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TRANSMISSION PATTERNS AND SEROEPIDEMIOLOGY OF KAPOSI'S SARCOMA ASSOCIATED HERPES VIRUS – KSHV (HUMAN HERPES VIRUS 8 – HHV-8) IN SOUTH AFRICA

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2012

Declaration

I **Babatyi Innocentia Malope-Kgokong** declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the field of Community Health at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature of Candidate: _____

Date: _____ day of _____

Dedications

My mother Lucia Lasea Malope, if all daughters and sons had a mother like you, always asking for nothing but the best for their children, the world will be blessed and South Africa a very wealthy country. This PhD is for you.

To my mother in law, Kedibone Priscilla Kgokong who always made sure I have as much time as possible to myself to try and work on my studies. Thank you for being supportive and understanding.

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Publications and Presentations Arising from the Thesis

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1. Malope BI, Pfeiffer RM, Mbisa G, Stein L, Ratshikhopha EM, O'Connell DL, Sitas F, MacPhail P, Whitby D. Transmission of Kaposi sarcoma-associated herpesvirus between mothers and children in a South African population. J Acquir Immune Defic Syndr. 2007 Mar 1;44 (3):351-5. - (Chapter 5)

2. Malope BI, MacPhail P, Mbisa G, MacPhail C, Stein L, Ratshikhopha E M, Ndhlovu L, Sitas F and Whitby D. No evidence of sexual transmission of Kaposi's sarcoma herpes virus in a heterosexual South African population. AIDS 2008 Feb 19:22(4): 2073-8. - (Chapter 3).

3. Malope-Kgokong B I, MacPhail P, Mbisa G, Ratshikhopha E, Maskew M, Stein L, Sitas F and Whitby D. Kaposi's Sarcoma Associated-Herpes Virus (KSHV) Seroprevalence in Pregnant Women in South Africa. Infectious Agents and Cancer 2010, 5:14 (http://www.infectagentscancer.com/content/5/1/14) (Chapter 4)

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1. Malope BI, MacPhail P, Mbisa G, MacPhail C, Stein L, Ratshikhopha EM, Ndhlovu L, Searle C, Sitas F, Whitby D. Kaposi's Sarcoma Herpes Virus (KSHV) is not Associated with Sexually Transmitted Infections or High Risk Sexual Behavior in a South African Heterosexual Population. 14th Conference on Retroviruses and Opportunistic Infections (CROI 2007): February 25-28, 2007: Los Angeles Convention Center: Los Angeles, CA.

2. Malope B I , Pfeiffer R M, Mbisa G, Stein L, Ratshikhopha E M, O'Connell D, Sitas F, Macphail A P, Whitby D. Transmission of Kaposi sarcoma herpes virus (KSHV) between South African mothers and children. 9th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI): October 16-17, 2006: Bethesda, Maryland, USA.

Abstract

Factors associated with the transmission of Kaposi's sarcoma-associated herpesvirus (KSHV) are inconclusive. In countries where KS and KSHV are confined to men who have sex with other men (MSM), KSHV is associated with sexual risk factors. In countries where KSHV is endemic, it affects adults and children of all ages and irrespective of sexual orientation, suggesting the existence of non-sexual risk factors for KSHV infection.

In this thesis, three distinct cross sectional studies aiming to define the seroprevalence of KSHV in South African populations and to identify plausible risk factors for KSHV infection were undertaken. The studies measured KSHV seropositivity in relation to sociodemographic factors and HIV status. In children, factors associated with horizontal mother to child transmission were also explored. In adults KSHV seropositivity was also measured in relation to sexually transmitted infections and/or measures of sexual behaviour. Calculated risk factors were expressed as odds ratios (95% confidence interval) for KSHV.

Methods

Mother to Child KSHV seroepidemiology Study: KSHV seroprevalence (reactive to either lytic K8.1 or latent Orf73) was measured in 1287 children and their 1179 biological mothers. Association between KSHV seropositivity in children was measured against KSHV seropositivity and HIV status of their mothers.

KSHV seroepidemiology in women attending antenatal clinics: Antibodies to KSHV lytic K8.1 and latent Orf73 antigens were tested in 1740 pregnant women attending

antenatal clinics in South Africa in 2001. Information on HIV and syphilis serology, age, education, residential area, gravidity, and parity was anonymously linked to evaluate risk factors for KSHV seropositivity. Clinics were grouped by municipal regions and their proximity to the two main river catchments defined.

Carletonville Community KSHV seroepidemiology Study: Sera from 2103 South African individuals (862 miners, 95 sex workers, 731 female and 415 male township residents) were tested for antibodies to KSHV lytic K8.1 and latent Orf73, HIV gonococcus, herpes simplex virus type 2 (HSV-2), syphilis and chlamydia. Information on social, demographic and high-risk sexual behaviour was linked to laboratory data.

Results

Mother to Child KSHV seroepidemiology Study: KSHV seroprevalence (reactive to either lytic K8.1 or latent Orf73) was 15.9% (204 of 1287 subjects) in children and 29.7% (350 of 1179 subjects) in mothers. The risk of KSHV seropositivity was significantly higher in children of KSHV seropositive mothers compared with those of KSHV-seronegative mothers. The HIV status of mothers was marginally associated with an increased risk of KSHV seropositivity in their children (AOR = 1.6, 95% CI: 1.0 to 2.6; P = 0.07). KSHV seroprevalence was significantly higher in HIV-infected subjects (P = 0.0005), and HIV-infected subjects had significantly higher lytic and latent KSHV antibody levels than HIV-negative subjects.

KSHV seroepidemiology in women attending antenatal clinics: KSHV seroprevalence was nearly twice that of HIV (44.6% vs. 23.1%). HIV and syphilis seropositivity was 12.7% and 14.9% respectively in women without KSHV, and 36.1% and 19.9% respectively in those with KSHV. Women who were KSHV seropositive were 4 times more likely to be HIV positive than those who were KSHV seronegative (AOR

4.1 95%CI: 3.4 - 5.7). Although, women with HIV infection were more likely to be syphilis seropositive (AOR 1.8 95%CI: 1.3 - 2.4), no association between KSHV and syphilis seropositivity was observed. Those with higher levels of education had lower levels of KSHV seropositivity compared to those with lower education levels. KSHV seropositivity showed a heterogeneous pattern of prevalence in some localities.

Carletonville Community KSHV seroepidemiology Study: Overall KSHV and HIV prevalences were 47.5 and 40%, respectively (P<0.43). The risk of HIV infection was highest in sex workers followed by female residents and miners, compared with male residents (P<0.001). HSV-2 infection was highly prevalent (66%) and lower, but still substantial, prevalence (6–8%) was observed for other sexually transmitted infections (STI). No significant difference in KSHV infection was observed among the residential groups (P>0.05). KSHV was not associated with any of the STI or any measures of sexual behaviour.

Conclusion

The findings of these three studies contribute substantially to global KSHV seroepidemiology and show that in Southern African settings KSHV is associated with non-sexual mode of transmission. Firstly KSHV is common in very young children up to ten years of age and increases with age until adulthood. The high prevalence of KSHV in the South African populations remained evident in all populations. In children, the risk of acquisition of KSHV was higher among children of KSHV-seropositive mothers than if the mother was KSHV negative. The association between KSHV and HIV was also noted in the study of pregnant women attending antenatal clinics and in the mother to child study. However this association was not evident in the Carletonville population where both KSHV and HIV were highly prevalent.

In both the adult studies the lack of association between KSHV and syphilis was evident. KSHV infection was also not associated with other sexually transmitted infections and measures of sexual behaviour. As expected, the pattern of HIV and STI in sex workers suggests high rates of high-risk sexual behaviour in this population; however KSHV seropositivity was the same amongst sexworkers and all the other community groups. This pattern of the lack of association with high-risk sexual behaviour, particularly in sex workers and with any markers of STI strongly suggests that the sexual mode does not play a significant role in KSHV transmission in this South African population. This may also suggest that KSHV transmission may involve geographical and cultural factors other than sexual transmission.

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List of Abbreviations

Abbreviations	Full Names
ACTG	AIDS Clinical Trials Group
AIDS	Acquired Immunodeficiency Syndrome
AOR	Adjusted Odds Ratio
AVS	Ateline Herpesvirus-2
BCBL	Body Cavity Based Lymphoma
BSA	Bovine Serum Albumin
CD	Castleman's Disease
CERG	Cancer Epidemiology Research Group
95% CI	95% Confidence Interval
ddH20	Double Distilled Water
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic Of Congo
dsDNA	Double Stranded Deoxyribonucleic Acid
EBV	Epstein Barr Virus
ELISA	Enzyme-Linked Immunosorbent Assay
FITC	Fluorescein Isothiocyanate
HCMV	Human Cytomegalovirus
HHV-1	Human Herpesvirus 1
HHV-2	Human Herpesvirus 2
HHV-3	Human Herpesvirus 3
HHV-4	Human Herpesvirus 4
HHV-5	Human Herpesvirus 5
HHV-6	Human Herpesvirus 6
HHV-7	Human Herpesvirus 7
HHV-8	Human Herpesvirus 8
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
HSV-1	Herpes Simplex Virus-1
HSV-2	Herpes Simplex Virus-2
HV	Herpesvirus
HVS	Herpesvirus saimiri
IARC	International Agency or Research on Cancer
ICTV	International Committee on the Taxonomy Of Viruses
IFA	Immunofluorescence Assays
IgG	Immunoglobulin G
IL6	Interleukin 6
KS	Kaposi's Sarcoma

Abbreviations	Full Names
KSHV	Kaposi's Sarcoma Herpesvirus
LANA	Latency Associated Nuclear Antigens
MCD	Multicentric Castleman's Disease
MEIA	Microplate Enzyme Immunoassay
Mg	Magnesium
MHV-4	Murine Herpesvirus
MMWR	Morbidity and Mortality Weekly Report
MM	Multiple Myeloma
MNC	Mean of the Negative Controls
MPC	Mean of the Positive Controls
MSM	Men who have sex with other men
NaN_3	Sodium Azide
NaOH	Sodium Hydroxide
NCI	National Cancer Institute
NGS	Normal Goat Serum
NHLS	National Health Laboratory Service
OD	Optical Density
OR	Odds Ratio
Orf	Open Reading Frames
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEL	Primary Effusion Lymphoma
PNP	Para-Nitrophenylphosphate
POR	Prevalence Odds Ratio
PVDF	Polyvinylidene Fluoride
RDA	Representational Difference Analysis
RFLP	Restriction-Fragment Length Polymorphism
SAIC	Science Applications International Corporation
SD	Standard Deviation
SIR	Standardized Incidence Ratios
SHV-2	Herpesvirus Saimiri
STI	Sexually Transmitted Infections
sVCA	Small Viral Capsid Antigen
TMB	Tetramethylbenzidine

Thesis Structure Overview

This thesis is structured to cover seven chapters that review the epidemiology of KSHV, describe the methodology and demonstrate results of the three KSHV studies conducted. The discussion and conclusion include a consolidated description of the study findings taking into consideration any concordant and discordant findings and the shortfalls of the studies. The overall contribution of the studies to the understanding of KSHV epidemiology in South Africa and its contribution to the global KSHV scientific literature are deliberated. The chapters are divided as follows:

Chapter 1 **-** Literature review

Chapter 2 - Study objectives, materials and methods

Separate chapters on description and findings of the three studies conducted

Chapter 3 - The Mother to Child KSHV seroepidemiology study

Chapter 4 - Seroepidemiology of KSHV in South African Females Attending Antenatal Clinics

Chapter 5 - The Carletonville Community KSHV Sero-epidemiology Study

Chapter 6 - Discussion

Chapter 7 – Conclusion

1 Literature Review

1.1 Introduction

In 1994 a herpes-like virus was identified using representational difference analysis (RDA) from Kaposi's sarcoma (KS) lesions of homosexual males infected with Human Immunodeficiency Virus (HIV) (Chang et al, 2004; Chang et al, 1994; Birchall et al, 1994). The DNA sequences of this virus showed characteristics of a gamma (γ) Herpesvirus and was later indisputably confirmed as the causative agent of KS (Kedda et al, 1996; Mbulaiteye et al, 1995; Ambroziak et al, 1995; Aluigi et al, 1996) and was named Kaposi's Sarcoma-associated Herpesvirus (KSHV). Following the nomenclature endorsed by the International Committee on the Taxonomy Of Viruses (ICTV), KSHV was also designated human Herpesvirus 8 (HHV-8). However, in this thesis the acronym KSHV will be used to refer to the virus.

The discovery of KSHV followed more than a century after KS was described in 1872 by the distinguished Hungarian dermatologist Dr Moritz Kaposi (Oriel, 1997). However, as herpesviruses are known to co-evolve with their host species, it is likely that KSHV existed long before this discovery (Hayward & Zong, 2007; Hayward, 1999).

Prior to the HIV/AIDS epidemic, KS was generally rare worldwide and even unnoticeable in various African regions including the sub-Saharan African region. This is because KSHV infection alone is not usually sufficient to cause the development of KS. The risk is also influenced by KSHV viral load, immunosuppression status in the host and other host factors not yet well understood (Souza et al, 2004; Thanos et al, 2004; Cesarman, 2002; Pellet et al, 2006). Following the HIV/AIDS epidemic, drastic increases in the risk of developing KS was noted in African countries. What used to be a relatively rare cancer in many parts of Africa is now the leading cancer in HIV/AIDS endemic regions

Table 1-1: Different Types of KS summarising cutaneous, visceral and clinical characteristics.

KS Type	Populations Affected	Cutaneous presentation	Visceral Involvement	Clinical Course
Classic KS	Middle Aged Elderly men of Mediterranean, Central Eastern European origin or from the Middle East	Minor skin lesions confined to the distal lower extremities Lesions may be easily removed by simple surgical procedures	Uncommon	Usually indolent Rarely aggressive and disseminated
Endemic KS	African men, young male and female children mainly from Central Africa	Visible lesions on the torso, legs and feet. Lesions may persist with oral, surgical and localised and systemic treatment required	Internal organ involvement reported in a subset of adults Lymph node and visceral involvement common in children	Indolent to locally invasive in adults Occasional rapid progression reported in adults with visceral disease Aggressive in children
Iatrogenic KS	Immunosuppressed patients following organ transplantations Common in elderly patients Increased risk with use of cyclosporin A	Minor skin lesions confined to the torso. Lesions relapse when immunosufficient state reverts	Relatively common	May be aggressive Reversible/may regress with immune reconstitution
AIDS KS	Acquired immunosuppressed patients due to HIV Affects all populations in endemic countries Confined to MSM in Europe, Australia and America	Lesions affect the whole body and internal organs Life threatening	Common in severe HIV conditions, reversible by good HAART adherence	Aggressive or indolent May lead to death Improved by good adherence to HAART

(Sitas et.al, 1999; Mayama et.al, 1998). A summary of clinical characteristics and presentation of the different types of KS is given in Table 1.1 above.

The epidemiology of KS and KSHV varies from place to place, with the lowest prevalence reported in America and Europe (Dal Maso et al, 1996; Ebrahim et al, 1997; Cu-Uvin et al, 1996) and the highest prevalence in African (Hladik et al, 2003; Adjei et al, 2008; Malope et al, 2008; Cattani et al, 2003; Sitas & Newton, 2001; Sitas et al, 1999a) and Mediterranean countries (Whitby et al, 1998; Cattani et al, 2003; Cattani et al, 2003; Serraino et al, 2001).

In countries with a lower prevalence, the occurence of KSHV is elevated in men who have sex with men (MSM) and evidence of sexual modes of transmission exits (Albrecht et al, 1994; Martin et al, 1998; Engels et al, 2007; Sosa et al, 1998). However, a different pattern is seen in countries with a high prevalence, especially African and Mediterranean countries where KSHV infection is detected amongst children and is very common in heterosexual populations (Whitby et al, 2000; Amir et al, 2001; Athale et al, 1995; Baillargeon et al, 2002). In these countries although still ill-defined, evidence of non-sexual modes of transmission is strong (Mbulaiteye et al, 2004; Campbell et al, 2009; Dedicoat et al, 2004; Mbulaiteye et al, 2004; Sitas et al, 1999b). Furthermore, KS has also been noted in American and European children (Anderson et al, 2008; Baillargeon et al, 2002; Serraino & Franceschi, 1996; Stiller et al, 2001)

Evidence suggesting that both the sexual and nonsexual modes of transmission may play a role in the spread of KSHV infection continues to emerge. This mainly depends on the populations studied, within and between continents geographical setting and the overall HIV/AIDS impact that clearly segregates the KSHV patterns of endemic and non-endemic countries. These factors complicate the exact definition of the modes of transmission of KSHV, which remains uncertain.

This thesis attempts to provide some local knowledge about the epidemiology of KSHV in South Africa. A series of three separate studies were designed to explore aspects of the seroepidemiology of KSHV in the South African populations. The main objectives of this thesis are to describe the prevalence of KSHV, to define some of the modes of transmission of KSHV, and to identify risk factors that may be associated with transmission in South African mothers and their children, pregnant women and amongst the heterosexual community groups.

1.2 Herpesviruses

Herpesviruses are a large family of enveloped, linear double-stranded DNA viruses with relatively large complex genomes (IARC Working Group, 1997; Arvinet al; 2007). Their genomic sequences and proteins vary according to species and family. They infect a wide range of vertebrate hosts, including humans (International Agency or Research on Cancer (IARC Working Group, 1997). They encode a variety of enzymes involved in nucleic acid metabolism, DNA and protein synthesis. Approximately 130 herpesviruses species have been isolated, of which 8 infect humans (Hudnall et al, 2004), and which are known as human herpesviruses (HHVs) (IARC Working Group, 1997).

