIV-KS lesions shrunk substantially, the residual lesions were excised, and she did not experience recurrence during the observation period.

Oral HIV-KS in children

Two patients (5%) were children, aged 10 and 11 years. Their average CD4+ T cell count at the time of oral HIV-KS diagnosis was 212 cells/mm^3.

The course of oral HIV-KS

In the 28 patients who were not lost to follow-up, oral HIV-KS lesions increased in number and/or in size in 21 patients (75%), remained stable or shrunk in four patients (14%) and resolved in three patients (11%).

All 21 patients who died, invariably experienced worsening of their oral HIV-KS and their average CD4+ T cell count at oral HIV-KS diagnosis was 64 cells/mm^3 (Table 4). Of those who survived (seven patients), in three there was resolution of the oral HIV-KS (one had IRIS-associated HIV-KS and was treated with HAART in combination with systemic cytotoxic chemotherapy and surgery; one was treated with HAART and systemic cytotoxic

chemotherapy; and one with HAART and surgery). In four patients the oral HIV-KS lesions remained unchanged or shrunk. These patients were treated with HAART or with HAART in combination with local cytotoxic chemotherapy.

Mortality related to oral HIV-KS

Nine patients (24%) were lost to follow-up. Twenty one (11 males and 10 females) of the 28 patients (75%) who were not lost to follow-up died, on average within 13.6 weeks from the time of oral HIV-KS diagnosis; and seven patients (two males and five females) (25%) survived (average period of follow-up of 91 weeks) (Table 4). Out of the 28 patients who were not lost to follow-up, 85% of the males and 66% of the females died. Statistically the difference between the percentage of males and females who died was not significant (*chi-squared test:* p=0.274).

Table 4: Mortality and survival in relation to oral HIV-KS

	Males	Females	Total
Mortality			
Number of patients who died	11 (85%)	10 (66%)	21 (75%)
Average time of death from oral HIV-KS diagnosis	15 weeks	12.1weeks	13.6 weeks
Average CD4+ T cell count (cells/mm³) at oral HIV-KS diagnosis	75	54	64
Survival			
Number of patients who survived	2 (15%)	5 (33%)	7 (25%)
Average follow-up time	76 weeks	106 weeks	91 weeks
Average CD4+ T cell count (cells/mm³) at oral HIV-KS diagnosis	258	129	166

Eleven of the 21 patients (52%) who died did not receive HAART nor any other treatment for their oral HIV-KS. The average time from oral HIV-KS diagnosis to their death was 20 weeks. Eight of the 21 patients (38%) who died were on HAART as a sole modality of treatment, for an average of 4.4 weeks. Two patients (9%) were concurrently treated with

HAART (for an average of 20.5 weeks) and with local cytotoxic chemotherapy (Figure 2).

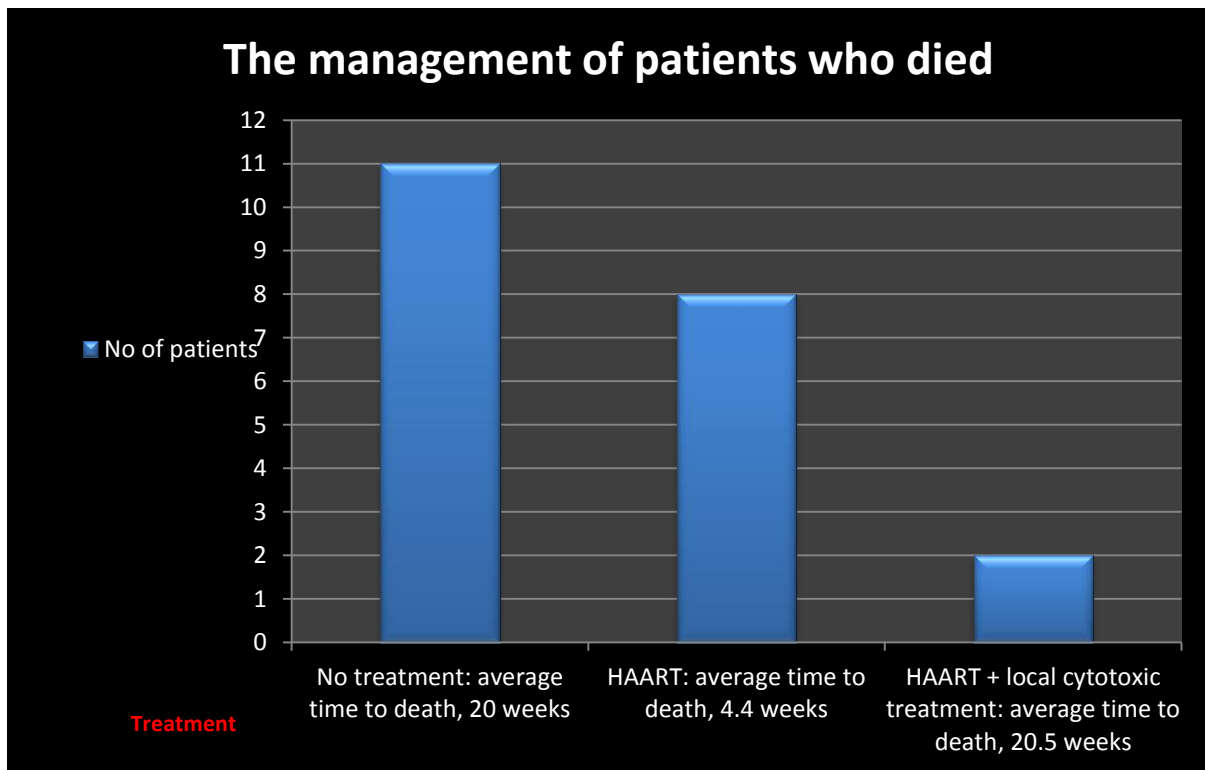All patients with oral HIV-KS who developed facial lymphoedema died.

The management of patients who died

Treatment categories: No treatment: average time to death, 20 weeks (11 patients); HAART: average time to death, 4.4 weeks (8 patients); HAART + local cytotoxic treatment: average time to death, 20.5 weeks (2 patients)

In none of the patients who died, could the cause of death be established. The average CD4+ T cell count of the patients who were alive at the end of the study observation period, was 166 cells/mm^3 at oral HIV-KS diagnosis (Table 4), while the average CD4+ T cell count of the patients who died during the observation period was 64 cells/mm^3, at the time of oral HIV-KS diagnosis. Statistically, the difference in the CD4+ T cell count between these two groups of patients was significant (*t-test:* p=0.016).

Of the seven patients who were alive at the end of the observation period, three were on HAART as the sole mode of treatment; one was treated with HAART and systemic cytotoxic chemotherapy; one with HAART in combination of systemic cytotoxic chemotherapy and surgery; one with HAART in combination with local cytotoxic chemotherapy; and one with HAART in combination with surgery (Figure 3).
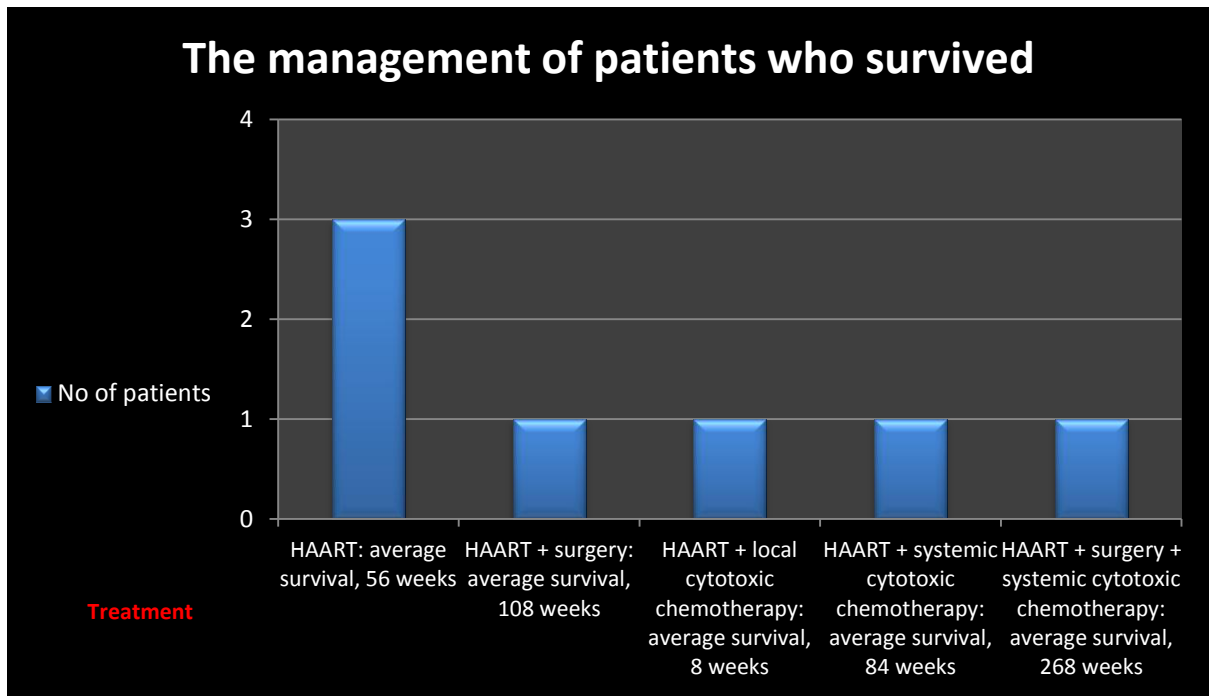
The management of patients who survived

| (x-axis) | Treatment | | | | |
| No of patients | | | | | |

(bar chart values)
- HAART: average survival, 56 weeks — 3
- HAART + surgery: average survival, 108 weeks — 1
- HAART + local cytotoxic chemotherapy: average survival, 8 weeks — 1
- HAART + systemic cytotoxic chemotherapy: average survival, 84 weeks — 1
- HAART + surgery + systemic cytotoxic chemotherapy: average survival, 268 weeks — 1

Figure 3

Medical history

Of the 37 patients with oral HIV-KS, 19 (51%) had concurrent infection with *Mycobacterium tuberculosis* (TB), one (3%) had a sexually transmitted disease (gonorrhoea), and one (3%) had bronchitis. Of the 19 patients who had TB, two were lost to follow-up, and 16 of the remaining 17 patients died during the observation period.