Herpesviruses are named according to the nomenclature endorsed by the ICTV, which entails the serial Arabic numbers and the family or subfamily of the host (IARC, 1997). Following these criteria, the 8 defined herpesviruses that infect humans are designated human herpesviruses 1 to 8 (HHV-1 to HHV-8). In addition to the traditional name, except for HHV-7, human herpesviruses are also given descriptive names. In many cases, one herpesvirus species causes a spectrum of different diseases in the infected host (IARC, Working Group, 1997). HHV-8 for example has been linked to KS, Multicentric Castleman's disease (MCD) and Primary Effusion lymphomas (PEL) (see section 1.5). HHV infections are usually endemic, with sexual contact as a common mode of transmission. However, other modes of transmission have been described.

1.2.1 General Structure of Herpesviruses

All identified herpesviruses have a common viral structure, consisting of the core, capsid, tegument and the envelope (Figure 1.1) (Mettenleiter, 2002).

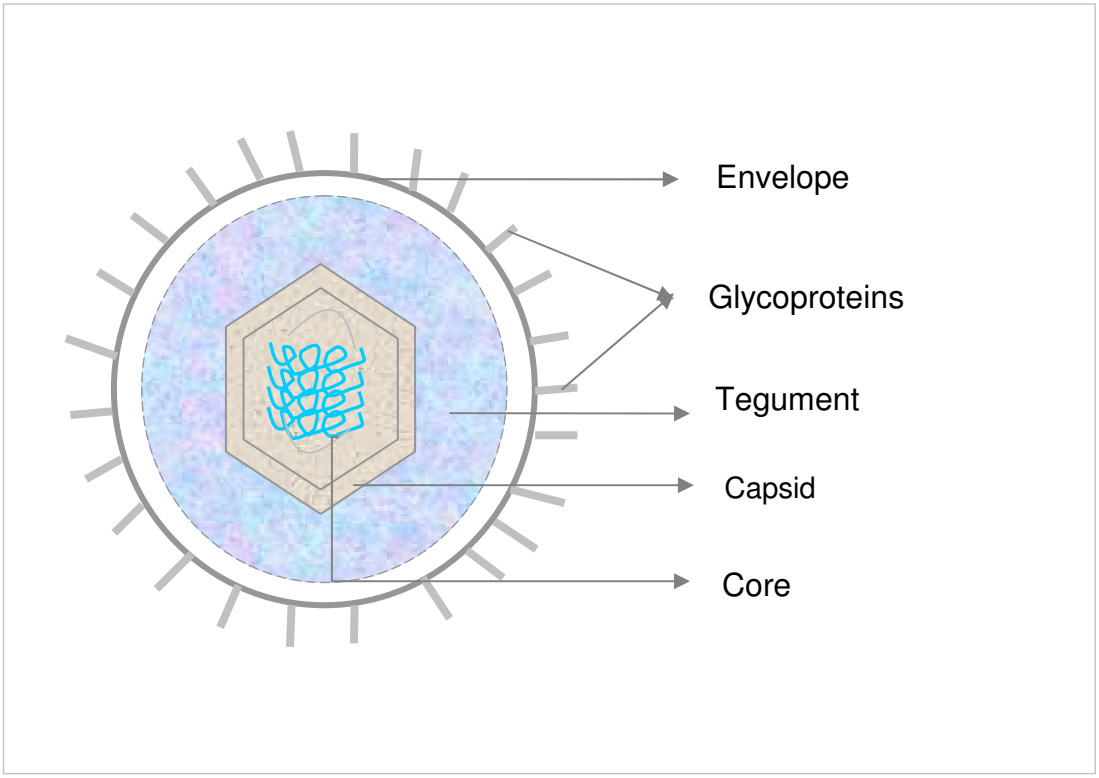


Figure 1-1: General structure of Herpesviruses (Reproduced from – http://stdgen.northwestern.edu/stdgen/bacteria/hhv2/herpes.html on the 12th Oct 2011)

The envelope is the outer layer of the virion and is composed of altered host membrane and a dozen unique viral glycoproteins. These surface glycoproteins appear in electron micrographs as short “spikes” embedded in the envelope (IARC Working Group, 1997). The tegument is distributed asymmetrically between the envelope and the capsid and its thickness varies depending on the location of the virus within the infected cell.

The capsid is icosahedral (T = 16) in shape, made up of 162 hexagonal capsomers and has a diameter of ~95 – 105nm (IARC, 1997). It is protein filled and appears shapeless in electron micrographs. The capsid covers a doughnut shaped core of ~75nm in

diameter. It consists of viral enzymes, necessary for the biological and biochemical functioning of the virus.

1.2.2 Classification of Herpesviruses

Herpesviruses are classified under 3 subfamilies designated - alpha (α), beta (β) and gamma (γ) ((Spear & Longnecker, 2003) (Table1.2). The three subfamilies have different biological properties and tissue tropism. Further subdivision was based on the similarities in genomic sequence arrangement and relation of viral proteins (Roizman & Baines, 1991). The alpha (α) herpesviruses include the genera Simplexvirus, Varicellovirus, Mardivirus and Illovirus (http://ictvonline.org/virusTaxonomy.asp?version=2009&bhcp=1). They have a broad host range including mammals, reptiles and birds and are characterised by a short reproductive cycle in epithelial cells. The human α-herpesviruses species are HSV-1, HSV-2 and HHV-3 (Table 1.2).

The beta (β) herpesviruses include the *genera Cytomegalovirus, Murogelovirus, Roseolovirus and Proboscivirus* and the identified human species are HHV-5, HHV-6 and HHV-7 (http://ictvonline.org/virusTaxonomy.asp?version=2009&bhcp=1). They replicate in vivo in a variety of cell types, including epithelial cells (McGeoch, 2001; McGeoch 1997).

The gamma (γ) herpesviruses include the genera *Lymphocryptovirus*, *Rhadinovirus, Marcavirus and Percavirus (Moore* et al*, 1996; http://ictvonline.org/virusTaxonomy.asp?version=2009&bhcp=1)*. This includes HHV-4/EBV and the latest KSHV (Figure 1.2). They are characterised by their tropism for lymphoid cells and their capacity to induce cell proliferation in vivo (Kikuta et al, 1997; Staskus et al, 1999). They have a restricted natural host and replicate efficiently in haemopoietic cells as well as replicate in epithelial

cells and fibroblasts. A distinct characteristic of gamma herpesviruses is that they cause malignancies, a feature not noted for alpha and beta herpesviruses.

Table 1-2: Summary of the classification & biological characteristics of the human herpesvirus (HHV) family

Family	Subfamily	Abbreviated ICTV Name	Common Descriptive Name	Related Diseases	Genome Size* (Kb)
Alpha (α)	*Simplex virus*	*HHV-1*	Herpes Simplex Virus 1	Facial, labial & ocular lesions	~152
	Simplex virus	*HHV-2*	Herpes Simplex Virus 2	Genital lesions	~155
	Varicellovirus	*HHV-3*	Varicella-Zoster Virus	Chickenpox & shingles	~125
Beta (β)	*Cytomegalo-virus*	*HHV-5*	Human Cytomegalovirus	Congenital infection, mononucleosis, pneumonia & hepatitis	~230
	Roseolovirus	*HHV-6*	Exanthema Subitum Virus	Exanthem subitum, heterophil myeloma, infectious mononucleosis, pneumonia, encephalitis & retinitis	~162
	-	*HHV-7*	Human Herpesvirus 7	Exanthem subitum & iosidu excite, Pityriasis rosea	~145
Gamma (γ_1)	*Lymphocrypto-virus*	*HHV-4*	Epstein-Barr Virus	Infectious mononucleosis & oral hairy leukoplakia	~172
Gamma (γ_2)	*Rhadinovirus*	*HHV-8*	Kaposi's Sarcoma Human Herpesvirus	Kaposi's sarcoma, Castleman's disease, body cavity based lymphoma, etc.	~ 170 to 210

*Genome size as per Knipe & Howley, 2007; ICTV, 1997.

1.2.3 Genome Characteristics of Herpesviruses

Herpesvirus genomes are characterised on the basis of size, base composition and structural arrangement in unique and repeated base composition. The length of the Herpesvirus dsDNA genome ranges from 120 to 230 kilobases (Kb) (Roizman et al, 1981). They have a base composition of about 31% to 77% guanine + cytosine (G+C) content and encode between 70 and 200 genes (Pellet & Roizman, 2007).

All herpesvirus genomes contain identifiable terminal repeats resulting from their base sequence arrangements. Larger terminal-repeat arrangements of 100 base pairs or

more have been identified. Thus, herpesviruses are also divided into six structurally distinct groups, designated A – F, which are based on the pattern and reiteration of these repeat base sequences (ICTV, 1997; Pellet & Roizman, 2007). KSHV, together with the primate herpesvirus saimiri

(HVS/SHV-2), ateline herpesvirus-2 (HVA-2) and the murine herpesvirus 4 (MHV-4), are members of group B (Figure 1.2). In this group a large sequence from one terminus is directly repeated numerous times at both termini.

1.2.4 Biological Properties of Herpesviruses

Four distinct biological properties have been identified for the herpesvirus family. First, herpesviruses have been shown to express a large number of enzymes involved in nucleic acid metabolism, DNA synthesis and processing of proteins (Ablashi et al, 2002; Pellet & Roizman, 2007).

Secondly, replication of herpesviruses (i.e. assembly of the DNA and capsid) takes place inside the nucleus. Therefore herpesviruses take advantage of the host's transcription machinery and DNA repair enzymes to support a large genome with complex arrays of genes. The genes are characterised as either essential or dispensable for growth in cell culture. The essential genes regulate transcription and, therefore, assembly of the virion while the dispensable genes enhance the cellular environment for virus production, necessary for viral defence from the host immune system and to promote cell-to-cell spread. The large numbers of dispensable genes are required for a productive *in vivo* infection (Pellet & Roizman, 2007).

Thirdly, productive viral infection leads to the host cell destruction. Lastly, herpesviruses can remain in a latent state in their host and reactivate following cellular stress. Herpesvirus latency involves stable maintenance of the viral genome in the nucleus with limited expression of a small subset of viral genes. Most human herpesviruses are

ubiquitous in most populations. Due to persistence of the latent infections and asymptomatic shedding of the virus, herpesviruses are commonly associated with horizontal person-to-person transmissions. KSHV is an exception to this rule as its prevalence varies by population groups and has an uneven geographical distribution (Ablashi et al, 2002; Pellet & Roizman, 2007, ICTV, 1997). KSHV is also shed asymptomatically in saliva and spread horizontally.

1.2.5 Hypothesis on the Origins of Human Herpesviruses

The origin of herpesviruses is not clear. However, herpesviruses are closely related to bacteriophages (viruses that target and infect bacteria), (Homa and Brown, 1998). Several studies used HSV-1 as a model for studying the origin of herpesviruses. They are thought to be evolutionary ancient infections and are found throughout mammals, birds, reptiles etc. They have been shown to have co-evolved with their host species. In 1996, Trus and co-workers showed that, like bacteriophages, the HSV-1 protein assembly resembles roundish “procapsids”, which mature into polyhedral capsids. This relationship is further supported by Baker and Jiang (2005), who proposed that the lineages of herpesviruses and Caudovirales (prokaryote infecting tailed DNA bacteriophages) are structurally related.

KSHV genes have also been shown to be closely related to herpesviruses when isolated in Macacques and baboon species (Whitby et al, 2003; Bruce et al, 2005; Locher et al, 1998a; Locher et al, 1998b; Whitby et al, 2003; Lacoste et al, 2000c). In 2003, Whitby and co-workers identified a baboon novel rhadinovirus, PapRV2, with substantial sequence identity to two essential KSHV genes. Hayward, 1999, suggested that KSHV may be an ancient human virus that reflects the migrationary divergence of modern human populations over the past 35,000-60,000 years (Hayward, 1999)

1.3 Kaposi's Sarcoma-associated Herpesvirus (KSHV)

KSHV was the latest of the human herpesviruses to be discovered. Fragments of the KSHV genome were first identified in 1994 in the disseminated tissues of KS patients with AIDS. These were reported to have some DNA homology to the Epstein-Barr virus and herpesvirus saimiri (Chang et al, 1994; Cathomas, 2000). Subsequent studies confirmed these findings and a whole virus was isolated in KS lesions and described (Neipel et al, 1997b; Neipel et al, 1997a; Nicholas et al, 1997; Thielen et al, 2006; Whitby et al, 2004; Whitby et al, 2003).

KSHV is classified as a member of the gamma-2 herpesvirus subfamily, which is a group of lymphotropic herpesviruses. Like other herpesviruses, KSHV can maintain a lytic and latent phase. The virus codes for several proteins homologous to cellular proteins that could eventually lead to disturbances in the regulation of cellular proliferation and apoptotic mechanisms resulting in the development of KS (Boulanger, 1998; Belanger et al, 2001; Boshoff & Chang, 2001; Esteban et al, 2003). However, KSHV alone was shown not to be sufficient to cause KS, it exists in a dormant stage and is well controlled in an immunosufficient host for many years, but its pathogenic progression will take advantage of the immunosuppressed system. The relationship between KSHV, immunosuppression and development of KS has led to rapid increases in the incidence of KS in countries where HIV infection is endemic.

1.3.1 Structure of KSHV

Like all herpesviruses, the basic structure of KSHV consists of a nuclear envelope with protruding spikes, a tegument and a capsid surrounding double stranded DNA (Figure 1.1). The capsid of the KSHV is composed of 12 pentons, 150 hexons, and 320 triplexes arranged on a T=16 icosahedral lattice (Wu et al, 2000). The inner radius of the KSHV capsid is identical to that of the HSV-1 capsid but is smaller than that of the HCMV capsid, which is consistent with the relative sizes of the genomes they enclose (Wu et al,

2000). The structure of KSHV is reported to be similar to that of the herpes simplex virus-1 (HSV-1) and human cytomegalovirus (HCMV) (Wu et al, 2000; Trus et al, 2001). However, the KSHV capsid was reported to differ from that of the HSV-1 capsid in two major ways: first, the KSHV hexons lack the "horn-shaped" VP26 densities bound to the HSV-1 hexon subunits and the KSHV triplexes appear smaller and less elongated than those of HSV-1 (Wu et al, 2000; Trus et al, 2001). Also, the KSHV inner radius is smaller than that of the HCMV (Wu et al, 2000).

1.3.2 Classification of KSHV

KSHV is the first known human member of the genus Rhadinovirus (Chang et al, 1994; Neipel et al, 1998). Rhadinoviruses share a typical genome structure, which contain numerous sequences that appear to be sequestered from cellular DNA. Subsequent sequencing of KSHV classified the virus within the Gammaherpesvirinae subfamily, which also includes two other DNA tumour viruses: Epstein-Barr virus (EBV) and Herpesvirus saimiri (HSV) (Moore, 1998).

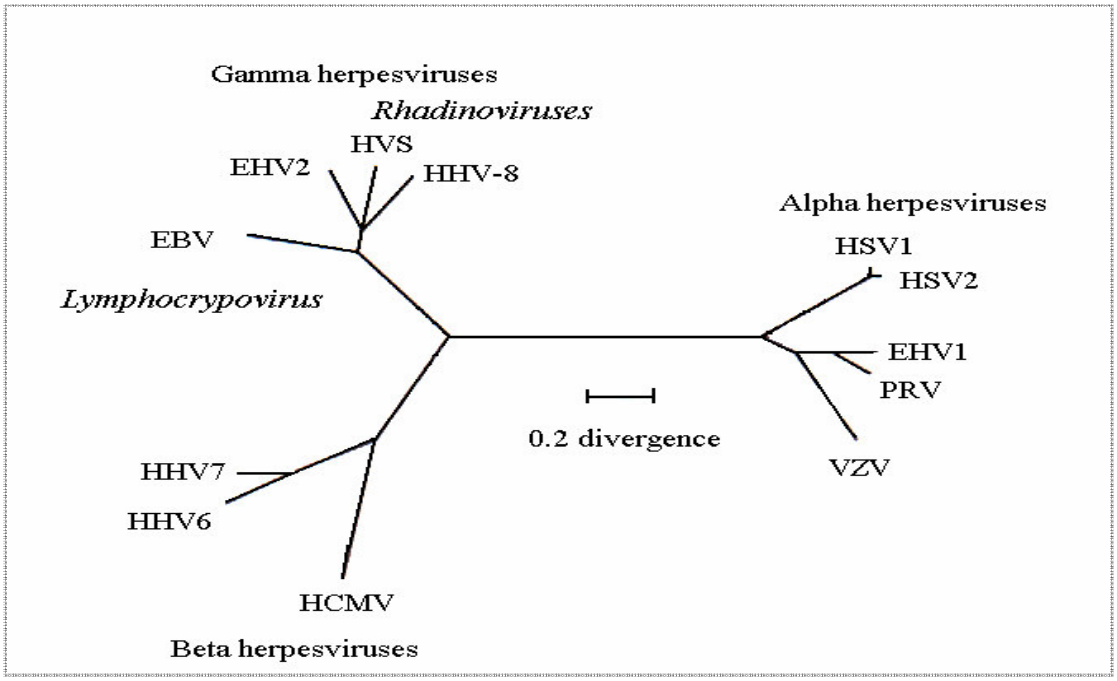


Figure 1-2: Phylogenic tree of the classification of human herpesviruses. Abbreviations: EHV2 - , EBV- Epstein Barr virus, HVS-, HCMV- PRV, HHV- Human Herpesvirus, HSV – Human Simplex Virus)] (Figure supplied by Whitby D, National Cancer Institute, Fredericks, MD, USA, Chang, 2002).