DISCUSSION

Figure 4: A macular-nodular lesion on the dorsum of the tongue in a 44-year old female with a CD4+ T cell count of 13 cells/mm^3.

Figure 5: An exophytic confluent oral HIV-KS lesion on the hard palate in a 27-year old female with a CD4+ T cell count of 136 cells/mm^3.

Figure 6: An exophytic oral HIV-KS lesion on the lower right retromolar area extending into the oropharnyx in a 29-year old female with a CD4+ T cell count of 49 cells/mm^3.

Many of the findings of this study (Table 1) conform with other reports in the literature regarding oral HIV-KS including that oral HIV-KS may be unifocal or multifocal; range in colour from pink to red to bluish-purple to deep brown, may manifest as macules, papules, nodules or exophytic masses, may vary in size from a few millimetres to several centimeters (Figures 4-9) and most frequently affects the hard palate, gingiva and the dorsum of the tongue of patients in their fourth decade of life (Lager et al., 2003). Most of the patients in this study were in their third (35%) or fourth (24%) decades of life (Figure 1), similar to the findings which were reported by Lager et al., 2003.

There was a statistically significant higher number of patients with multiple oral lesions at the time of oral HIV-KS diagnosis than patients with single lesions (Table 1); and with decreasing order of frequency, the gingiva, hard palate, oropharynx (upper and lower retromolar area, and soft palate), alveolar mucosa and the dorsum of the tongue were the sites most

Figure 7: Exophytic oral HIV-KS lesion on the buccal and upper labial mucosa of a 54-year old male with a CD4+ T cell count of 258 cells/mm³.

Figure 8: Exophytic oral HIV-KS lesion on the buccal gingiva and upper labial mucosa of a 30-year old female with a CD4+ T cell count of 29 cells/mm³.

Figure 9: Exophytic oral HIV-KS lesion on the lingual gingiva of a 54-year old male with a CD4+ T cell count of 258 cells/mm³.

commonly affected (Table 2). In none of the 37 patients included in this study was the floor of the mouth or the ventral/lateral surface of the tongue affected. It is unknown why HIV-KS has the tendency to affect certain oral sites but not others.

Forty six percent (46%) of the patients of this study cohort did not know their HIV-serostatus at the time of oral HIV-KS diagnosis, implying that oral KS in the Ga-Rankuwa area in South Africa, may serve as an indicator of HIV infection.

Fifty four percent (54%) of patients were diagnosed with HIV infection, on average 52 weeks before the appearance of oral HIV-KS, and although not statistically significant, these patients had a higher average CD4+ T cell at the time of HIV diagnosis than at the time of oral HIV-KS diagnosis (Table 3). Although the prevalence of HHV-8 infection in South Africa is relatively high (Malope et al., 2007; Whiby et al., 2006), there were no recorded cases of oral KS in HIV-seronegative subjects (data not shown) suggesting that endemic African KS is

not frequent in the Ga-Rankuwa area of South Africa.

Seven patients were on HAART atthe time of oral HIV-KS diagnosis. Although at the time of oral HIV-KS diagnosis their average CD4+ T cell count (90 cells/mm^3) was lower than the average CD4+ T cell count of the HAART-naïve patients (114 cells/mm^3) (Table 3), the difference was not statistically significant. This seemingly surprising finding that the patients on HAART had a low CD4+ T cell count could be attributed to the facts that in South Africa, HIV-seropositive persons who rely on provincial (governmental) services for their medical care have to abide by an official policy that HAART can be provided only when their CD4+ T cell count has dropped below 200 cells/mm^3; and that some people who have medical conditions suggestive of HIV disease are reluctant to undergo serological testing for HIV and often prefer to be treated by traditional healers. As a result, HIV infection is often diagnosed and HAART is often introduced late in the course of their advanced HIV disease when the CD4+ T cell count has already fallen substantially below 200 cells/mm^3.

Therefore, as a consequence of HAART being introduced when the CD4+ T cell count is already very low, there will be a lower level of reconstitution of the CD4+ T cell number compared to the level of reconstitution of the CD4+ T cell numbers achieved when HAART is started when the CD4+ T cell counts are at a higher level (Valdez et al., 2002; Resino et al., 2006; Feller and Lemmer, 2008; Kithata et al., 2009; Portsmouth, 2010). In addition, in provincial (governmental) medical facilities in South Africa, as in other countries in sub-Saharan Africa, monitoring the effectiveness of HAART is not always as efficient as it is in developed countries (Sutcliffe et al., 2008; Uldrick and Whitby, 2011). This may result in HIV-seropositive patients having a low level of CD4+ T cell counts despite HAART. All these factors may explain why the average CD4+ T cell count of our study cohort was low at the time of oral HIV-KS diagnosis, why the number of HAART-naïve patients was high, and why the CD4+ T cell counts of patients on HAART was also relatively low.

It has been reported that in 22% of HIV-seropositive subjects with KS, the initial presentation of HIV-KS is in the mouth; and in up to 70% of subjects with HIV-KS, the mouth will sooner or later be affected (Fauci and Lane, 2005; Epstein et al., 2005). As our study was designed to include only patients with oral HIV-KS regardless of whether or not there was KS of other body systems, and did not include patients with KS who had no oral involvement, our findings cannot be compared to the findings of other studies reported in the literature in which the inclusion criterion was that of patients having HIV-KS, but who may or may not have had oral HIV-KS.

From the medical history and from the physical examination of our patients it is evident that in 21 patients (57%) the initial presentation of HIV-KS was in the mouth, and that in six patients (16%) the initial presentation of HIV-KS occurred concurrently in the mouth and on the skin (Table 1). Ten patients (27%) developed cutaneous HIV-KS before oral HIV-KS diagnosis (Table 1), and three patients (8%) developed cutaneous HIV-KS after oral HIV-KS diagnosis. In total 19 of the 37 patients (51%) with oral HIV-KS developed cutaneous HIV-KS. The difference between the average CD4+ T cell count of the patients who had cutaneous HIV-KS at the time of oral HIV-KS diagnosis (113 cells/mm^3) and the average CD4+ T cell count of those who did not have cutaneous HIV-KS at the time of oral HIV-KS diagnosis (103 cells/mm^3) was not statistically significant.

As HIV-KS may affect any body system (Aboulafia, 2010; Pantanowitz, 2010) it is probable that some of our patients could have had undiagnosed internal HIV-KS at the time of oral HIV-KS diagnosis, or may have developed internal HIV-KS at any time later during the observation period of the study. However, we could not investigate this possibility because

of administrative reasons and lack of medical capacity. For these reasons autopsy information of demised patients was also not available.

Lymphoedema may precede the development of HIV-KS; may present at the time of diagnosis of HIV-KS; or may develop after the diagnosis of HIV-KS, in parallel with the progression of HIV-KS disease (Feller et al., 2008b). Lymphoedema in association with HIV-KS is an indicator of poor prognosis (Krown et al., 1997; Pantanowitz et al., 2010) and facial lymphoedema which develops concurrently with rapid enlargement of exophytic oral lesions (Figures 10 and 10b) foretokens death (Feller et al., 2008b; Feller et al., 2010a).
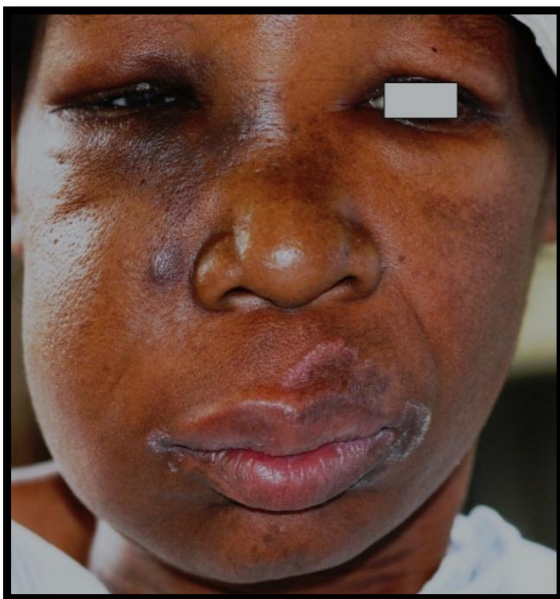

Figure 10a

Figure 10b

Figures 10a & 10b: A 44-year-old female with facial and oral HIV-KS, and associated facial lymphoedema. Her CD4+ T cell count was 16 cells/mm^3 at the time of oral HIV-KS diagnosis and she died two-weeks later.

In this study, nine patients (24%) had facial lymphedema. Three patients presented with facial lymphoedema at the time of oral HIV-KS diagnosis and six patients developed the

facial lymphoedema on average 2.3 weeks after oral HIV-KS diagnosis. All the patients with facial lymphoedema had extensive exophytic oral lesions and their average CD4+ T cell count at the time of oral HIV-KS diagnosis was 28 cells/mm^3 compared to 133 cells/mm^3 of those patients who did not develop facial lymphoedema (Table 3). This difference in the average CD4+ T cell counts is statistically significant, indicating that the development of facial lymphoedema is associated with very low CD4+ T cell counts.

During the observation period of the study, in all the nine patients with facial lymphedema, the facial lymphoedema rapidly enlarged in parallel with the rapid progression of established advanced oral HIV-KS. All these nine patients died very soon after the diagnosis of oral HIV-KS, on average within two weeks, regardless if they were on HAART. As some of these patients with facial lymphoedema were terminally ill with severe immunosuppression and multiple metabolic abnormalities, it is not possible to determine if facial lymphoedema in association with rapidly progressive oral HIV-KS is directly implicated in the rapid death of these patients, but it is evident that it is a strong prognostic indicator (Feller et al., 2008b; Feller et al., 2010a).

The pathogenic mechanisms that cause facial lymphoedema in association with HIV-KS are obscure. However, as oral KS lesions and oral fluids of HIV-seropositive subjects carry a high HHV8 load, and as advanced exophytic oral HIV-KS lesions have a higher HHV8 load than initial maculopapular lesions (Pak et al., 2007), it is possible that in the presence of exophytic oral lesions, lymphatic obstruction secondary to HHV8 induced proliferation of endothelial cells, and/or compression of lymphatics by rapidly progressing oral HIV-KS, will bring about leakage of protein-rich fluid into the interstitial spaces, promoting the development of facial lymphoedema (Feller et al., 2008b; Feller et al., 2010a). Therefore, it is

likely that treating exophytic oral HIV-KS lesions with systemic cytotoxic chemotherapy may result in the shrinkage of the oral lesion with the subsequent decrease in HHV8 load in the affected tissues, thus reducing the risk of developing facial lymphoedema. This possibility needs further investigation.