Figure 1.2 shows a phylogenetic tree of the classification of KSHV and other human viruses based on comparison of aligned amino acid sequences between herpesviruses for the major capsid protein gene and for a concatenated nine-gene set (Moore et al, 1996). The comparison of major capsid protein sequences was obtained by the neighbour joining method and is shown in an unrooted type, with branch lengths proportional to divergence (mean number of substitution events per site) between the nodes bounding each branch (Moore et al, 1996). The phylogenetic tree of gamma herpesvirus sequences demonstrates that KSHV is most closely related to the gamma-2 herpesvirus sub lineage, genus Rhadinovirus (Figure 1.2). Recently, two new viruses with more homology to KSHV than any other previously known herpesvirus have been isolated from monkeys (Desrosiers et al, 1997).

1.3.3 Genomic characteristics of KSHV

The KSHV genome size is about 165 to 170 kb (Renne et al, 1996; Zhong et al, 1996) which is slightly larger than that of the herpes simplex virus-1(HCV-1), ~152-kb, (Roizman, 1996) and smaller than that of the human cytomegalovirus (HCMV) which is ~230-kb (Russo et al, 1996). The KSHV genome contains at least 85 open reading frames, some of which are homologous to those of other herpesviruses and some unique to KSHV. The DNA sequences unique to KSHV are designated with the prefix K (Neipel et al, 1997b; Russo et al, 1996).

Members of the rhadinoviruses share a common genome structure in which multirepetitive high GC DNA flanks a central segment of low GC DNA on both sides (Neipel et al, 1997b; Russo et al, 1996). The KSHV genome has a central unique region ~145 Kb, which contains all the viral open reading frames (ORF's) (Russo et al, 1996; Burysek et al, 1999;Neipel et al, 1997a; Lee et al, 1998; Talbot et al, 1999; Nicholas et al, 1998).

1.3.4 KSHV strains

The KSHV genome has been subtyped into different strains by sequencing the ORF-K1 gene (Zong et al, 1999; Zong et al, 1999). Several KSHV strains labelled subtypes A – E, M, N and Q have been identified globally (Cassar et al, 2007; Zhu et al, 2008; Kadyrova et al, 2003; Biggar et al, 2000; Poole et al, 1999; Zong et al, 1999). Subtype A is most common in western Europe and north America (Dadke et al, 2003; Kouri et al, 2005; Stefanou et al, 1998; Biggar et al, 2000).

The subtypes are further subdivided into different variants, which subdivide the groups into more specific KSHV variants. KSHV subtype A has the variants A1 – A5, subtype C is subdivided into variants C1 to C6. Subtype A variants are widespread to other continents including Africa, Australia (A1 only) and the Mediterranean region (A1 - A5) (Nascimento et al, 2005; Lacoste et al, 2000b). Subtype B is mainly restricted to Africa and in those of African lineage (Kajumbula et al, 2006; Treurnicht et al, 2002; Treurnicht et al, 2002).

Subtype C is subdivided into variants C1 – C4, which are commonly distributed along the Middle East and Mediterranean regions. Subtypes C1 and C3 have also been described in northern America and Australia (C3 only), a non-sequenced Subtype C has also been described in South America and Asia. Subtype D is the least described and has been identified in the Brazilian Amerindians of different tribes (Biggar et al, 2000; Ishak et al, 2007; Poole et al, 1999). Subtype E was described much later in the South America population (Biggar et al, 2000; Zong et al, 1999; Hayward, 1999; Boshoff & Weiss, 2001).

1.4 Laboratory Detection of KSHV

Although KSHV was only discovered a decade and a half ago, a number of laboratory serological and molecular assays for the detection of KSHV in blood, body secretions

and body tissues have already been established. The available methods can be used to identify antibodies in selected proteins or the whole KSHV particle. Sero-epidemiological studies apply IFA (Gao et al, 1996b; Kedes et al, 1996; Simpson et al, 1996; Inoue et al, 2000), immunoblotts (Zhu et al, 1999) or ELISA[i] (Mbisa et al, 2010) to detect KSHV antibodies to lytic and latent cycle proteins. These include proteins encoded by ORF's[ii] such as ORF65 and K8.1, antibodies against latency associated nuclear antigens (LANA) and recombinant capsid proteins ((Simpson et al, 1996; Andre et al, 1997; Lang et al, 1999). The KSHV DNA can be detected using in situ hybridization PCR[iii] methods, (Cesarman et al, 1995; Soulier et al, 1995; Schalling et al, 1995; Reed et al, 1998; Boshoff et al, 1995a). Several studies have compared different laboratory assays and techniques to try and describe the best approach to the detection of KSHV infection (Zhu et al, 1999; Spira et al, 2000; Pellett et al, 1999). The use of a combination of two or more serological assays is regarded as more appropriate as they increase sensitivity and specificity in detection of KSHV infection(Lang et al, 2000; Rabkin et al, 1998; Topino et al, 2001) .

1.4.1 Molecular detection of KSHV viral particles

Molecular assays for detection of KSHV DNA are mainly used for validation of serological assays to monitor disease development, progression and ascertain effects of antiretroviral therapies (Pak et al, 2005; Dittmer et al, 2003). The widely used method is PCR for detection of viral DNA in various body tissues and fluids (Neipel et al, 1997b; Newton & Rybak et al, 1998; Broccolo et al, 2002; Fujimuro et al, 2006; Nascimento et al, 2005).

PCR is mainly used for detection of KSHV DNA in infected samples and to determine viral load (Boivin et al, 2002; Cathomas et al, 1996; Pellett et al, 1999). This procedure has been successfully used to detect KSHV DNA particles in PBMC, lymph node biopsy specimens, blood specimens, oral and nasal secretions, prostate biopsy and semen

specimens (Howard et al, 1997, Taylor et al, 2004; Pauk et al, 2000; Blackbourn et al, 1998; Diamond et al, 1998; Viviano et al, 1999).

Using PCR, KSHV DNA has been isolated in all biopsy sample types of KS (Deback et al, 2008; Dittmer, 2003) and other KSHV related diseases (Hammock et al, 2005; di Gennaro et al, 2001). The PCR product can be enhanced by southern blotting or can be embedded in paraffin. It was noted that although these procedures promote detection of KSHV DNA, they pose possibilities of KSHV viral contamination. More recently quantitative real time PCR (qPCR) has been used for KSHV DNA detection and viral load determination. This technique has the advantage of additional sensitivity and specificity without contamination risks. Zhang et al, 2000, used PCR-RFLP genotyping to identify LANA genetic variations and to differentiate individual KSHV isolates (Zhang et al, 2000). In another study, analysis of RFLP was undertaken using nested ORF73 internal repeat domain PCR products derived from the blood and mouth rinse samples of individuals in Malawian family groups (Cook et al, 2002). The resulting RFLP patterns were unique to an individual and could be compared intra- and extra- familiarly.

1.4.1.1 Serological detection of KSHV

Serological assays are widely used to establish the epidemiological patterns of KSHV and its relation to KS and other related diseases. Serological assays are also used to understand the modes of KSHV transmission and emerging incidence patterns of KSHV. Different serological assays are used to detect both the lytic and the latent stages of KSHV. Using serological and PCR methods, KSHV infection has been shown to be uncommon in the general population of the United Kingdom and the United States, but common in groups at increased risk for KS such as HIV infected homosexual men (Gao et al, 1996; Kedes et al, 1996; Simpson et al, 1996). However standardised reference cut-offs for KSHV serological assays have not been established and in most cases in-

house assays are used with varied methods describing the presence or lack of infection (Mbisa et al, 2010; Rabkin et al, 1998; Spira et al, 2000; Zhu et al, 1999).

1.4.1.2 Immunoblot assays

Immunoblot assays have been used for the detection of a variety of viral KSHV proteins (Zhu et al, 1999; Gao et al, 1996a; Miller et al, 1996; Katano et al, 1999). Western blotting assays are commonly used as rapid and sensitive tests for detection and characterization of viral immuno-proteins, including identification of specific antigens recognized by polyclonal and monoclonal antibodies (Gallagher & Chakavarti et al, 2008). They involve the solubilization and separation of proteins, glycoproteins or lipopolysaccharides by gel electrophoresis, followed by quantitative transfer and irreversible binding of nitrocellulose, PVDF[iv] and nylon membranes (Gallagher & Chakavarti et al, 2008). Western blots are regarded as being more sensitive in detecting of selected KSHV lytic and latent proteins than IFA's. Immunoblot assays using ORF 73 and ORF57 proteins were shown to be more sensitive than IFA in the detection of KSHV seropositivity (Yang et al, 2009; Wang et al, 2002; Zhu et al, 1999). Combination of western blot assays and other serological assays in detection of KSHV, may prove to be more sensitive and reliable(Yang et al, 2009; Wang et al, 2002).

1.4.1.3 KSHV Immunofluorescence Assays

Immunofluorescence assay (IFA) is a laboratory technique which uses microscopy for colourful visualization of targeted proteins or antigens in cells or tissue biopsies, by binding chemically conjugated antibodies to a fluorescent dye, i.e. FITC[v]. The IFA methods are mainly based on BCBL cell lines and have been used for detection of antibodies to lytic and latent KSHV antigens (Rabkin et al, 1998; Couty et al, 1999; Martin et al, 2000; Smith et al, 1997; Inoue et al, 2000). IFA's are more suitable and reliable for describing KSHV serology in populations with low probabilities of infection (Perez et al, 2006; Chohan et al, 2004). IFA tests are also used as confirmatory assays

in seropositive tests pre-screened by less sensitive serological assays (Chohan et al, 2004).Upgrades of the original IFA KSHV methods have been attempted (Minhas et al, 2008); (Perez et al, 2006; Inoue et al, 2000).

IFA are reputable assays that have proved to be useful in the description of KSHV epidemiology (Inoue et al, 2000). In a South African study in which an IFA method was used for detection of latent KSHV antibodies, the seroprevalence of KSHV antibodies was 83% in patients with KS and significantly higher than in those without KS (Sitas et al, 1999a). The main disadvantage of IFA compared to ELISA is that IFA is not suitable for large high throughput studies and it also has greater variability (Mbisa et al, 2010).

1.4.1.4 KSHV Enzyme-linked Immunosorbent Assays

Enzyme linked immunosorbent assays – (ELISA's) are commonly used to detect the presence of antibodies or antigens in samples. They are used for the detection of whole viral lysates, synthetic peptides or recombinant peptide carrier proteins (Chatlynne et.al, 1998; Davis et.al, 1997; Pau et.al, 1998). They can easily be used to analyse large batches of samples and are thus commonly utilised in epidemiological studies. Several ELISA methods for the detection of the KSHV infection in infected individuals have been developed, with or without Kaposi's sarcoma(Peri et al, 1994; Juhasz et al, 2001; Davis et al, 1997). KSHV serology is still new and, attempts to improve the current serological and molecular methods are ongoing (Mbisa et al, 2010; Juhasz et al, 2001). ELISA's based on the detection of the recombinant capsid related protein have been developed (Davis et al, 1997; Tedeschi et al, 1999; Lebbe et al, 1999).

1.4.2 Concordance between serological KSHV assays

Several laboratory assays are used for detection of antibodies to KSHV antigens. Expression of latent and lytic antibodies in the infected subjects may vary (Lan et.al, 2004), so that not all KSHV infected subjects express both the lytic and latent antibodies

at a particular point. Thus some methods detect antibodies to lytic KSHV antigens while others detect antibodies to latent KSHV antigens. Epidemiological and comparative studies that have applied more than one method for estimation of KSHV seroprevalence have consistently indicated that using only one method for detection of KSHV antibodies might exclude some subjects, thus underestimating KSHV seroprevalence in a community (Sergerie et al, 2004; Enbom et al, 2000; Diociaiuti et al, 2000).

In one study, in European and Ugandan subjects using 18 different assays for detection of KSHV antibodies, only IFAs for detection of antibodies against KSHV lytic or latent (LANA) antigens and two ELISAs for detection of antibodies against KSHV structural proteins were found to be highly concordant, specific and sensitive, with odds ratios that indicated a high predictive value. The best results were noted when the two IFA were used together, as indicated by their combined sensitivity (89 %) and specificity (95 %) (Schatz et al, 2001).

In another study that compared different serological assays to detect KSHV antibodies in KS subjects and a PCR method to confirm serologic assay results, it was concluded that no single serological assay is completely sensitive and specific to the detection of KSHV antibodies. This might be because single assays detect antibodies against a single KSHV protein fragment. In another study that compared seven IFA's and ELISAs KSHV antibodies were most frequently detected in sera of subjects with classic (≥ 80 %) and AIDS-related (67 - 91%) Kaposi's sarcoma, followed by human immunodeficiency virus-seropositive patients (27 - 60%), and least frequently in healthy blood donors (0-29%). However, there was a high serodiscordance between the assays for individual sera (Rabkin et al, 1998). It is therefore probable that applying at least two assays to detect lytic KSHV antibodies and latent antibodies might prove to be more valuable due to an increase in specificity and sensitivity for KSHV antibodies (Schatz et al, 2001; Pellett et al, 2003; Rabkin et al, 1998).

1.5 Diseases related to KSHV

KSHV was initially identified from two novel herpesvirus-like DNA fragments isolated from lesions of homosexual males with AIDS-associated KS (AIDS-KS) (Chang et al, 1994). Subsequent studies have consistently detected KSHV DNA in all types of KS tissues (Dupin et al, 1995b; Boshoff et al, 1995b; Boshoff et al, 1995b; Moore & Chang, 1995; Schalling et al, 1995). Serological studies have also reported high KSHV seroprevalences in patients without KS in areas where KS is endemic (Sitas et al, 1999a; Whitby et al, 1998). Additional evidence for causation came from studies indicating that KSHV could be detected prior to disease (Mbulaiteye et al, 1995) and also that it is detected in the spindle cells (Boshoff et al, 1995).

KSHV has thus been shown to be the necessary causative agent for KS. However, KSHV infection alone is not sufficient for development of the disease (Moore & Chang, 1998; Iscovich et al, 2000). This is because the development of KS appears to be under strict immunological control, with KS occurring mainly in subjects who are immuno-compromised (Taylor et al, 1986; Jacobson et al, 2000; Rezza et al, 1999). Pre the HIV epidemic, increased risks of KS were noted in patients under immunosuppressive therapy after undergoing organ transplantations or iatrogenically immunosuppressed patients (Penn, 1997; Penn, 1993; Doutrelepont et al, 1996; De Paoli, 2004; Rady et al, 1998). KS was common in renal transplant patients and very rare in the general population. In the HIV-AIDS era, the association between KS and immunosuppression is undisputed as more and more KS cases are noted in HIV immunosuppressed subjects.

This is even more true in Africa were HIV is very common. An exception is the confined classic and African endemic KS which occurs in otherwise healthy subjects without immunosuppression (Friedman-Kien & Saltzman, 1990; Giraldo et al, 1984; Atzori et al, 2004). Classic KS patients are usually elderly and, while not overtly immune suppressed are likely to have waning immune systems.

In addition to KS, KSHV DNA has also been detected in some of the relatively rare haematological lympho-proliferative disorders. There has been detection of KS DNA in multicentric Castleman's disease (MCD), primary effusion lymphoma (PEL) or body cavity based lymphoma (BCBL), and other non-KS skin diseases (Soulier et al, 1995; Carbone et al, 2000b; Carbone et al, 2000a; Gessain et al, 1996; Cesarman et al, 1995; Memar et al, 1995; Geraminejad et al, 2002; Inagi et al, 1996; Nishimoto et al, 1997; Berenson, 1999; Berenson & Vescio, 1999; Abdulla et al, 2000; Cathomas et al, 1998; El Kassimi et al, 2003; Rettig et al, 1997). KSHV was once also linked to multiple myeloma but this has been disproved by multiple further studies. Some of these KSHV related diseases are briefly discussed in the next sections.

1.5.1 Kaposi's Sarcoma

Kaposi's sarcoma (KS) is defined as a multicellular, mesenchymal neoplasm characterised by the presence of spindle-shaped tumour cells, angiogenesis (uncontrolled growth of blood vessels in tumours), extravasated erythrocytes, oedema and a mononuclear inflammatory cell infiltrate (Foreman, 2001; Kang et al, 1998). It was discovered in 1872 and described as an idiopathic multiple pigmented sarcoma (Rothman, 1962; IARC, 1996). The affected patients were commonly elderly males of Jewish or Mediterranean origin and presented with cutaneous lesions mainly on the lower extremities. All of these patients eventually died within 3 – 5 years of diagnosis (Rothman et al, 1962), however the disease is generally described as indolent and rarely aggressive. For several decades thereafter, the disease was thought to affect predominantly elderly men of Mediterranean and eastern European origin in their 5^{th} to 7^{th} decade of life (Rothman, 1962) and this type was termed "classic KS" (Classic-KS).

In the 1950s another type of KS, known as the African endemic KS (endemic-KS), was reported to be a common cancer in parts of Central Africa (Cook-Mozaffari et al, 1998). Unlike classical KS, endemic-KS was more aggressive and occurred mainly in children

and young adults in sub-Saharan Africa (Ziegler & Katongole-Mbidde, 1996). In addition to skin lesions, endemic-KS may involve lymph nodes and other organs, especially in children, where skin lesions were not obvious (Davies & Lothe, 1962). Later, another type of KS became apparent as a common complication in immunosuppressed patients who had undergone solid organ transplants (Doutrelepont et al, 1996; el Maghraoui et al, 2003; Pasini & Bubic-Filipi, 1999; Nagy et al, 2000). This was termed iatrogenic KS.