Immune reconstitution inflammatory syndrome (IRIS) can be defined as a paradoxical resurgence of pre-existing subclinical or mildly symptomatic infection, or of a pre-existing inflammatory condition, in parallel with an improvement in the immune status, during the initial months of host immune-reconstitution (Feller et al., 2007c; Feller and Lemmer, 2008; Feller et al., 2008a). In HIV-seropositive subjects IRIS may occur shortly after the introduction of HAART and can be associated with several conditions including *M.tuberculosis*, hepatitis B and C and KS. The course of IRIS in the context of HIV infection depends on the burden of the pre-existing pathogen and the extent of the dysregulated immune-inflammatory response that is generated during the process of immune reconstitution (Feller et al., 2007c; Feller and Lemmer, 2008; Feller et al., 2008a).

HIV-seropostive subjects who are of younger age, those who have a CD4+ T cell count of <100 cells/mm^3 and those with a CD4+ T cell percentage of <10% at the time of starting HAART are at greater risk of developing IRIS (Ratnam et al., 2006; Feller and Lemmer, 2008). It has been suggested that there is also an association between the magnitude of the increase in CD4+ T cell count or in the percentage of CD4+ T cell increase, and the plasma load decrease after HAART, and the risk of developing IRIS (Shelburn et al., 2005; Breton et al., 2004).

IRIS-associated HIV-KS is the consequence of a dysregulated immune-inflammatory reaction to HHV8 antigen, usually occurring within eight weeks after the initiation of HAART (Leidner and Aboulafia, 2005). About 6.6% of HIV-seropositive subjects will develop IRIS-associated HIV-KS after the initiation of HAART (Bower et al., 2005), however the characteristics of oral HIV-KS as an IRIS phenomenon are not well defined.

To the best of our knowledge this is the first report in the literature documenting the prevalence of IRIS associated oral HIV-KS in a population of patients who have oral HIV-KS. One patient (3%) had IRIS associated HIV-KS. This patient was treated with systemic cytotoxic chemotherapy and surgical excision and the oral HIV-KS was cured. A comprehensive description of the case report of this patient had been published previously (Feller et al., 2008a). At the time of writing this research report, the patient is still alive 5.5 years after the treatment of IRIS-associated oral HIV-KS, and currently her CD4+ T cell count is 383 cells/mm^3. This is in line with reports in the literature documenting that IRIS associated oral HIV-KS responds well to conventional therapy (Papagastsia et al., 2009; Letang et al., 2010).

During the observation period, 21 of the 37 patients (57%) died, on average within 13.6 weeks from the time of oral HIV-KS diagnosis; nine (24%) were lost to follow-up; and 7 patients (19%) survived (average period of follow-up of 91 weeks). All the 21 patients who died, invariably experienced worsening of their oral HIV-KS, and their average CD4+ T cell count at oral HIV-KS diagnosis was 64 cells/mm^3. The average CD4+ T cell counts of the patients who were alive at the end of the study observation period, was 166 cells/mm^3 at oral HIV-KS diagnosis. The difference in the CD4+ T cell counts at the time of oral HIV-KS diagnosis between those who died and those who survived was statistically significant (*t-test*:

p=0.016). This suggests that the low CD4+ T cell count at the time of oral HIV-KS diagnosis, is an indicator of poor prognosis.

Of those who survived, in three patients the oral HIV-KS was completely resolved. One of these patients was treated with HAART in combination with systemic cytotoxic chemotherapy and surgery, one with HAART in combination with systemic cytotoxic chemotherapy, and one patient with HAART and surgery (Figure 3). In four patients the oral HIV-KS remained unchanged or shrunk. These patients were treated with HAART or with HAART in combination with local cytotoxic chemotherapy.

This conforms with many reports in the literature that the course of HIV-KS is unpredictable. HIV-KS may be either a mild or a life threatening disease but without treatment the overall prognosis is poor (Feller et al., 2008b). Exophytic oral HIV-KS and oedema are independently associated with poor prognosis of HIV-KS disease (Krown et al., 1997; Pantanowitz et al., 2010), and when occurring concurrently, as it is evident from the results of this study, are indicators of rapid fatality.

HAART is used to treat HIV infection. Although effective HAART does not directly influence HHV8 replication, by reducing HIV load with subsequent improvement in the host-immune status, it indirectly brings about a decrease in the incidence and prevalence of HIV-KS and an improvement in the clinical manifestation of pre-existing HIV-KS disease (Uldrich and Whitby, 2011). However, HAART does not ensure that HIV-KS will not develop, and despite HAART, KS remains the most frequent HIV-associated neoplasms (Feller et al., 2008b).