In 1981, a fourth type of a clinically more aggressive KS was reported in homosexual subjects with AIDS (Aquino et al, 1986; Muggia & Lonberg, 1986; Safai et al, 1985). This discovery was made in the USA when young homosexual males with immunosuppression presented with cutaneous KS lesions (MMWR, 1981). This was later to be known as AIDS associated KS (AIDS-KS). Thus, at present, 4 types of Kaposi's sarcoma, termed Classic-KS, endemic-KS, iatrogenic-KS and AIDS-KS have been described.

In general, KS is more common in males than in females. Children and AIDS patients tend to develop more virulent disease. Jewish and Mediterranean males have the highest incidence of classic Kaposi's sarcoma. Unlike the American black population in which KS incidence remain low, black Africans have the highest incidences of African Kaposi's sarcoma. Also, KS is also more common in homosexual than heterosexual males. Although clinical manifestations may differ for some of the KS types, all types are histologically indistinguishable and evolve through a chronological sequence of patch, plaque and nodule formation (O'Connell et al, 1977; Simonart et al, 2000; Boshoff & Weiss, 2001; Chow et al, 1989). The different types of KS are discussed in the next sub-sections.

1.5.1.1 Classical Kaposi's sarcoma

In 1872, Classic-KS was the first type of KS to be described. Further cases were reported in Italy in 1882, in which a detailed analysis of affected patients was given

(Ronchese, 1958; Schiavo, 1996). Classic-KS usually presents with reddish-brown to brown-violet plaques or nodules on the skin of the lower limbs in elderly men, often from Mediterranean heritage, and progresses slowly (Rothmans et al, 1962, Schwartz et al, 2004). Although all the initially diagnosed patients died within 3 - 5 years of diagnosis (Rothmans et al, 1962, Schwartz et al, 2004), for most of the first 3 quarters of the 20th century, Classic-KS was viewed as a slow growing cancer that was not life threatening (Rothman et al, 1962). Those with Classic-KS often lived with the disease for 10 years or more and were expected to die with, rather than of, KS (Hengge et al, 2002). Considerable pain, particularly in areas with oedema is also common.

In the United States and Europe, classic KS has a peak incidence between 40-70 years, with a wide range of up to 89 years. In Italy and, most notably in southern Italy, between 1976-1984, prior to the AIDS epidemic, KS incidence rates, especially classic-KS, were two-to-three-fold higher than in the United States and Sweden and much higher than in England, Wales and Australia (Franceschi & Geddes, 1995; Biggar et al, 2000; Dictor and Attewel, 1988; Grulich et al, 1992). Incidences and seroprevalence of Classic-KS in Italian males increases with age after 50 years of age (Santerelli et al, 2001).

A high frequency of classic KS also occurs in Israel, Greece, Turkey (Iscovich et al, 2000; Schwartz, 1996; Stratigos et al, 1999) and, in low-risk countries, it occurs in individuals born in southern Europe and the Middle East. In Italy between 1985 and 1998, classic KS accounted for 97% of all KS in elderly people from 65 years of age and above and only 42% of men between 39 and 64 years (Dalmaso et al, 2005).

1.5.1.2 African Endemic KS

Endemic-KS presents with lymphadenopathy and cutaneous lesions in both adults and children of African origin (Rothman, 1962; Davies and Lothe,1962). Before 1980 and pre-HIV AIDS, endemic-KS represented the most common type of KS in the African continent and was of greater geographic variation. Although it is very common in East

African countries such as Uganda and Tanzania and also Cameroon and the Democratic Republic of Congo, endemic-KS was fairly common in Southern African countries such as South Africa but barely occurred in other African countries (Figure1.6) (Davies and Lothe, 1962; Oettle, 1962; Cook-Mozaffari et al, 1998, Dedicoat and Newton, 2003). Before 1980, the incidence of endemic-KS in endemic East African countries was 6 – 9/ 1000 males and ranged between 0 –3 / 1000 males in the Southern African regions and other African countries such as Nigeria, Gambia and the Ivory coast (Cook-Mozaffari et al, 1998, Dedicoat and Newton, 2003).

Between 1964 and 1968, the incidence of endemic-KS in Ugandan males was 14.6% per million per year and was about 5% of all diagnosed tumours (Taylor et al, 1972). Increased incidences of KS in Zaire were reported between 1957 and1982 (Gigase et al, 1984). In 1957, KS was diagnosed in 9 % of all cancer cases confirmed by biopsy and then, in 1960, KS was diagnosed in 14% of all male cancers and 17 % of all male cancers in 1969 – 1983 in eastern Zaire (Gigase, 1984). KS was rare in women frm Zaire with only 1 case diagnosed between 1971 and 1980 (Coker & Wood, 1986). In Zambia, KS represents up to 25% of childhood cancers and has an average male-to-female ratio of 1.76:1, with male predominance higher in children older than 5 years (2.5:1) than in children younger than 5 years (1.4:1).

The geographical variations in the incidence of KS within the African continent before the onset of the HIV epidemic in the 1980s are clearly shown in Figure 1.3. At that time, KS incidence was described by Cook-Mozaffari and co-workers as occurring in extreme narrow belts of relatively high incidence stretching westward across Zaire to the coast of Cameroon and southward down the rift valley to Malawi. This endemic KS was more common in men than in women (Cook-Mozaffari et al, 1998), but was similar in female and male children. The pattern of KS in Africa changed in the HIV era and is discussed in the next sections

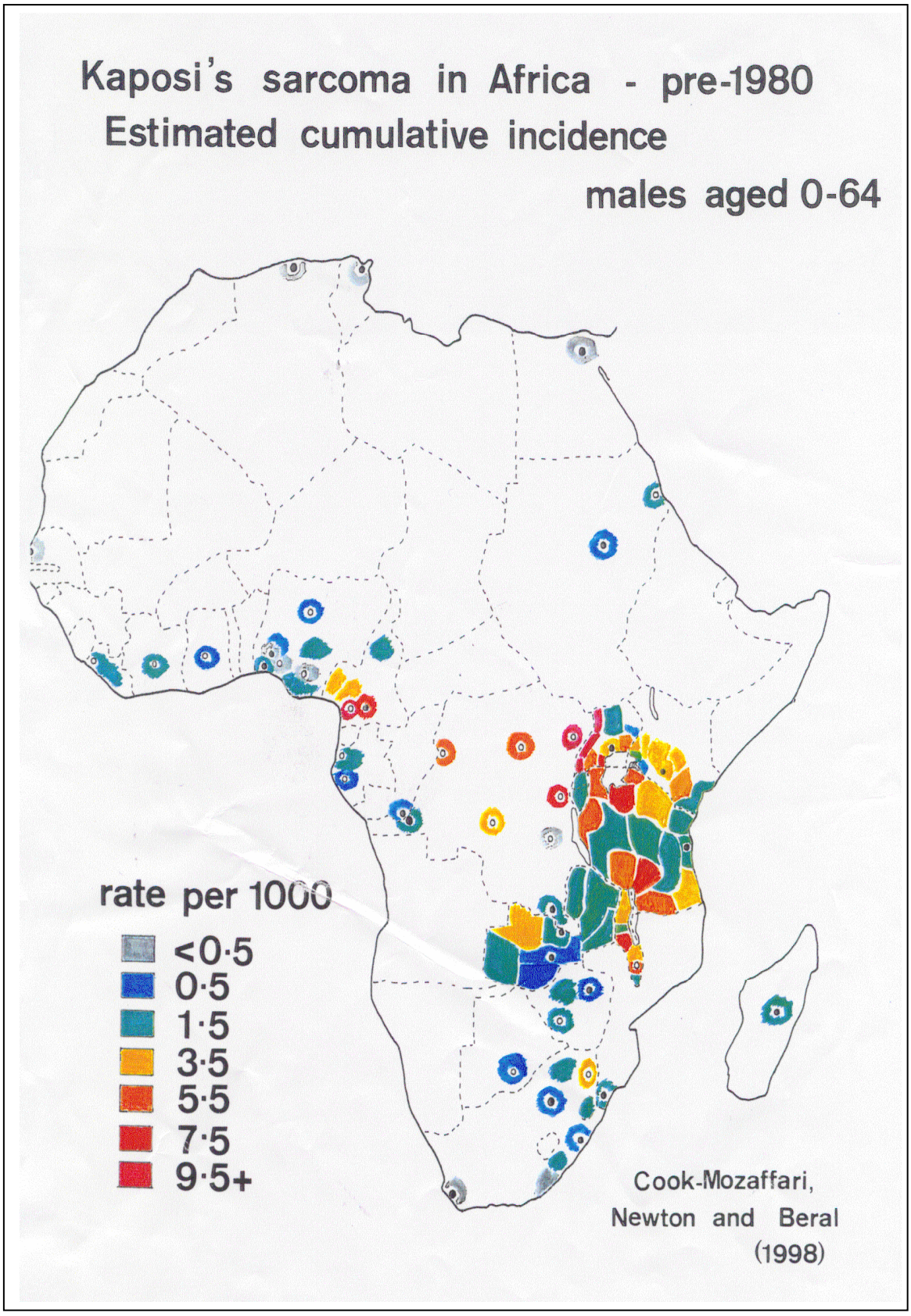


Figure 1-3: Incidence of Kaposi's sarcoma within the African continent before the onset of the HIV epidemic in the 1980s (Figure supplied by Whitby D, National Cancer Institute, Fredericks, MD, USA, courtesy of Cook- Mozaffari et al, 1998)

1.5.1.3 Iatrogenic Kaposi's sarcoma

Long before the discovery of HIV, immunosuppression was already implicated in the development of Kaposi's sarcoma. Iatrogenic-KS occurred in immunosuppressed patients who had undergone solid organ transplants (Geraminejad et al, 2002; Simonart et al, 2000). Iatrogenic-KS represented about 6% of all tumours post-transplantation (Penn et al, 1993). In 1979 Howard and co-workers, reported that, in patients of European, Jewish or African origin, the risk for KS increased by up to 500-fold post-transplantation (Harwood et al, 1979). Iatrogenic-KS has also been shown to occur in 0.4% of patients in the United States and Western Europe (Penn, 1987; Farge et al, 1993) but in up to 5% of renal transplant patients in Saudi Arabia, with KS representing up to 88% of tumours in renal transplants patients in one of the hospitals in Saudi Arabia (Penn, 1997; Qunibi et al 1988).

Iatrogenic KS, lesions present chronically or with rapid progression (Hengge et al 2002). The lesions are generally limited to the skin and oral mucosa and typically regress with discontinuation of immunosuppressive therapy (Penn et al, 1993). High incidences of iatrogenic-KS post-transplantation have also been reported in other countries (Moosa, 2005; Andreoni et al, 2002; Nagy et al, 2000). In Cape Town, South Africa, between 1976 and 1999, 41 (7.6%) patients who had undergone renal transplants developed cancer and iatrogenic-KS was the most common cancer in non-white populations, in whom it accounted for almost 80% of all cancers (Moosa, 2005).

Recurrence of iatrogenic-KS occurs when immunosuppressive therapy is re-introduced and complete remission is noted in only 20% of patients with visceral involvement (Penn et al, 1993; Frances et al, 1997). Patients with iatrogenic-KS tend to have bleeding in the gut resulting from KS, although termination or reduction of immunosuppression often, but not always, results in regression of KS.

1.5.1.4 AIDS associated KS

HIV/AIDS was first diagnosed in 1981 in the United States in homosexual patients with KS (MMWR, 1982). The type of KS described was regarded as more aggressive, disseminated and resistant to treatment than all other known types of KS (Schwartz, 1996). The AIDS-KS lesions are widespread on the skin and often involve the internal organs, particularly the lungs and gastrointestinal tract. This AIDS-KS is widely regarded as more aggressive and visceral invasion can lead to organ dysfunction and mortality (Hermans et al, 1998; Dourmishev et al, 2003; Manji et al, 2000; Nasti et al, 1999). The prognosis is poor, with a substantial proportion of AIDS-KS patients dying as a result of complications related to KS (Ansari et al, 2002; Jougla et al, 1996) and the remaining patients suffering significant morbidity.

The aggressive course originally noted by Kaposi in 1872, has become part of the devastation of the AIDS epidemic. In all diagnosed cases that Kaposi described, the patients died within 5 years. However, AIDS-KS is generally more aggressive than the classic-KS in which the disease behaviour is generally benign. Patients with AIDS-KS usually die from associated opportunistic infections or from gastrointestinal KS with haemorrhage. In the United States and Europe, AIDS-KS is mainly noted among homosexual and bisexual males. Drastic increases in the prevalence and incidence of KS are noted in the African continent, with increased rates noted even in countries where KS was previously very rare. For example in Uganda, the incidence of KS in children has increased by about 40 fold (Ziegler & Katongole-Mbidde, 1996), and rapid increases were noted in South African females rather than males, such that, in 1988, the male to female ratio of KS was 7: 1 declining to 2:1 by 1996 (Sitas and Newton, 2001).

Even though AIDS-KS is a rapidly progressive disease, it can be controlled by the use of highly active antiretroviral therapy (HAART) (Pellet et al, 2001). In the US, the mean survival rate of patients with AIDS-KS is extended through administration of HAART

(Jones et al, 2000; Horster et al, 2004). Introduction of antiretroviral treatment has improved KS progression in affected patients and has contributed to remission of the disease in those adhering to treatment (Ramirez-Amador et al, 2003; Villasis-Keever et al, 2001; Barillar et al, 2003; Bihl et al, 2007).

1.5.2 Other Haematological malignancies

1.5.2.1 Multicentric Castleman's Disease

Castleman's disease (CD) is a rare atypical lymphoproliferative disorder (Palestro et al, 1999). CD was first described in 1956 by Benjamin Castleman and, like KS, had remained idiopathic until the discovery of KSHV (Frizzera et al, 1988). It is subdivided according to its morphological presentation into a hyaline vascular, plasma cell and mixed type (Palestro et al, 1999; Bradamante et al, 1998). CD is associated with lymph node enlargement, hepatosplenomegaly and fever (Aaron et al, 2002). It is described as either localised or multicentric. Multicentric Castleman's disease (MCD) affects multiple systems and is histologically characterised to be of mixed type with a predominantly lymphadenopathic presentation consistently involving peripheral nodes (Frizzera et al, 1988). MCD is common in HIV positive patients with AIDS (Horster et al, 2004; Cazorla et al, 2005; Dayyani et al, 2007), although it is still sometimes diagnosed in HIV negative people (Suda et al, 2001; Sukpanichnant et al, 2002).

KSHV DNA has been detected in most cases of MCD (Soulier, 1995 et al; Gessain et al, 1996; Hayashi et al, 1999; Dupin et al, 1995; Karcher et al, 1995; Briz et al, 1998), however there are several reported cases of MCD in which KSHV could not be detected (Kwong et al, 1997). KSHV has been shown to encode a homologue of viral interleukin 6 (vIL6) which plays a significant role in the pathogenesis of MCD (Parravicini et al, 1997; Menke et al, 2002). This is because MCD has been shown to be precipitated by excessive production of the cytokine interleukin-6 (IL-6) and that successful treatment may lead to a decrease in IL-6 expression (Newsom-Davis et al, 2004).

1.5.2.2 Primary Effusion Lymphoma (PEL)

Nador and co-workers (1996) identified primary effusion lymphoma (PEL) as an unusual subset of AIDS-related lymphomas that grow mainly in the body cavities as lymphomatous effusions without an identifiable contiguous tumor mass (Nador et al, 1996).PEL was previously referred to as Body Cavity Cell Lymphoma (BCBL). PEL presents as a diffuse large B-cell lymphoma with neoplastic cells that mainly proliferate within major body cavities as lymphomatous effusions without a detectable solid tumour (Wakely et al, 2002; Fan et al, 2005). PEL has also been identified in HIV negative patients (Said et al, 1996; Carbone et al, 1997; Codish et al, 2000; Cobo et al, 1999).

KSHV has been shown to play a causative role in the pathogenesis of PEL (Fan et al, 2005; Horenstein et al, 1997). In most cases of PEL co-infection by both KSHV and EBV[vi] are common (Horenstein et al, 1997; Mack et al, 2008). The role of EBV in the pathogenesis of some PELs is unclear however KSHV is always detected in PEL. (Carbone et al, 2010; Cobo et al, 1999; Jones et al, 1999; Brimo et al, 2007; Wies et al, 2008)

1.5.2.3 Multiple Myeloma

Multiple Myeloma (MM) is a cancer of the bone marrow caused by abnormal proliferation of plasma cells sometimes simultaneously at different sites. KSHV has been isolated in some MM samples (Brousset et al, 2000; Wies et al, 2008; Vescio et al, 2000; Yi et al, 1994). However, the association between KSHV and MM is very uncertain. The majority of studies have failed to detect KSHV DNA in bone marrow biopsies of patients diagnosed with MM (Cesarman et al, 1999; Olsen et al, 1995; Ablashi et al, 2000). Unlike KS, in most MM studies KSHV is detected in only a fraction of samples. A South African study successfully detected KSHV DNA sequences in 40% of bone marrow adherent cell cultures and in only 5% of bone marrow aspirates (Patel et al, 2001). Serologic detection of KSHV antibodies in MM samples has also been unsuccessful

(Ablashi et al, 2000). However, the link between KSHV as a causative agent of multiple myeloma has been disproved by multiple further studies (Pan et at, 2000, Olsen et al, 1998).

1.6 Changing Patterns of KS

Even though KS was discovered more than a century ago, prior to 1981 it was generally considered a globally rare and confined disease. In Africa, endemic KS was confined to East African countries such as Uganda and Tanzania and as well as in Cameroon and the Democratic Republic of Congo (DRC). Lower incidences were noted in the lower southern African regions, while in other African regions KS remained rarely reported. In the US and European populations, classic KS was confined to older males 40 – 70 years, similar to what was noted in the Jewish and Mediterranean populations. Iatrogenic-KS was also only common in immunosuppressed subjects, who had undergone organ transplants, but this was a small confined population and KS remained generally a rare disease in non-endemic areas.

Then, post 1981, after the appearance of AIDS, AIDS-KS became the most common cancer on the African continent. As Africa was widely affected by the HIV-AIDS epidemic, the incidence of KS increased drastically. KSalso became a problem in African countries such as Botswana, Ghana, Egypt and Gambia in which KS of any type previously been rarely seen. AIDS-KS was also to infiltrate the American and European homosexual, bisexual and needle drug abusing population. Currently, reports show that KS is now well contained in the American and European cases as fewer cases are now reported (Mocroft et al, 2004; Shiels et al, 2008), largely due to the introduction of highly active antiretroviral (HAART) therapy. However, in Africa and other affected developing countries, increased incidences of AIDS-KS are consistently reported in children, female and male adults. This section will discuss the changing patterns of KS in the two settings.

1.6.1 Epidemiology of Kaposi's sarcoma in African Countries

1.6.1.1 KS in Sub-Saharan African Countries

Before 1981, few articles described KS incidence in Southern Africa. In 1975, Macrae and Cook described KS as one of the rare cancers in Botswana. Cassidy and co-workers (1982) could not identify cases of iatrogenic KS in their review of malignant tumours in renal transplant patients admitted between 1967 and 1979 at one of the major hospitals in the Western Cape, South Africa. However, iatrogenic KS incidence was 5.4% with diagnosis in 3 of the 53 South African patients who had cardiac transplants and accounted for 30% of all diagnosed malignant cancers (Lanza et al, 1983). Several studies managed to trace few cases of classic and endemic KS between 1978 and 1990, in patients reported to have been treated for endemic KS in one of the largest hospitals++ in South Africa (Stein et al, 1994). During the pre-Aids era, KS was probably an unusual occurrence in these regions with no detrimental outcomes and could easily have been overlooked.

Parallel to the constantly growing HIV/AIDS epidemic in the southern African countries, AIDS-KS spread quickly. In South Africa, KS incidence was reported to have increased by up to 3 fold within an 8 year period post the HIV/AIDS era (1988 – 1996) (Sitas et al, 2001) and continued to rise in subsequent years. While standardized incidence rates of KS increased from <1 to 15:100,000 between 1990 and 2006 in the KwaZulu Natal province of South Africa, a shift in the peak age-specific incidence rates from the elderly population to the middle aged was also noted (Mosam et al, 2009). AIDS-KS in South African black and white populations also started to appear more frequently after the mid-1980s (Phillips et al, 1987; Becker et al, 1988; Glassman et al, 1995; Chetty et al, 1998; Chuck et al, 1996; Chetty et al, 1999). However, the rural areas within the Western Cape seemed to have a lower burden with KS incidences from 1998-2002, reported at 0.3 and 1.6 per 100 000 in females and males respectively (Somdyala et al, 2010).

AIDS-KS was also noted more commonly in South African children infected with HIV (Grant et al, 1999), proving to be more aggressive and sometimes fatal (Theron et al, 200; Marais et al, 2003). The clinical presentation of AIDS-KS showed a different pattern from the earlier types. AIDS-KS also became a common complication in South African oral health centres (Rudolph et al, 1999; Chetty et al, 1998; Hille et al, 2002; Lager et al, 2003; Coogan, 2005; Meer, 2006; Feller et al, 2006). Gastrointestinal (Chetty, 1999; Chetty et al, 2003) and pulmonary involvement were also frequent and sometimes terminal (Mosam et al, 2008; Ramdial et al, 2010; Cairncross et al, 2009). HIV was clearly associated with increased risk for KS in South African populations (Sitas et al, 2000; Stein et al, 2008).

Interest in KS grew and substantial reviews on AIDS-KS and non-AIDS-KS started appearing in other Southern African countries including Zimbabwe (Marks et al, 1995; Watts et al, 1997; Meditz et al, 2007; Lampinen et al, 2000; Chokunonga et al, 1999; Bassett et al, 1995; Malin et al, 1995), Botswana (Ansari et al , 2002; Cainelli et al, 2009; Whitby et al, 2004), Lesotho (Kamiru et al, 2002), Mozambique (Gamborino et al, 2000; Coras et al, 2004; Caterino-de-Araujo et al, 2009) and Namibia (Itula et al, 1997). KS incidence was also more noticeable in Zambian populations, with an increased recognition of an unusual non-endemic KS clinical pattern, which was non-responsive to treatment and usually led to the death of those affected, clearly fitting the multiple global descriptions of AIDS-KS (Bayley et al, 1984).

1.6.1.2 KS in Central African Countries

Endemic KS was present in Central Africa long before the beginning of the AIDS epidemic. Zaire was one of the most affected countries with cases reported as early as 1948. By 1957, endemic KS was noted in 9% of all cancer biopsies, while, within the year period 1969 -1983, KS accounted for 17% and 2% of all male and female cancer biopsies in the north eastern regions of Zaire (Gigase et al, 1984). AIDS-KS has been

noted in Zairian populations although it was thought to have not changed the KS incidences as it has in the neighbouring African countries (Coker et al, 1986; Oates et al, 1986). Ocular KS manifestations have also been described in HIV infected patients in Zaire (Kaimbo Wa et al, 1994). AIDS-KS is also noted as one of the major complications in Congolese patients (M'Pele et al, 1986; Ondzotto et al, 2004).

Even before the AIDS epidemic, endemic KS was common in Cameroon. In a review of cancers between 1967 and 1973, skin cancers contributed to 30% and 20% of all 2758 histologically diagnosed cancer cases, with KS contributing to 31% of all skin cancers (Jensen et al, 1978). The same study indicated that KS was 11 times more common in males than females (Jensen et al, 1978). In 2006, Cameroon experienced an increase in HIV related skin lesion incidence with 68% of HIV positive patients reported to have skin lesions and KS seen most commonly as a complication in advanced HIV/AIDS disease (Josephine et al, 2006).

Endemic KS remains common in the Central African Republic but is now complicated by the increase in AIDS-KS with a substantially low life expectancy in the AIDS-KS group (Laroche et al, 1986; Lesbordes et al, 1988; Bouquety et al, 1989). AIDS-KS is also noted as one of the ocular complications that may compromise visual acuity in affected patients in this region (Chen, 2007).

Pre the HIV/AIDS epidemic, endemic KS was more common in Central African males and children than any of the other African countries. This was highlighted in the review by Gigase and co-workers (1984), which led to conclusions that KS was more common in Central African countries and lowest in countries further from the equator (Gigase, 1984). Later, Cook-Mozaffari and co-workers (1988), agreed with this theory, describing KS epidemiology between 1975 and 1982 as occurring in extreme narrow belts of relatively high incidence stretching westward across Zaire to the coast of Cameroon and southward down the rift valley to Malawi. Soon after the discovery of HIV in 1981,

notable spikes of AIDS-KS were increasingly reported in other central African countries (Piot, 1984; van De, 1984; Coker, 1986; Otu, 1986). Unlike Endemic KS which progressed slowly clinically and was very responsive to treatment, and common in males (Stein, 1993; Stein, 1994); this AIDS-KS was common in both sexes, more aggressive, resistant to the usual treatment methods and related to death (Coker, 1986; Lesbordes, 1988; Onwubalili, 1988; Bouquety, 1989; Ansari, 2002).

1.6.1.3 KS in North African Countries

Non-AIDS-KS seems to be more prevalent in North African Countries than the common AIDS-KS currently seen in central and sub-Saharan African countries (Costa et al, 2005; Weigert et al, 2004; Reis-Filho et al, 2002; El Agroudy et al, 2003; Hussein et al, 2008; Ben Tekaya et al, 2001;). In 1975 and 2002, classic KS affecting oral sites was described in Portugal (Farman et al, 1975; Reis-Filho et al, 2002). In 2000, Callaco and co-workers described a case of orbital AIDS-KS describing it as a rare finding in Portugal (Collaco et al, 2000). Childhood KS also seems to be rare in these regions (Hussein et al, 2008). Development of iatrogenic KS was more common in black Africans (15.4%) than Caucasians (0.8%) in a group of renal transplant patients in a hospital in Portugal, suggesting associations with ethnicity (Weigert et al, 2004).

In Egyptian kidney transplant recipients, iatrogenic KS seems to be the most common malignancy sometimes leading to death (El Agroudy et al, 2003). Iatrogenic KS is also a common complication in Sudanese renal transplant patients (Sabeel et al, 2003), however, in general, KS is rare in children (Wahab et al, 1986).

Classic KS was shown to be more common in Jewish immigrants coming from Algeria, Morocco and Tunisia than Jews born in Israel (Iscovich et al, 1998). In a review of 91 KS patients presenting between 1978 and 1998 in Tunisia, only 2% and 4% presented with AIDS-KS and iatrogenic KS respectively, while the rest had classic KS (Ben Tekaya, 2001). AIDS-KS was diagnosed in 3% of Tunisian young women infected with HIV

(Zouiten et al, 2002). In Morocco AIDS-KS was diagnosed in 7% of 696 patients hospitalised with AIDS between 1987 and 1999 (Chakib et al, 2003). AIDS-KS is commonly noted as an initial presentation in affected patients in Spain (Azon et al, 1990) with 20% of those with HIV/AIDS presenting with KS (Galceran et al, 2007; Perez-Blazquez et al, 2008; Castilla et al, 1996).

1.6.1.4 KS in East African Countries

KS contributed between 1% and 8% of malignant cancers seen in East African missionary hospitals by 1969 (Burkitt et al, 1969). Between 1979 and 1994, KS was the third most common cancer in Kenyan children at 6.1% per 100 000 children (Makata et al, 1996). Between 1997 and 1999, KS cases increased with AIDS-KS overshadowing other forms of KS (Mwanda et al, 2005). In a study conducted in KS patients in Burundi, AIDS-KS was described as more common and more often fatal than the other forms of KS (Laroche et al, 1986). The severity of the clinical manifestation of AIDS-KS was also noted in other East African countries. Like other East African countries, Ethiopia is also affected by AIDS-KS (Getachew et al, 1997), although it seems to be confined to other areas within the region (Lindtjorn et al, 1987).

One of the earliest descriptions of KS was done in the late 1970s in Malawian populations (O'Connell et al, 1977). In the AIDS era, KS was described as one of the most common malignancies in Malawian children between 1985 and 1993 (Mukiibi et al, 1995; Sinfield et al, 2007) while, in a later study, it was shown to be the second most common cancer type in Malawian populations (Banda et al, 2001). KS incidences have also been reported to have increased drastically in Madagascar, including in children (Seraphin et al, 1984; Coulanges et al, 1984). In the periods just before the outbreak of war in Rwanda, HIV malignancies were noted to be at an increase with KS noted in 6% of those affected (Newton et al, 1996; Ngendahayo et al, 1989; Ngendahayo et al, 1989).

Like all the other East African countries, Uganda and Tanzania were also affected by the increase in AIDS-KS incidence (Craighead et al, 1988; Bakari et al, 1996; Wabinga et al, 1993; Ziegler et al, 1997). Overall, East African countries are drastically affected by the Aids epidemic, with recognition of increased and odd KS clinical manifestations. The AIDS-KS is more common in males, but women and children are also affected on a much larger scales than ever before. What is more worrying is the severity and sometimes fatal clinical course of the disease, leading to substantial reduction in the life expectancy of those affected, which seems to cripple the nation of its younger populations.

1.6.1.5 KS in West African Countries

In general KS remains fairly uncommon in West African countries. In the Ivory Coast KS was one of the least common cancers and occurred in 7.7% of cases reported in men and 2.1% in women (Echimane et al, 2000). While in Burkina Faso, KS was noted in 2.5% of all 1024 cancers diagnosed between 1992 and 1996 in a National Hospital (Barro-Traore et al, 2003).

However, AIDS-KS was reported as the most common cancer diagnosed in Nigerian patients (Asuquo et al, 2008), and is the sixth most common cause of AIDS related deaths (Sani et al, 2006). It remains more common in Nigerian males that females (Kagu et al, 2006) (Mbah et al, 2008). KS was diagnosed in 0.3% of Nigerian patients with HIV/AIDS diagnosed between 1992 and 1996 (Akinsete et al, 1998).

1.6.2 Epidemiology of Kaposi's sarcoma in non-African Countries

1.6.2.1 Mediterranean regions

The earliest descriptions of classic KS were done in middle aged men from the Mediterranean region and KS is very prevalent in this region. The age standardized incidence rates of classic KS were reported at 2.5 – 5.0/100000 and 0.7 – 2.8/100000 in

Northern Italian males and females between 1989 and 1998, respectively (Ascoli et al, 2001). Corresponding incidence rates were reported in Italian adult populations between 1998 and 2002 (Atzori et al, 2004). In addition in 2009 KS was reported to be the fourth most common complication in organ transplant patients in Spain (Marques et al, 2009; Garcia-Astudillo et al, 2006). AIDS KS has increased the KS burden in Mediterranean regions with cases reported in MSM and other heterosexual populations.

1.6.2.2 Kaposi's sarcoma in the United States, Europe, Australia and Asia

With the exception of the African continent and the Mediterranean regions, KS remains very rare worldwide. The earlier descriptions of KS in the American, European and Australian populations were of Classic-KS noted mainly in middle aged men of Mediterranean origin with the odd American case of endemic KS reported in African American males (Fusonie et al, 1967; Rothman et al, 1962; Laor et al, 1979). In Australia only 26 cases of KS were recorded by the New South Wales Central Cancer Registry between 1972 and 1982, with a total annual incidence of 0.47 per million (Kaldor et al, 1994). Higher incidences were reported in American populations during the same era, with 0.29.and 0.07 cases/100,000 per year reported in males and females, respectively (Biggar et al, 1984). Iatrogenic KS was also seen in organ transplant patients (Veness et al, 1999; Myers et al, 1974) with KS noted as one of the common complications following immunosuppressive therapy.

Following the AIDS epidemic the face of KS changed in these regions, with more and more cases of KS cases being diagnosed (Whyte et al, 1989; Elford et al, 1993; Boyle et al, 1993). In the general population KS remained rare. However, AIDS-KS plagued the homosexual population who were at a high risk for sexually transmitted infections including HIV (Haverkos et al, 1982; Soullard et al, 1982; Myskowski et al, 1982; Serraino et al, 1992; Casabona et al, 1990; Casabona et al, 1991; Simard et al, 2010; Elford et al, 1993). AIDS-KS became recognised as a more aggressive cancer with more

fatal outcomes than classic KS (Myskowski et al, 1982; Sears et al, 1983). AIDS KS also presented ocular anomalies (McCluskey et al, 1985).

Between 1981 and 1983, KS was diagnosed in 28% of the first reported cases of 1000 American homosexual with HIV/AIDS (Jaffe et al, 1983), and with time even more cases were reported (Franceschi et al, 1997).

Drastic increases in AIDS-KS were noted with more cases reported in European than American homosexual males (Franceschi et al, 1997). Overall the AIDS-KS incidence rates in European countries varied from 2.2 to 18.4 between 1987 and 1989, increasing to between 3.7 and 24.3 between 1990 and 1992 per million (Franceschi et al, 1997). The incidence rates started stabilizing thereafter with ranges between 3.1 and 25.2 between 1993 and 1994 per million (Franceschi et al, 1997). KS was more common in France, Italy, Germany, Spain, and the United Kingdom (Serraino et al, 1992; Franceschi et al, 1997).

Between 1987 and 1989, reported AIDS-KS cases were mainly white and 8 times higher in American white homosexual or bisexual men (10122 cases) than American blacks (1259 cases), (Franceschi et al, 1997). Drastic decreases were noted between 1993 and 1994 with 2338 KS cases reported in whites and 545 in blacks. AIDS-KS remained uncommon in American heterosexual men and women (Francesch et al, 1997). Decreases in Standardized incidence ratios (SIR[vii]) for AIDS-KS persisted between 1996 and 2004 (Simard et al, 2010). These constant declines in KS as an AIDS defining illness are being attributed to the successful introduction of HAART as an effective treatment for AIDS (Simard et al, 2010; Franceschi et al, 2010).

In Asian countries, KS has also increased following the AIDS epidemic (Misra et al, 1998), but the trends are reported seem to be lower than in European and American homosexual populations (Lanjewar et al, 2011). KS is noted in children, HIV positive

homosexuals and HIV uninfected patients in Taiwan (Chao et al, 1993; Lin et al, 1987; Lee and Lee, 1992). Although classic KS seems to be common in some areas within China, affecting mainly males, (Dilnur et al, 2001) oral KS manifestations are very rare in Chinese HIV infected patients (Tsang et al, 1999).

1.6.3 Treatment strategies for AIDS-KS

Where KS is associated with immunosuppression, the return of immunocompetence will lead to remission of the KS lesions in most patients with non-invasive disease (Monticelli et al, 2000; Murdaca et al, 2002). This pattern is noted most frequently in iatrogenic patients who have stopped treatment and have successfully recovered (Moosa et al, 1998). In AIDS patients, successful treatment with HAART in adhering patients has been shown to improve KS in affected patients (Bower et al, 2009; Martinez et al, 2006; Murdaca et al, 2002).

In their study Bihl and co-workers (2007), reported better clinical outcome of KS lesions in patients treated with combined HAART and chemotherapy than those who were on HAART only. Treatment of HIV/AIDS patients with HAART has been shown to decrease the incidence of KS (Ortega et al, 2009). KS incidence has also been shown to be lower in HAART patients, than newly diagnosed HIV infected patients who develop AIDS within a year of diagnosis (Dal Maso et al, 2009).