In this study, seven patients (19%) have been on HAART at least four weeks before their oral HIV-KS diagnosis, confirming that HIV-seropositive subjects on HAART are not immune to developing KS; and eight of the 21 patients (38%) who died during the observation period were on HAART as a sole modality of treatment. These patients died on average 4.4 weeks after their oral HIV-KS diagnosis (Figure 2). During this period they experienced worsening of their oral HIV-KS disease. This suggests that although introduction of HAART should be the first line of therapy for HAART-naïve HIV-seropositive subjects with oral KS, HAART by itself is not effective in controlling oral HIV-KS disease. Two of the 21 patients who died (9%) were concurrently treated with HAART and with local cytotoxic chemotherapy (Figure 2).

Eleven of the 21 patients (52%) who died during the observation period received neither HAART nor any other treatment for their oral HIV-KS. The average time from oral HIV-KS diagnosis to their death was 20 weeks. The paradoxical fact that in this study HAART-naïve patients lived longer than patients on HAART might be attributed to skewed statistics associated with the small number of patients, or to ineffective HAART that was started late in the course of oral HIV-KS disease when the CD4+ T cell count has already fallen very low.

As stated previously we were unable to determine the cause of death of our patients who died and therefore it is unknown if they died as a direct consequence of their HIV-KS disease, or as a result of any other HIV-associated causes.

The small number of patients who received treatment for HIV-KS in this study prevents drawing conclusions regarding what is the best treatment approach to control the progression of oral HIV-KS and to improve the prognosis of the patients. However, as reported

elsewhere (Feller and Lemmer, 2010) it seems that exophytic oral HIV-KS lesions are best treated with HAART and systemic cytotoxic chemotherapy, and once the lesions have shrunk and become surgically accessible, they should be excised

It is advisable to treat oral HIV-KS, particularly if the patients have cutaneous HIV-KS at the time of oral HIV-KS diagnosis, with systemic cytotoxic chemotherapy at an early maculopapular stage, as it may prevent the development of extensive oral HIV-KS and the development of facial lymphoedema (Feller et al., 2008a; Feller and Lemmer, 2010). From the experienced gained in treating the patients in this study, limited systemic cytotoxic chemotherapy is sufficient in bringing about resolution of oral HIV-KS.

Only two patients in this cohort were treated with systemic cytotoxic chemotherapy, in one case cure of oral HIV-KS was confirmed by histopathological examination, in the other case clinical resolution of the oral HIV-KS was evident. We would have wanted to treat all the patients with systemic cytotoxic chemotherapy but the limited capacity of the provincial (governmental) medical services in the Ga-Rankuwa area, South Africa precluded it.

In developed countries HIV-KS predominantly affects males. However in many countries in sub-Saharan Africa where HIV infection is endemic and young females aged 15-24 years are more frequently infected with HIV than males, there is almost an identical incidence of HIV-KS in males and females (Subbia et al., 2010; Stebbing et al., 2010).

It has been reported that at the time of HIV-KS diagnosis females present with more advanced disease, and more frequently than males they are affected by non-cutaneous HIV-KS disease (Nasti et al., 1999; Stebbing et al., 2010). This conforms with the results of our

study in which more females than males were affected by oral HIV-KS (M:F = 1:1.2). Although not statistically significant, in this study females had a lower average CD4+ T cell count (85 cells/mm^3) than males (141 cells/mm^3) at the time of oral HIV-KS diagnosis, in line with other studies documenting that females with HIV-KS have a more severe immunodeficiency than males with HIV-KS (Nasti et al., 1999). However in contrast to other studies reporting that females are younger than males at the time of HIV-KS diagnosis (Nasti et al., 1999; Meditz et al., 2007), the age of the females (33 years) and males (34 years) in our cohort was very similar.

In spite the fact that the CD4+ T cell counts of females was lower than males at oral HIV-KS diagnosis, the differences between the percentage of males who survived and females who survived, (and males who died, and females who died) was not statistically significant. That conforms with some reports in the literature that gender differences does not influence survival of patients with HIV-KS (Stebbing et al., 2010).

The difference between females and males regarding the number and the phenotype of the oral HIV-KS lesions was not significant (table 1). However, females had a statistically significant higher percentage of lesions ≥ 10 mm ≤ 50 mm than males, while males had a statistically significant higher percentage of lesions < 10 mm than females (Table 1). The percentage of lesions > 50 mm was similar in both sexes. It seems that these differences and similarities do not have a particular clinical significance.

Five percent (5%) of the study population (two patients) where children. Both were boys, aged 10 and 11 years. This conforms with data from sub-Saharan Africa that children are not infrequently affected by HIV-KS. In fact, in Zimbabwe, the most frequent malignancy in

children between 1-14 years of age is HIV-KS (Subbiah et al., 2010). Oral involvement in HIV-seropositive children with KS is common in sub-Saharan Africa and this is probably owing to the high prevalence of HHV8 and HIV co-infection in African children in this part of the world. HIV-seropositive children with advanced oral KS have a particularly aggressive HIV disease with a poor prognosis (Feller et al., 2010b).

In this study one child developed facial lympoedema and died four weeks after his oral HIV-KS diagnosis. The other child had only limited oral involvement, he started HAART, his oral lesions have been stable and he is still alive.

Thirty eight percent (38%) of the patients in this study had common HIV-associated oral diseases which presented concurrently with oral HIV-KS (Table 1). It is probable that these diseases were associated with the low CD4+ T cell counts of the patients, and not with the oral HIV-KS. However, one cannot exclude the possibility that some HIV-associated oral diseases may further dysregulate the cytokine milieu in the affected oral tissues, thus promoting KS tumourigenesis (Webster-Cyriaque, 2002; Panatanowitz and Dezube, 2007).