There are no clear recommendations for treatment of KS in the HIV/AIDS setting particularly in resource poor countries. While limited cutaneous disease will respond to HAART alone, when to add chemotherapy and which chemotherapy is most effective, is not clear. These questions will be addressed by AIDS Clinical Trials Group (ACTG) (A5263 and A5264) due to start in 2012 (Prof A P MacPhail – personal communication). However, combination chemotherapy (Vincristine and Bleomycin) and pegylated liposomal Doxorubicin are effective treatments for advanced KS while radiotherapy is

effective in treating localised cutaneous lesions (Dedicoat et al, 2003; Bodsworth et al, 2001).

1.7 Epidemiology of KSHV

The current global epidemiology pattern of KSHV clearly follows that of KS, with increased prevalence of KSHV reported in countries at high risk of KS and *vice-versa*. KSHV prevalence in the adult population varies widely between countries. The lowest prevalence of less than 5% is reported in the USA and European populations. The described trends show that while KSHV infection is infrequent in the general American and European populations, it is common in groups at increased risk for developing KS (Gao et al, 1996; Kedes et al, 1996; Simpson et al, 1996). In these regions, KS is rarely described in young children and heterosexual men and women and is elevated in people with high risk sexual behaviour, especially the homosexual and bisexual populations.

In contrast, KSHV prevalence is high in the Mediterranean and African regions where the infection is ubiquitous and has been described in children and adults across all age groups, using serological and molecular laboratory techniques (Kasolo et al, 1997, Malope et al, 2007). KSHV is very common in African regions where increased incidences of endemic Kaposi's sarcoma have been reported (Gao et al, 1996; Mayama et al, 1998; Olsen et al, 1998; Simpson et al, 1996) and also in African regions were HIV-AIDS associated related KS is now very common (Sitas et al, 1999a, Olsen et al, 1998).

The seroprevalence of KSHV, especially in African countries, has been shown to increase steadily from birth to adulthood. This suggests a continuous acquisition effect, which may involve circulation of the virus amongst people in close contact. Probable transmission of KSHV between family members, between mothers and their children and within clustering conditions seems very plausible. KSHV infection rates in these populations increase with increasing age.

1.7.1 Epidemiology of KSHV in African Countries

The African continent is greatly affected by HIV/AIDS, with the highest rates reported in the Southern African regions. This was followed by increases in KS, which has become one of the leading cancers within this region. The discovery of KSHV in 1994 and its confirmation as the necessary causative agent of KS, led to increased studies trying to understand the epidemiology, biology and pathology of this virus. The sub-Saharan region is mostly endemic for KSHV, with prevalences ranging from 2% in young children up to 87% or more in adults, varying by country and depending on population groups studied and the study settings (Butler et al, 2009; Dedicoat & Newton, 2003; Sitas et al, 1999; Dollard et al, 2010a; Caterino-de-Araujo et al, 2009). The African continent derives benefit from studies with larger sample sizes, which leads to statistical inferences that are comparable and add significance to studies with smaller sample sizes within the same regions.

1.7.1.1 KSHV in sub-Saharan African Countries

Several studies looking at the seroepidemiology of KSHV have been conducted in the sub-Saharan region. A KSHV seroprevalence of 35% was reported in 136 adult and paediatric patients at a hospital in the KwaZulu Natal province of South Africa and was not significantly different between females and males. The seroprevalence ranged from 38% in children less than 18 months of age to 63% in adults greater than 35 years of age (Wilkinson et al, 1999). Lower KSHV seroprevalence was reported in rural children in the same province, with overall seroprevalence of 14% reported in 556 children. The seroprevalence was higher in children of mothers with high KSHV antibody titers (29%), than if the mother had no KSHV antibodies detected (13%) [Odds ratio (OR), (2.6; 95% confidence interval (CI), 1.1-6.2)] (Klaskala et al, 2005).

Similar KSHV seroprevalences have been reported in the Gauteng province of South Africa. In a larger study of 2191 HIV negative cancer patients without KS admitted at

hospitals in the Gauteng province of South Africa, a seroprevalence of 39% was reported. The seroprevalence increased with increasing age (Wojcicki et al, 2004). Lower KSHV seroprevalences ranging from 8% to 9% were reported in 427 South African children aged less than 9 years but was not associated with age (Butler et al, 2009). More studies within the Gauteng province have reported increasing KSHV seroprevalence by age group (Malope et al 2008; Sitas et al, 1999; Sitas et al, 2001; Malope et al, 2007).

Higher KSHV seroprevalences have also been reported in Malawi, Mozambique, Zambia and Zimbabwe with the highest being reported in Botswana. Campbell and co-workers (2009), reported prevalences of 28% in 2750 males, with a lower prevalence (13%) reported in children (Dollard et al, 2010). The KSHV seroprevalence was 48% in Zambian pregnant women (Olsen et al, 1998; He et al, 1998) while another study reported prevalences of 40% in 3160 women attending antenatal clinics (Klaskala et al, 2005). Olsen et al, 1998 reported a seroprevalence of 58% in individuals 14 to 84 years of age (Olsen et al, 1998). While the youngest Zambian children have a high incidence rate of KSHV seroconversion with 13.8 infections per 100 child-years reported by the time they reach 48 months of age (Minhas et al, 2008).

Very high seroprevalences have been reported in black populations (76%) in Botswana and the San populations (87%) (Engels et al, 2000). In Malawian healthy volunteers, a KSHV seroprevalence of 54% was reported compared to 67% in hospitalised patients and was related to increasing age ($P<0.001$) (DeSantis et al, 2002). In Mozambique, variable KSHV seroprevalence ranging from 2% to 50% have been reported in adults (Caterino-de-Araujo et al, 2009 &, 2010 a & b; Caterino-de-Araujo et al, 2010). Obviously the above studies describe common sero-epidemiological KSHV infection patterns within the sub-Saharan regions. This is despite the use of different KSHV diagnostic laboratory methods. However, all studies described have used a combination of at least 2

laboratory methods that detect antigens to lytic and latent antigens, to increase specificity and sensitivity for defining KSHV detection.

1.7.1.2 KSHV in Central African Countries

There are very few studies describing the sero-epidemiology of KSHV in Central African populations. However, in a hospital based study, KSHV seems to be very common in Cameroon, ranging from prevalences of 40% reported in children 5-10 years increasing to up to 62% in adults 30 - 40 years of age (Rezza et al, 2000). High seroprevalence of human Herpesvirus-8 are also reported in pregnant women and prostitutes in Cameroon (Bestetti et al, 1998) .The high KSHV prevalences is also confirmed by another study in the same country which reported an overall prevalences of 27.5% in children and adolescents with peak incidences of 48% after 15 years of age, similar to those of pregnant women within the region (Gessain et al, 1999).

1.7.1.3 KSHV in North African Countries

Varying KSHV seroprevalences have also been described in North African countries. KSHV seroprevalence is very high in Egyptian children with antibodies being detected in up to 42% of children up to 4 years of age (Andreoni et al, 2002), and more than 50% in children older than 6 years (Andreoni et al, 1999). Lower prevalences are reported in Tunisian populations. KSHV seroprevalences ranging between 12% and 14% have been reported in Tunisian children, pregnant women and blood donors respectively (Hannachi et al, 2011). Another study in Tunisia reported significantly high KSHV seroprevalence in schizophrenic patients compared to healthy controls at 28.7% and 14.8%, respectively (Kissi et al, 2011).

1.7.1.4 KSHV in East and West African Countries

KSHV infection rate of 43% have been reported among 1061 Kenyan men working in the trucking industry (Baeten et al, 2002) and was similar to the seroprevalence reported in

female prostitutes in the same country (Lavreys et al, 2003). KSHV Lana antibodies were detected in 26% of Ethiopian immigrants residing in Israel (Margalith et al, 2003) and in 53% of pregnant women attending antenatal clinics in Ethiopia (Lemma et al, 2009). The overall seroprevalence of KSHV was 30.7% (96/313) in 2 Tanzanian hospitals and was more than 3 fold higher in one hospital compared to the other (14.4% in Tosamaganga *vs*. 46.3% in Pemba Island (Meschi et al, 2010).

KSHV seroprevalence is higher in children and adolescents in Uganda (Wawer et al, 2001; Mayama et al, 1998), with seroprevalences ranging from 9% to 36%. High seroprevalences are reported in Ugandan children, adults and blood donors (Butler et al, 2009; Hladik et al, 2003). In Ugandan, general and clinic population based studies showed a KSHV seroprevalence increase from about 9% in children 2 years of age to 36% in children 2 to 8 years age (P(trend) < .001) (Butler et al, 2009). In another study, which compared KSHV seroprevalences in 2375 Zimbabwean, South African and Ugandan population groups, Ugandans were 2- and 3 times more likely to be infected by KSHV than ZImbabweans and South Africans, respectively (Dollard et al, 2010). In another study high prevalence (74%) of KSHV antibodies was reported in 114 HIV-negative Ugandan blood donors, (Kakoola et al, 2001).

In West Africa, high KSHV prevalences of up to 42% have been described in Zambian populations (Klaskala et al, 2005). In a study in pregnant women from Burkina Faso, seroprevalences of 10% to 12% were described (Ilboudo et al, 2007 & 2009). KSHV seroprevalence was 23% in Ghananian HIV negative blood donors, but was higher (66%) in HIV infected individuals (Adjei et al, 2008).

1.7.2 Epidemiology of KSHV in non-African Countries

KSHV seems to be a common infection even in countries where a low KS prevalence is reported. In America, KSHV seroprevalences ranging between 3% and 15% have been reported in blood donors (Cannon et al, 2009; Baillargeon et al, 2001), with a similar

seroprevalence reported amongst transfusion recipients and surgical control patients (Qu et al, 2010; Engels et al, 2007).

KSHV infection has also been detected in children in America and Europe. In one study prevalence as low as 1% was reported in 4166 American children aged between 6 and 17 years of age (Anderson et al, 2008). However, Baillargeon et al, 2002 showed that at least 1:4 (26%) south Texan American children are infected. KSHV seroprevalence of 12.5% has also been reported in European children (Viviano et al, 2009).

In some Asian countries KSHV seroprevalences are comparable to those in sub-Saharan African regions. KSHV seroprevalences varied from 13% to 48% amongst four regions in China within the same district of Xinjiang which is in the north-western area of China (Du et al, 2000; Dilnur et al, 2001) and was associated with increasing age (Fu et al, 2009). A prevalence of 4% has been reported in Saudi Arabia (Almuneef et al, 2001).

1.8 Transmission patterns of KSHV

KSHV infection is reported in varying geographic locations and seroprevalence scales worldwide. There is general agreement that transmission is via saliva in all groups and populations. Sexual risk factors in MSM are probably markers for saliva exposure. The way in which this virus is acquired is still not clear, with different opinions coming from countries of low KS prevalence as well as those in which KS and KSHV infections are common in the general populations. In the countries were KSHV infection is not common and KS is confined to the homosexual and bisexual populations, there is a strong existing principle that the virus is sexually transmitted (Kedes et al, 1996; Martin et al, 1998). To support this concept, several studies in these regions and globally have reported strong associations between KSHV and high risk sexual behaviour, heterosexual partners of infected individuals, sexually transmitted infections and HIV

(Rezza et al, 1998; Sosa et al, 1998; Martin et al, 1998; Dupuy et al, 2009; Engels et al, 2007)

In African and Mediterranean regions, where KSHV infection and KS are common, it appears that the virus may not be acquired via sexual routes. Non sexual transmission patterns can be the only explanation for increased KSHV infections noted in children 18 months of age increasing steadily up to teenage years (Mayama et al, 1998; Mbulaiteye et al, 2005 & 2008; Whitby et al, 2000). In very young children, it is clear that sexual routes cannot be implicated in any way as the mode of KSHV infection. Obviously, non-sexual routes in which KSHV infection is acquired exist. Implicated routes for the non-sexual routes of KSHV transmission to children include: vertical and horizontal transmission from an infected mother to her children, intra and extra familial person-to-person transmission, clustering, water sources and oral transmission (Mbulaiteye et al, 2004 & 2005; Whitby et al, 2000). Transmission through oral body fluids such as saliva has been shown as possible risk factors, with insignificant detection rates in colostrum and breastmilk (Brayfield, 2004).

Uncertainty on the route of transmission of KSHV infection within these high KS regions is raised when the sexually active populations are considered. As in America, Australia and Europe, several studies conducted in African and Mediterranean adult heterosexual populations support the sexual transmission routes. On the other hand contrasting studies conducted in adult heterosexual populations have failed to show any association between KSHV infection and sexually transmitted infections and high risk sexual behaviours. With recent studies continuing to report contrasting information, it remains uncertain whether KSHV is a sexually transmitted infection or not.

1.8.1 Is KSHV sexually transmitted?

The transmission patterns of KSHV remain uncertain and are part of a continuing debate. In Australia, Europe and America, it has been postulated that KSHV is

transmitted sexually (Elford et al, 1993; Engels et al, 2007). The idea that KS is caused by a sexually transmitted infection was suspected even before KSHV was discovered (Beral et al, 1990; Elford et al, 1993). These suggestions were based on the fact that, in these regions, KS remains rare in the general population but is very common in those who are HIV positive. This group has been repeatedly shown to take part in high risk sexual behaviour that exposes them to an increased risk of acquiring STIs and HIV infection. However, in these low risk countries, KSHV infections have been reported in heterosexual males and females.

In support this , some studies have shown that antibodies to KSHV were more frequently detected in men who have sex with men and STD clinic attendees than in the general population and blood donors (Gambus et al, 2001; Regamey et al, 1998; Simpson et al, 1996). The risk for KSHV infection has been shown to correlate with an increasing number of both homosexual and heterosexual partners and HIV infection (Renwick et al, 2002; Goudsmit et al, 2000; Sitas, 1999). KSHV infection was also more likely to be detected in populations with high risk sexual behaviour, such as homosexual males (Regamey et al, 1998) and female and male sex workers (de Sanjose et al, 2002; Perna et al, 2000; Tedeschiet al, 2000; Nawar et al, 2005). Furthermore, before the discovery of KSHV, an increased risk for KS infection was reported in women who had sexual contacts with bisexual males than those who had sexual contact with low risk individuals (Beral et al, 1990 & 1992). This led to assumptions that the causative agent for KSHV may be transmitted sexually. Kouri and co-workers reported identical KSHV strains in sexual partners claiming to have been in monogamous relationships for at least one year (Kouri et al 2007). The elevated prevalence of KSHV infection in MSM, led to suggestions that KSHV may be transmitted preferentially through oro-anal sexual contact (Casper et al, 2006; Beral et al, 1992). However, evidence for transmission of KSHV among MSM via specific sexual practices is lacking.

KSHV DNA has been detected in semen from KSHV infected individuals, but the detection rate is variable, (Belec et al, 1998; Howard et al, 1997; Viviano et al, 1997 Bagasra et al, 2005; LaDuca et al, 1998), it may even lead to suggestions of possible contamination. Low detection rates were also reported from genital tract samples (Calabrò et al, 1999; Whitby et al, 1999; Lampinen, 2000), which may also suggest possible contamination. However, other studies failed to detect any KSHV DNA in these samples. KSHV DNA has been detected more commonly in samples from the prostate gland (Corbellino et al, 1996; Montgomery, 2006).

On the other hand, there are studies in African and Mediterranean countries which have also reported associations between KSHV infection, sexually transmitted infections and sexual behavioural factors. Some of the studies clearly show an increase in KSHV infections in people with high risk sexual behaviour, such as prostitutes, homosexual and bisexual males compared to low risk populations (Lavreys et al, 2003; Eltom et al, 2002), supporting the suggestion for a sexual transmission route. However, evidence is conflicting and information agreeing or disagreeing with all the factors mentioned above continue to emerge. Clearly more research studies are required to provide reasonable evidence that will help find the exact routes of KSHV infection.

1.8.2 Is KSHV non-sexually transmitted?

Stronger contrasting information pointing to non-sexual routes of transmission of KSHV exists in countries where KSHV is common such as the African and Mediterranean regions. In these regions, KS occurs in people of all age groups, with seroprevalence increasing with increasing age from very young children to the elderly (Mayama et al, 1998). KS and KSHV infection are also common in very young children (Sarmati et al, 2004; Whitby et al, 2000). Moreover, associations between KSHV have been reported between mothers and their biological children and other people such as siblings and

relatives who are in frequent contact with the children (Mbulaiteye et al, 2006; Sitas et al, 1999; Dedicoat et al, 2004).

Lately, emerging studies conducted on American and European populations report KSHV infection in very young children who are not yet sexually active (Anderson et al, 2008; Baillargeon et al, 2002). Detection of KSHV antibodies in children from south Texas, America, particularly among those under the age of 12 years, indicates that non-sexual transmission of this virus is likely to occur among this population (Baillargeon et al, 2002). Although the notion that KSHV is sexually transmitted in these regions still persists, emerging biological, behavioural and environmental factors continue to throw doubt on this (Whitby et al, 2007; Mbulaiteye et al, 2005; Mayama et al, 1998). Future investigations involving larger study samples will be necessary to develop an understanding of specific routes and risk factors of HHV-8 transmission among children with low KSHV infection rates.

KSHV has been detected in up to 80% of salivary samples of KS or KSHV infected individuals (de Souza et al, 2007; Andreoni et al, 2002; Vieira et al, 1997). KSHV prevalences were reported to be higher in children of mothers with KSHV detected in their saliva (Dedicoat et al, 2004) and salivary exposure leading to possible person to person transmission within a household has also been implicated (Mbulaiteye, 2004 & 2006). Butler and co-workers, reported different practices in parents, relatives and or caregivers that expose African children to saliva, (Butler et al, 2011). The practices include pre-mastication of food, sharing of sweets and pre-mastication of medicinal plants given to the child. Breast milk is not implicated as a possible source of KSHV infection to children (Brayfield et al, 2004).

Of significance, and pointing to non-sexual transmission routes, are some studies looking at sexual practices amongst men who have sex with men. These studies have suggested that salivary exposure during oro-anal or oro-genital sex may be the possible

route of KSHV acquisition in these high risk groups (Grulich et al, 1997; Bagni and Whitby, 2009). In a study of 293 MSM, 87% reported ever applying saliva as a lubricant during anal intercourse (Butler et al, 2009). Saliva is regarded as the most important source of KSHV infection rather than semen or stool in sexual transmission (LaDuca et al, 1998).

In contrast to the above findings, there are studies in Africa which have shown no association between KSHV and sexual behaviour, sexually transmitted infection and HIV infection. A Zimbabwean study reported in 2009 showed no association between KSHV and the number of recent sexual partners, condom use, prior sexually transmitted infections, prostitution, chronic hepatitis B infection, or incident HIV-1 infection (Campbell et al, 2009). In the same study, marital status was not associated with KSHV infection, with KSHV seropositivity in wives not associated to seropositivity in husbands (Campbell et al, 2009).

1.8.3 KSHV epidemiology and HIV

HIV is a sexually transmitted infection and is clearly associated with other STIs. Immunosuppression due to HIV is certainly the driving force behind the global increase in KS incidence. HIV complicates the overall picture of KSHV transmission as it may contribute to many factors that confuse the inferences about KSHV infection. It is important to understand the role that HIV plays in KSHV epidemiology and pathogenesis and how immunosuppression affects the acquisition of KSHV infection.

KSHV infection has been reported to be higher in individuals with HIV infection than in HIV negative groups (Chatlynne & Ablashi, 1999; Adjei et al 2008; Larocca, 2005). HIV type-1 infection has also been reported to increase the risk of development of Kaposi's sarcoma (Varthakavi et al, 2002). In one study conducted amongst immunosuppressed individuals, the risk of KS was significantly higher in those with HIV-1 infection than among those with other types of immunosuppression. This suggested a direct action of

HIV-1 on KSHV replication and therefore in the onset of KS in co-infected individuals (Merat et al, 2002).

1.9 Aim of this thesis

KSHV was confirmed as the causative agent of Kaposi's sarcoma (KS) soon after its discovery in 1994. However, the challenge remained to establish molecular and immunological laboratory techniques that can routinely be used to unravel the biological, pathological and epidemiological properties of this virus. The studies in this thesis aimed to add knowledge to the understanding of the epidemiological patterns of KSHV in the South African populations that may help describe the probable seroepidemiology and transmission patterns of KSHV in the populations studied.

To achieve this, three epidemiological studies were planned. For the study outcomes to provide statistically significant inferences, large numbers of samples were required. Thus, because the studies aimed to understand the general seroepidemiology of KSHV and the modes of transmission in the South African populations, we identified suitable previous local cross-sectional studies with available information and samples stored. The studies looked at sexually transmitted infections, HIV, information on sexual risk behaviour and had available sociodemographic information from participants. Permission was sought to utilize information and samples from these retrospective studies, which had already collected sera, sociodemographic information and other comparable information.

Permission was obtained from the South African Department of Health, Gauteng province, to utilise sera and information collected in 2001 for the estimation of seroprevalence of HIV and sexually transmitted infections (STIs) in women attending antenatal clinics. Permission was also sought from the Mothusimplilo project in which house to house and room to room (in mining hostels) interviews were conducted and

sera obtained from various Carletonville community groups. The study aimed to estimate the seroprevalence of HIV and STIs in community groups and to determine associated socio-economic and behavioural risk factors. The other study involved obtaining permission to collect available samples from the National Health Laboratory Service (NHLS), Department of Human Genetics. The department collects samples from mothers and their children undergoing paternity disputes; these samples were collected prospectively.

To maintain confidentiality, all available samples and information from the three studies were anonymously unlinked, relabelled and could, therefore, not be traced to any of the subjects in any of the three studies. Ethical Clearance to conduct the studies was obtained from University of the Witwatersrand Committee for Research on Human Subjects (Medical) (Appendices A – D). For convenience, the proposed studies were named to relate to the original study and are now referred to as follows:

i. The Antenatal Clinics KSHV Sero-epidemiology Study
ii. The Mother to Child KSHV Sero-epidemiology Study
iii. The Carletonville Community KSHV Sero-epidemiology Study

Although conducted in different populations groups, these three studies explore a major hypothesis – describing the sero-epidemiology of KSHV in South African populations. The studies were designed to answer complementary objectives. Each study has additional specific objectives that could not be attempted in the other complementary studies. For this purpose only the broader aims of this thesis are provided in this section. The direct objectives related to each study are described separately in chapters 4, 5 and 6.

The studies aim to provide information on:

i. The sero-epidemiology of KSHV in South African children and adults.

ii. The association between KSHV seropositivity and socio-economic status.

iii. The association between KSHV seropositivity and HIV infection.

iv. The association between KSHV seropositivity and other sexually transmitted infections.

v. The association between KSHV seropositivity in children and their biological mothers.

vi. The differences in the seroprevalence of KSHV amongst different community groups grouped by gender and risk for HIV or other sexually transmitted infections.

vii. To identify risk factors of KSHV seropositivity in the different community groups.

2 Materials and Methods

As a KSHV serology method was not yet available in South Africa, KSHV testing was done in the United States, at the Viral Oncology Section, AIDS and Cancer Virus Program, Science Applications International Corporation (SAIC-Frederick), National Cancer Institute (NCI-Frederick), Frederick, Maryland, USA. The researcher obtained a 3 month travelling grant to visit the Viral Oncology Section at NCI-Frederick and was trained on the laboratory techniques used.

Serum samples were stored at -20˚C and shipped on dry ice to the United States for KSHV antibody testing. As described in *Chapter 1: Section 1.4.3,* the recommendation that at least two or more assays, one detecting antibodies to lytic and the other to latent KSHV infections, was followed to describe the sero-epidemiology of KSHV in populations (Mbisa et al, 2010; Rabkin et al, 1998; Spira, 2000 et al; Zhu et al, 1999). Two in-house enzyme-linked immunoassays (ELISAs) for lytic and latent KSHV antibodies (lytic KSHV K8.1 glycoprotein and latent KSHV ORF73) were performed on each sample. These methods are described separately in sections 2.1 and 2.2 below.

Except for the “Mother to Child KSHV Sero-epidemiology Study” which also required testing for HIV, both the “Antenatal Clinics KSHV Seroepidemiology” and the “Carletonville Community KSHV Sero-epidemiology” studies already had information on HIV and other sexually transmitted diseases. HIV testing was done locally in South Africa at the Contract Laboratory Services (CLS) and the laboratory methods used are described in section 2.3 below.

2.1 Protocol for the lytic KSHV K8.1 ELISA assay

2.1.1 Reagents for manufacturing the lytic KSHV K8.1 ELISA plates

- Coating buffer:
    - 0.005 carbonate/bicarbonate buffer @ pH 10.0

- Blocking, conjugate and sample diluents:
    - 2.5 % BSA,
    - 2.5 % NGS,
    - 0.005 % Tween 20
    - 0.005 Triton X-100 in PBS
- 5 % BSA in 1x PBS:
    - BSA stock solution diluted 1: 6 in 1x PBS (Sigma chemical, # A-7284)
- Wash solution:
    - PBS with 0.05 % Tween 20, preserved with 0.1 % chloroacetamide
- Plates:
    - Non - sterile Dynex HBX4 plates (96 wells)
- Isolated KSHV K8.1 – (prepared by the NCI, Frederick, Maryland, USA)
- Preparation of the lytic KSHV K8.1 ELISA plates
    - The plates for the lytic K8.1 assay were prepared over a period of two days:

Day 1 – Coating phase

- KSHV K8.1 was diluted at 1:5, 000 in 0.005 M carbonate/bicarbonate buffer, pH 10.0.
- 100 µl of the diluted K8.1 was added to each well of the 96 – well microtiter plate.
- Each completed plate was covered with a plate sealer and incubated overnight at 4℃.

Day 2 – Blocking phase

- The next morning, the plates were removed from the refrigerator and the seals removed.
- Using the automatic plate washer, the plates were washed 3x with at least 350µl of the wash solution per well. The washed plate were inverted and tapped dry on a clean paper towel.

- Thereafter 280µl of the blocking solution was added.
- The plates were then covered with a plate sealer and incubated for three hours at 37˚C.
- After three hours the plates were transferred to a - 80℃ freezer and stored until needed

2.1.2 Reagents for the lytic KSHV K8.1 ELISA assay procedure

- Blocking, conjugate and sample diluents:
  - 2.5 % BSA,
  - 2.5 % NGS,
  - 0.005 % Tween 20
  - 0.005 % Triton X-100 in PBS
- 5 % BSA in 1x PBS:
  - BSA stock solution diluted 1: 6 in 1x PBS (Sigma chemical, # A-7284)
- Wash solution:
  - PBS with 0.05 %Tween 20, preserved with 0.1 % chloroacetamide.
- Conjugate:
  - Goat anti human IgG – AP (Roche # 605 – 480)
- Stop Solution:
  - 3N NaOH
  - 10 % Diethanolamine substrate buffer @ pH 9.8 (1L): the following were added in a 1L bottle
  - 800 ml ddH2O
  - 97 ml diethanolamine
  - 200 mg NaN3
  - 100 mg Mg – 6 H2O
  - The pH was adjusted to 9.8, the volume brought to 1L with ddH20 and the solution stored in a dark bottle at 4˚C.
- Substrate Solution: 1mg/ml Para-nitrophenylphosphate (PNP):

- The PNP tablets were dissolved in diethylamine buffer 15 – 20 minutes prior to use as follows:
    - 1 tablet per 5ml for 5mg tablet or
    - 1 tablet per 40 ml for 40 mg tablet

2.1.3 Procedure for the lytic KSHV K8.1 ELISA assay

- The K8.1 plates were thawed in a 37˚C incubator until at room temperature
- The plates were washed 3x with at least 350 µl of wash solution per well, using an automatic plate washer. The washed plates were inverted and tapped dry on a clean paper towel.
- Thereafter 50µl of the sample diluents was added to the plate a
- The serum samples were diluted as follows:
- 1:10 samples in sample diluent in the master plate
- Re - diluted 1:2 to make a final dilution of 1:20.
- Each plate was covered with a plate sealer and incubated for 90 minutes at 37℃.
- The plates were taken out of the incubator and the seals removed.
- The plates were washed 5 times with 350µl of wash solution per well using an automatic plate washer.
- The plates were tapped dry on clean paper towels.
- The goat antihuman IgG-AP was diluted at 1:3000 in conjugate buffer.
- 100µl of the diluted goat antihuman IgG-AP was added to the appropriate wells
- Each plate was covered with a plate sealer and incubated for 30 minutes at 37˚C.
- The last two steps were repeated.
- This was followed by addition of 100ul of substrate solution to each well.
- Each plate was covered with a plate sealer and incubated for another 30 minutes at 37˚C.
- Lastly, 50µl of stop solution was added to each well
- The plates were read at 405nm with blanks on wells A1and H1.
- The cut-off value of the assay was calculated as an average of negative controls x 0.750.

2.2 Protocol for the latent KSHV Orf73 ELISA assay

2.2.1 Reagents for manufacturing the latent Orf73 ELISA plates

- Coating Buffer:
    - 1X PBS, pH 10.
    - Blocking, conjugate and sample dilution buffer:
    - 2.5% BSA,
    - 2.5% NGS,
    - 0.005% Tween 20,
    - 0.005% Triton X-100 in PBS.
- 5% BSA in 1X PBS:
    - 30% BSA stock solution diluted 1:6 in 1 x PBS (Sigma Chemical, #A-7284).
- Wash Solution:
    - PBS with 0.05% Tween 20. Preserved with 0.1% chloroacetamide.
- Isolated KSHV ORF73 – (prepared by the NCI, Frederick, Maryland, USA)
- Plates:
    - Non-sterile Dynex HB x 4 plates (96 wells).
    - Preparation of the latent KSHV Orf73 ELISA plates

Day 1 –Coating Phase

- ORF73 protein was diluted 1:500 in 1M PBS buffer, pH 10.0.
- each plate was covered with a plate sealer and incubated overnight at 4°C.

Day 2 – Blocking phase

- The plates were washed 3x with at least 350µl of wash solution per well, using an automatic plate washer, inverted, tapped and left to dry on paper towels.
- 280ul of blocking solution was added to each well.
- Each plate was covered with a plate sealer and incubated for 3 hours at 37°C.
- Plates transfered to a –80°C freezer and stored until needed.

2.2.2 Reagents for the latent KSHV Orf73 ELISA assay procedure

- Blocking, conjugate and sample dilution buffer:
    - 2.5% BSA
    - 2.5% NGS
    - 0.005% Tween 20
    - 0.005% Triton X-100 in PBS*
- 5% BSA in 1X PBS:
    - 30% BSA stock solution diluted 1:6 in 1X PBS (Sigma Chemical, #A-7284).
- Wash Solution:
    - PBS with 0.05% Tween 20. Preserved with 0.1% chloroacetamide.
- Conjugate:
    - Goat anti-Human IgG-AP (Roche #605-480).
- Substrate:
    - 1-stepTM PNPP (Pierce Biotechnologies #37621)
- Stop Solution:
    - 3N NaOH.

2.2.3 Procedure for the latent KSHV Orf73 ELISA assay

- ORF73 coated plates were thawed in a 37°C incubator until at least room temperature.
- Plates were washed 3x with at least 350µl of wash solution per well, using an automatic plate washer, inverted, tapped and left to dry on paper towels.

- A 1:100 dilution of each test sample and control was prepared.
- 100µl of the diluted samples & controls was added to the appropriate wells in the plate.
- Each plate was covered with a plate sealer and incubated for 3 hours at 37°C.

  .
- The plates were washed 5x with 350µl of wash solution per well using an automatic plate washer, inverted, tapped and left to dry on paper towels.

  .

- Goat anti-Human IgG-AP was diluted 1:3000 in conjugate buffer.
- 100µl of diluted goat anti-human antibody was added to each well.
- Each plate was covered with a plate sealer and incubated for 30 minutes at 37°C.
- The last two steps above for the goat anti-Human IgG-AP step above were repeated.
- 100µl of 1-step PNPP Substrate Solution was added to each well.
- Each plate was covered with a plate sealer and incubated for 30 minutes at room temperature in the dark.
- 50ul of Stop Solution was added to each well.
- An automated ELISA plate reader was used to read the plates at 405nm, blanking on wells A1 and H1.

2.2.4 Quality Control and standardization for KSHV assays

For each study and for both the lytic and latent KSHV assays, additional quality control measures were included because of the large sample sizes. The studies were tested separately using similar standardized procedures. Three positive and 3 negative standard controls were included on each testing plate and a titration of positive controls was also included for each batch of 6 plates.

As the assays were developed and standardized in the US, additional controls were included to standardise the assays for South African populations. To achieve this, blood samples were collected from South African subjects with clinically confirmed KS. Sera from these subjects were previously tested and confirmed to be KSHV-seropositive by a latent KSHV ORF73 immunofluorescence assay. Sera obtained from KS patients in South Africa were also shipped and tested as additional controls within the analysed KSHV batches. Overall, 510 negative and positive controls were used for the studies. Blood samples were also obtained form healthy volunteers at the NHLS laboratory workplace sent to the US to be used for calibration of KSHV serological assays. Overrall, blodd samples were obtained from 52 volunteers. Approval and ethical clearance to collect

these samples was obtained from University of the Witwatersrand Committee for Research on Human Subjects (Medical) (Protocol Number - M02-10-18) (Appendix D).

2.2.5 Cut-off Points for KSHV Assays

No standardised cut-off points for either the lytic K8.1 or LANA ORF73 were available. The cut-off points for each assay were previously defined using a panel of well-characterised samples from patients with AIDS and patients with classic KS, MSM, HIV-infected subjects with haemophilia, and blood donors. Cut-off points for each assay were adjusted to take account of plate-to-plate and day-to-day variation by including variations observed in the positive or negative controls in the calculation. Calculations for cut-offs are indicated after each assay description below.

The laboratory cut-off points for the lytic K8.1 and LANA ORF73 assays were calculated for each plate, taking into consideration the plate to plate and day-to-day variations of the assays. Thus, the cut-off points for each assay were calculated on a day to day basis and per plate from the performance of the negative and positive controls.

Table 2-1: Cut-off points for the KSHV lytic K8.1 and LANA ORF73 assays in the paternity study.

Control samples (n)	Lytic K8.1 Mean (±SD)	Lana Orf73 - Mean (±SD)
Negative (255)	**0.08 (0.03)***	0.02 (0.01)
Positive (255)	2.56 (0.25)	**2.14 (0.30)****
All (510)	1.32(1.25)	1.08(1.09)
Cut-off Points Used For The Assays		
Laboratory	0.83 (0.03)	0.43 (0.06)
Mixture Model		
Children	0. 80	0.40
Mothers	0.95	0.43

* Used to calculate the cut-off points for the lytic K8.1 assay.
** Used to calculate the cut-off points for the LANA orf73 assay

Table 2.1 shows the overall mean (±SD) for all the 510 (255 negative and 255 positive) controls used for the lytic K8.1 and LANA ORF73 assays. The laboratory cut-off points for the LANA ORF73 assay were defined as the mean of the negative controls (MNC) per plate plus 0.75 i.e. K8.1 cut-off = K8.1-OD MNC + 0.75. Using the formula for the pooled laboratory mean (±SD) for the lytic K8.1 assay was 0.83 (0.03).