The exact nature of KS is a matter of controversy. It is unclear whether KS is reactive phenomenon or a true malignancy (Pantanowitz and Dezube 2004; Feller et al., 2007a; Feller et al., 2007b; Feller and Lemmer 2010, Wood and Feller 2008; Sullivan et al., 2010; Pantanowitz et al., 2010). It is probable that HIV-KS starts as a reactive polyclonal angioproliferative response resulting from paracrine and autocrine action of locally produced inflammatory agents mediated by HHV8 in the background of HIV-associated profound immune suppression and cytokine-network dysregulation (Feller et al., 2007a; Feller et al., 2007b; Pantanowitz et al., 2010).

This pathological process brings about the initial maculopapular HIV-KS lesion. In a later stage of HIV-KS disease, specific HHV8 infected endothelial cells in the polyclonal population may undergo further transformation and subsequent clonal divergence evolving into either a few independent clones or a single clone (Feller et al., 2007b). Therefore, an advanced exophytic HIV-KS lesion may consist of polyclones, oligoclones and monoclones (Gill et al., 1998; Judde et al., 2000; Rabkin et al., 1997). It is reasonable to assume that the aggressive clinical course of most exopyitic oral HIV-KS, as it is evident from this study, is owing to the uncontrolled cell proliferation and prolonged survival of the monoclonal and oligoclonal cell populations within the lesions (Feller et al., 2007b). If this is indeed the chain of pathogenic events in the development of oral HIV-KS, the introduction of systemic cytotoxic chemotherapy in the early maculopapular stages will eliminate the bulk of HHV8 infected polyclonal endothelial cells. This will reduce the risk of evolution of expanding oligoclones and monoclones. If and when an effective anti-HHV8 therapeutic drug will be available, such a drug may be the treatment of choice for all epidemiological variants of KS.

As HHV8 infection is similarly prevalent in Asia as it is in Europe and the United States, but oral HIV-KS is very rare in China, India, Thailand and Cambodia (Tsang et al., 1999; Anil and Challecombe 1997; Nittayanata et al., 1997); and as the prevalence of HIV and HHV8 co-infection is high in some Ethiopian population groups, but the prevalence of HIV-KS is very low in these groups (Grossman et al., 2002; Lindtjorn 1987; Getachew et al., 1997; Figueroa et al., 1998; Kassa et al., 1999; Reichart et al., 2003), it is probable that some ethnic groups may possess specific genetic factors which make them resistant to the development of HIV-KS (Feller et al., 2007b; Feller and Lemmer, 2010). On the other hand genetic variants of immune-modulating genes and of pro-inflammatory cytokines may predispose specific ethnic groups to the development of classic KS (Nguyen and Casper 2010). It is probable that

genetic factors also play an important role in bringing about the marked male predilection of classic KS (Stebbing et al., 2010).

It is also possible that some ethnic groups are exposed to specific extraneous factors in their geographic locations which may promote the development of KS. These risk factors include chemicals (amyl nitrite, specific plant extracts), living in areas with iron oxide-rich volcanic soils or with blood-sucking insects, and possibly specific nutritional elements (Whitby et al., 2006; Subbiah et al., 2010; Simonart et al., 2010).

Although tobacco smoking is associated with an increased risk of various cancers, it appears that tobacco smoking is associated with a decrease risk of developing HIV-KS (Simonart, 2010). In our study 24% percent of the patients smoked. This figure is comparable with the prevalence of smoking in the general black population in South Africa (Stein et al., 2008), suggestive that in our cohort, smoking did not play any role in the development of oral HIV-KS.

CONCLUSIONS

In the Ga-Rankuwa area in South Africa:

1. Oral HIV-KS affects females more frequently than males (M:F = 1:1.2)

2. Five percent (5%) of patients with oral HIV-KS are children.

3. Three percent (3%) of patients with oral HIV-KS develop IRIS associated oral HIV-KS; and IRIS-associated oral HIV-KS responds well to conventional therapy.

4. Twenty four percent (24%) of patients with oral HIV-KS developed facial lymphoedema during the course of their HIV-KS disease. In all the cases, facial lymphoedema was associated with extensive oral HIV-KS, and it is an indicator of poor prognosis.

5. Patients with lower CD4+ T cell counts at the time of oral HIV-KS diagnosis have a poorer prognosis than patients with higher CD4+ T cell counts at the time of oral HIV-KS diagnosis.

6. Oral HIV-KS can be successfully treated with systemic cytotoxic chemotherapy.

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APPENDIX 1: THE STATISTICAL TESTS USED TO EXPLORE ASSOCIATIONS BETWEEN THE VARIOUS PARAMETERS OF ORAL HIV-KS.

Test	Test statistic	Significance	Comment
Testing for the difference between the mean age of males and the mean age of females at oral HIV-KS diagnosis	T-test	P = 0.76	No significant difference
Testing for the differences between the percentage of males who used tobacco and the percentage of the females who used tobacco at oral HIV-KS diagnosis	Chi-squared	P = 0.06	No significant difference
Testing for the differences between the percentage of males with cutaneous lesions and the percentage of females with cutaneous lesions, at oral HIV-KS	Chi-squared	P = 0.296	No significant difference
Testing for the difference between the percentage of the males who presented with other oral lesions and the percentage of females who presented with other oral lesions, at oral HIV-KS diagnosis.	Chi-squared	P= 0.286	No significant difference
Testing for the difference between the average CD4+ T cell count of males and the average CD4+ T cell count of females, at oral HIV-KS diagnosis	T-test	P = 0.11	No significant difference
Testing for the difference between the percentage of males and the percentage of females diagnosed with HIV infection, at oral KS presentation	Chi-squared	P = 0.15	No significant difference
Testing for the difference between the percentage of males and the percentage of females who contracted HIV infection before the diagnosis of oral KS	Chi-squared	P = 0.15	No significant difference
Testing for the difference between the percentage of patients diagnosed with HIV infection at oral KS presentation and the percentage of patients who contracted HIV infection before oral KS diagnosis	Binomial distribution	P = 0.319	No significant difference
Testing for the difference between percentage of males and the percentage of females with single oral HIV-KS lesions	Chi-squared	P = 1.13	o significant difference

Testing for the difference between the percentage of males and the percentage of females with multiple oral KS lesions	Chi-squared	P = 0.29	No significant difference
Testing for the difference between the percentage of patients with single oral lesions and the percentage of patients with multiple oral lesions	Binomial distribution	P = 0.003	Significant difference: higher percentage of multiple oral lesions.
Testing for the difference between the percentage of males and the percentage of females with macular lesions	Chi-squared	P = 0.68	No significant difference
Testing for the difference between the percentage of males and the percentage of females with papular lesions	Chi-squared	P = 0.1	No significant difference
Testing for the difference between the percentage of males and the percentage of females with nodular lesions	Chi-squared	P = 0.988	No significant difference
Testing for the difference between the percentage of males and the percentage of females with exophytic lesions	Chi-squared	P = 0.75	No significant difference
Testing for the difference between the percentage of males and the percentage of females with lesions < 10mm	Chi-squared	P = 0.007	Significant difference: males patients had a greater number of lesions < 10mm than females
Testing for the difference between the percentage of males and the percentage of females with lesions ≥10mm≤50mm	Chi-squared	P = 0.004	Significant difference: female patients had a greater number of lesions ≥10mm≤50mmthan males
Testing for the difference between the percentage of males and the percentage of females with lesions >50mm	Chi-squared	P = 0.44	No significant difference

Test	Test statistic	Significance	Comment
Testing for the difference between the average CD4+ T cell count of males and the average CD4+ T cell count of females, at oral HIV-KS diagnosis	T-test	P = 0.11	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell count of females who were simultaneously diagnosed with HIV and oral HIV-KS diagnosis	T-test	P = 0.35	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell count of females who contracted HIV infection before oral HIV-KS diagnosis, at HIV diagnosis	T-test	P = 0.38	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell of females who contracted HIV infection before oral HIV-KS diagnosis, at oral HIV-KS diagnosis	T-test	P = 0.31	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell of females who were receiving HAART for some time, at oral HIV-KS diagnosis	T-test	P = 0.34	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell of females who were HAART-naïve, at oral HIV-KS diagnosis	T-test	P = 0.25	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell of females who developed facial lymphoedema during the course of HIV-KS	T-test	P = 0.54	No significant difference
Testing for the difference between the average CD4+ T cell of males and the average CD4+ T cell of females who did not develop facial lymphoedema during the course of HIV-KS	T-test	P = 0.40	No significant difference

Test	Test statistic	Significance	Comment
Testing for the difference between the average CD4+ T cell count of patients who developed lymphoedema and the average CD4+ T cell of the patients who did not develop lymphoedema during their course of HIV-KS	T-test	P = 0.01	Significant difference: the patients with lymphoedema had a lower average CD4+ T cell count than those who did not develop lymphoedema.
Testing for the difference between the average CD4+ T cell count of patients who were on HAART at oral HIV-KS diagnosis, and the average CD4+ T cell of the patients who were HAART-naïve, at oral HIV-KS diagnosis	T-test	P = 0.606	No significant difference
Testing for the difference between the average CD4+ T cell count of patients who were simultaneously diagnosed with HIV and oral KS and the average CD4+ T cell count of patients who contracted HIV infection before developing oral HIV-KS, at the time of oral HIV-KS diagnosis	T-test	P = 0.11	No significant difference
Testing for the difference between the average CD4+ T cell counts of patients who contracted HIV infection before developing HIV-KS, at the time of HIV diagnosis and at the time of oral HIV-KS diagnosis	T-test	P = 0.11	No significant difference
Testing for the difference between the average CD4+ T cell count of patients who were alive at the end of the observation period and the average CD4+ T cell count of the patients that died during the observation period	T-test	P = 0.016	Significant difference: patients who were alive at the end of the observation period had a higher average CD4+ T cell count at oral HIV-KS diagnosis than those who died.
Testing for the difference between the average CD4+ T cell count of patients who had cutaneous lesions at oral HIV-KS diagnosis and the average CD4+ T cell count of patients who did not have cutaneous lesions at oral HIV-KS diagnosis	T-test	P = 0.77	No Significant difference