The cut-off for the lytic K8.1 assay was defined as the mean of the positive controls (MPC) per plate divided by 5 (or multiplied by 0.2) i.e. Orf73 cut-off = Orf73-OD MPC ÷ 5). Using this formula, the calculated mean (±SD) for the latent KSHV assay was 0.43 (0.06) (Table 2.1). Simple calculation of the cut-offs for the lytic K8.1 = MNC + 0.75 = 0.08 + 0.75 = 0.83 and for the LANA Orf73 **=** MPC ÷ 5 = 2.14 ÷ 5 = 0.43.

Taking the above result into consideration, another set of cut-off points were calculated using the mixture statistical model as explained by Pfeiffer and co-workers in 2000. Although the method was developed for determining cut-off for H.pylori, it was adapted to fit the KSHV lytic K8.1 and LANA Orf73 assays. Using this method, a new set of cut-off points was deduced, with separate cut-off points for mothers and children. The new higher lytic K8.1 cut-off points of *0.95 vs. 0.83* for the mothers and a lower cut-off point of *0.80 vs. 0.83* for children were established. For the LANA Orf73 the new cut-off point of *0.43* vs. 0.43 was repeated for the mothers and lower cut-off 0.40 *vs. 0.43* for children.

The laboratory cut-off points were used as major cut-off points as they exclude possible plate to plate and day to day variations by taking this measure into account. The mixture model may be used for comparisons between the two tests and measures of agreement were made. Two assays were used to define the KSHV status of the study subjects, the lytic K8.1 and the LANA Orf73 assays. The results for each assay were interpreted separately for the lytic K8.1 only and the LANA Orf73 only. A third variable that took into consideration the subjects who are either seropositive for lytic K8.1 or LANA Orf73 was calculated and was used as an indicator of overall KSHV status.

2.3 Protocol for the HIV Testing

2.3.1 Principle of the IMX HIV-1/HIV-2 III Plus assay procedure

The IMx® HIV-1/HIV-2 III PLUS kit was used for detection of the HIV status of the participants in the "Mother to Child KSHV Sero-epidemiology Study". HIV Testing was done at the Contract Research Laboratory (CLS), of the NHLS, South Africa. HIV antibodies were determined using an Abbot Axysm System for HIV-1/HIV-2 ELISA assays (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois). The assay applies the Microplate Enzyme Immunoassay (MEIA) method for qualitative detection of antibodies to human immunodeficiency viruses type 1 and or type 2 (HIV-1 /HIV-2) in human serum or plasma. The assay does not differentiate between HIV-1 and or HIV-2 antibody reactivity.

2.3.2 Reagents for the IMX HIV-1/HIV-2 III Plus assay

- IMX HIV-1/HIV-2 III Plus reagent pack consists of

    - 7.8 ml bottle of HIV-1/HIV-2 antigen (E.coli, B megaterium recombinants) coated micro particles in 0.1 M sodium chloride in TRIS buffer with protein stabilizers. Minimum concentration: 0.005 % solids. Preserved with sodium azide. (Reagent Bottle 1)
    - 5.3 ml bottle of anti-Biotin (rabbit): alkaline phosphatase conjugate in 0.5 M sodium chloride in TRIS Buffer with protein stabilizers. Minimum Concentration: 0.03µg/ml, preserved with sodium azide. (Reagent Bottle 2).
    - 10 ml bottle of 4 methylumbelliferyl phosphate in buffer. Minimum concentration: 1.2 mM. Preserved with sodium azide. (Reagent Bottle 3).
    - 7.8 ml bottle of biotinylated HIV-1/HIV-2 (E.coli, recombinants and synthetic peptides) antigens in Tris buffer with protein stabilisers. Minimum concentration: 100ng/ml. Preserved with sodium azide. (Reagent Bottle 4).
    - IMX HIV-1/HIV-2 III Mode Calibrator
    - 7.5 % ml bottle of Imx HIV-1/HIV-2 plus mode calibrator, consisting of recalcified human plasma non-reactive for HbsAg, anti- HCV, anti HBs, and anti-HIV-1/HIV-2. Preserved with sodium azide.
    - IMX HIV-1/HIV-2 III Plus controls: negative control, HIV-1 Positive Control and HIV-2 positive control

- Three 9ml bottles of Imx HIV-1/HIV-2 Plus controls prepared with recalcified human plasma. Preserved with sodium azide.
- The HIV-1 positive control was inactivated and recalcified human plasma reactive for anti-HIV-1 and nonreactive for HbsAg and Anti-HCV, diluted into negative plasma.
- The HIV-2 positive control was inactivated and recalcified plasma reactive for anti-HIV-2, and nonreactive for HbsAg and anti-HCV, diluted into negative plasma.
- IMX HIV-1/HIV-2 III controls: negative control and HIV-1 Positive Control
- Two 9ml bottles of Imx HIV-1/HIV-2 Plus controls prepared with recalcified human plasma. Preserved with sodium azide.

- The negative control was nonreactive for HbsAg, anti-HCV, anti-HBs and anti – HIV-1/HIV-2.
- The HIV-1 positive control was inactivated and recalcified human plasma reactive for anti-HIV-1 and nonreactive for HbsAg and Anti-HCV, diluted into negative plasma.

2.3.3 Procedure for the IMX HIV-1/HIV-2 III Plus assay

The following procedure was followed as per manufacturer's instructions:

- The serum samples were added to the wells of the reaction cells, thereafter the IMx probe/electrode assembly performs all the other pipetting steps in the following sequence:
- The probe/electrode assembly delivered the sample and antigen coated microplates (HIV-1 envelope, HIV-1 core, and HIV-2 envelope) to the pre-incubation well of the reaction cell.
- Binding of the antibodies to the antigen coated particles formed an antigen-antibody complex.
- An aliquot of the reaction mixture containing the antigen antibody complex was transferred to the glass fibre matrix to irreversibly bind the micro-particles to the glass fibre matrix.
- The matrix was washed to remove unbound materials.
- The biotinylated recombinant antigens ((HIV-1 envelope, HIV-1 core, and HIV-2 envelope) and synthetic peptides corresponding to the HIV-1

envelope and HIV envelope were dispensed onto the matrix forming an Ag-Ab-Ag complex.

- The matrix was washed to remove unbound materials.
- The substrate, 4-methylumbellieryl Phosphate, was added to the matrix and the MEIA optical assembly measures the fluorescent product.
- The presence and absence of antibodies to HIV-1/HIV-2 was determined by comparing the rate of formation of fluorescent product to the cut-off, calculated from the MODE-1 calibrator rate. The specimen was considered reactive for anti HIV if the rate of the specimen is greater than or equal to the cut-off (≥ 1.00).

Table 2-2: Cut-off values specified for the IMx® HIV-1/HIV-2 Plus controls

Control	Colour	Anti-HIV Minimum titre	Control Range(S/CO)
Negative	Natural	N/NA	0.10 – 0.75
HIV-1 Positive	Blue	1:1	≥ 1.00
HIV-2 Positive	Red	1:1	≥ 1.00

2.4 Protocol for the HIV Confirmation Tests

Confirmatory HIV testing was requested for positive results in children and for a few discordant mother and child HIV results. The Vironostika® HIV UniForm II plus O was used as a second test to confirm HIV results obtained using the IMX® HIV-1/HIV-2 III PLUS kit. The test is ELISA-based on a one step sandwich principle. A mixture of HIV-antigens coupled to horseradish peroxidase (HRP) serves as the conjugate with tetramethylbenzidine (TMB) and peroxide as the substrate. Upon completion of the assay, the development of colour indicates the presence of antibody to HIV-1, HIV-2 and/or HIV-1 group O, while no or low colour development suggests the absence of antibody to HIV-1, HIV-2 and/or HIV-1 group O. Specifically, micro-ELISA wells are coated with a mixture of HIV of HIV – antigens: HIV p24, HIV-1 gp 160 (2), HIV-1 ANT70 peptide and HIV-2 env peptide (amino acids 592 –603). Each micro-ELISA well contains an HRP- labelled conjugate sphere of the same HIV-antigen mixture. The specimen well contains an HRP-l.

2.5 Data Preparation for Statistical Analysis

2.5.1 Data Clean-up

As described previously, the samples for the three studies were obtained from uncorrelated sources, with already available data and/or questionnaire information for two of the studies. Labels for the same variables were named differently, i.e Gender and Sex, Race and Ethnic group, Age and Years, HIV and Result were used interchangeably. Column attributes for character variables in each was either named differently for defined groups i.e. Black (B or AF), Coloured (C or Co), Indian (A or I). For laboratory results, column attributes were either numeric or characters, e.g. HIV results were either Negative or Positive or 1 and 0.

Although the studies were analysed separately, renaming of variable names and character variables grouping was crucial. Homogeneity in data attributes was to be maintained before the different data sets were merged and also before the data sets could be analysed.

Firstly, data was imported into the SAS 9.1 statistical programme (SAS Institute Inc, Cary, NC, USA.). Frequency tables were used to identify the row names and character column attributes. For descriptive variables, means (±standard deviations) and distribution plots were used to identify data distributions and outliers and possible data capturing errors. Following this, variables were renamed to our standard labelling, which was applied to all studies if similar variables were identified. Each column attribute was carefully studied and compared and was also renamed accordingly. Understanding of other STI laboratory results was necessary and inquiries were made before final groupings were done. For two of the studies, data from questionnaires and results for HIV and STD were already available. There was less control on steps taken to clean obvious typographical errors and outliers as we could not refer back to the previous study sources. Thus, obvious outliers and typographical errors were deleted and treated as missing values.

Once data was prepared, all 3 studies were merged to the KSHV results data. KSHV samples were labelled with custom made barcoded labels which were readable by the ELISA analysing instrument. During labelling, each re-assigned number was captured to match the available study identity (ID) for each sample. Thus all available data was merged to the KSHV data using the unique label numbering.

2.5.2 Data Analysis

For all 3 studies, descriptive statistical analysis and measures of effect were done using SAS 9.1 (SAS Institute Inc, Cary, NC, USA.). The Kappa coefficient (κ) was calculated to determine concordance between antibodies against the lytic K8.1 and latent Orf73 antigens. Logarithmic transformation of the antibody titres (expressed as optical densities) for lytic K8.1 and latent Orf73 allowed for the use of standard parametric statistical methods and results are expressed as the geometric mean represented as μ_g and standard deviation range represented as σ_g.

Comparisons of means were done using Bonferroni adjusted student t-test between groups or Analysis of Variance (ANOVA) amongst multiple groups. Trends were measured using the Cochran-Armitage Trend Test. Prevalence odds ratios (PORs) and 95% Wald confidence intervals (CIs) for KSHV seropositivity were obtained using logistic regression. Both univariate and multivariate logistic regression models were used. In a multivariate model, PORs were adjusted for different variables depending on the study.

For the Mother to Child KSHV Seroepidemiology Study, the calculated PORs were adjusted for the age of the mother ($\leq$-25, 26–29, 30–34, and $\geq$35 years) and the age of the child (1.6–3, 4–6, and 7–10 years). For the Antenatal KSHV Seroepidemiology Study in the multivariate logistic model, PORs were adjusted for age group (≤ 20, 21-25 26-30, ≥ 31), education level in years (< 2 years, 2-5 years, 6-12 years and >12 years (post matriculation/secondary school) of formal education, municipal region (East Rand, Soweto, Pretoria, Vaal Triangle and West Rand) and syphilis seropositivity.

For the Carletonville Community KSHV Seroepidemiology Study, multivariate analysis included adjustment for age groups 25 years and less, 26–35 years, 36–45 years, and 46 years and older, community groups, HIV or other STI.

Chi-square ($\chi 2$) tests for binary measures, trend and homogeneity were calculated. Two-sided p-values were used to measure the significance of the associations before and after adjustment for other covariates.

2.6 Sample size calculations

Sample size estimations were made for each of the three studies. These were based on the type of the study, the estimated KSHV prevalence and the estimated odds ratios. The estimated sample size calculations will be discussed in the relevant chapters.

3 The Mother to Child KSHV Sero-epidemiology Study

The appearance of an epidemic of KS in HIV infected adult males in the 1980's gave rise to the presumption that the causative agent was a sexually transmitted infection. It had been known for decades that both classical and African endemic KS occurs at all ages, including young children (Chapter 1, section 1.6.1), but this appears to have been overlooked when the possible transmission patterns of the KS infectious agent were considered. Defining the modes of transmission of the KS virus, KSHV, is complicated further by its association with HIV. In the countries where KS is very commonly reported in MSM, rather than in the general population, the mode of KSHV transmission is thought to be sexual (Beral et al, 1990; Elford et al, 1993; Kaldor et al, 1993). However, this does not explain how very young children, who are clearly not sexually active, acquire the virus.

The rising incidence of childhood KS, associated with immunosuppression due to HIV/AIDS, in African and Mediterranean populations illustrates that other modes of transmission are likely (Chapter 1, section 1.8). AIDS KS occurs in all race groups, regardless of age, gender, and socioeconomic status. Of significance are the increased incidences of AIDS KS reported in children across the world, including the United States and Europe (Serraino & Franceschi, 1996). In addition, although rare, classic KS occurs in very young children in the Mediterranean regions and is thought to be linked to autosomal recessive predisposition (Sahin et al, 2010).

Although it is clear that different patterns of KS exist in countries where KSHV is prevalent, the mode in which humans acquire the virus is not clear. This indicates the need for epidemiological studies to provide an understanding of these emerging non-sexual transmission patterns. Interestingly this concept is also supported by reports indicating KSHV seropositivity in American children and adolescents (Baillargeon et al, 2002; Anderson et al, 2008).Studies in Africa have suggested that, in South Africa, KSHV infection in children may be acquired from their mothers (Sitas et al, 1999; Dedicoat et al, 2004). The postulated routes of KSHV transmission to young children include both the horizontal and vertical transmission of KSHV

from mother to child (Andreoni et al, 1999; Bourboulia et al, 1998; Mayama et al, 1998). Sitas and co-workers (1999), showed that KSHV seropositive mothers with high antibody titres are about twice more likely to have KSHV seropositive children than mothers with low KSHV antibody titres. In addition, in a Zambian study of mother and child pairs, He and co-workers (1998) found that all children with Kaposi's sarcoma had mothers who were KSHV seropositive, while not all children whose mothers had Kaposi's sarcoma were infected.

Pilot studies conducted in several countries, including African and Italian countries, further suggested that familial transmission of the virus might also occur (Plancoulaine et al, 2004; Mulaiteiye et al, . Non-familial transmission routes are also suspected (Andreoni et al, 1999; Bourboulia et al, 1998; Mayama et al, 1998). A study performed in Ugandan children indicated an association between KSHV and hepatitis B infection, suggesting that transmission of KSHV in childhood might be associated with living conditions that may also facilitate infection with the hepatitis B virus (Mayama et al, 1998). A number of routes that may contribute to horizontal transmission of KSHV infection between mother and child are suggested. KSHV shedding in saliva is regarded the most probable route (Koelle et al, 1997; Vieira et al, 1997). This would match that of other herpes viruses, including EBV that are transmissible via saliva (Lucht et al, 1995). Studies of KSHV in saliva of immuno-competent KSHV seropositive participants in areas of high KSHV prevalence are necessary.

It is not clear whether HIV facilitates the spread of KSHV in the community. KSHV shedding will vary in relation to KSHV antibody titre (and thus, indirectly, viraemia) and in relation to HIV infection. Existing studies show a possible relationship but more information is required to understand this subject. Moreover, the relative importance of the above postulated routes of KSHV transmission to children requires further research.

This chapter focuses on describing the seroprevalence of KSHV in South African children and their mothers. The study will also describe the relationship between the KSHV status of the

children and of their mothers. Furthermore, the study will attempt to look at the mother and child relationship of KSHV status if the mother is HIV seronegative or HIV seropositive.

3.1 Objectives

The objectives of this study were

- To measure the seroprevalence of KSHV in South African females undergoing paternity testing and in their children.
- To measure the association between KSHV antibody titre in the mother's serum and the presence of KSHV antibodies in their children.
- To measure the seroprevalence of HIV in South African females undergoing paternity testing and also in their children.
- To measure the association between KSHV seropositivity and HIV seropositivity in mothers and in children.
- To measure the association between KSHV antibody titre and the presence of HIV antibodies in mothers and in children.

3.2 Study Design

This is a cross sectional study, using secondary demographic data and leftover sera allocated to this study. The NHLS, Serogenetics Laboratory, Department of Human Genetics, based in Braamfontein, Johannesburg, collects sera routinely from mothers, children and disputing fathers to determine the paternity of the child. Sera are also routinely collected by most of the NHLS laboratories across all the provinces of South Africa and are all sent to the Braamfontein branch to be tested. Collected samples are clearly labelled, grouping together the mother, child and father sets. Routine information collected from the participants includes age, date of birth, race group, gender of all the participants involved and relationship status i.e. mother, disputing father or child.

The study was conducted at the NHLS Cancer Epidemiology and Research Group (CERG), Braamfontein, Johannesburg. Bar-coded labels obtained from the NCI-Frederick, Maryland, USA were used to carefully label samples for KSHV ELISA testing. All leftover sera were de-identified and were anonymously de-linked to hide participant identifiers. KSHV data was captured on an excel spreadsheet and matched to the initial de-identified sample numbers.

Approval to conduct the study was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Medical) – (Protocol Number - M990808) (Appendix A). Approval to collect the de-identified and anonymously de-linked samples was also obtained from Professor Tony Lane, Head of the Department of Human Genetics, Serogenetics Laboratory, NHLS, and Braamfontein, South Africa.

3.3 Study Participants

Serum samples that were left over after paternity testing were collected consecutively from the Department of Serogenetics between September 1999 and May 2001 and all were de-identified. The common practice for collection of samples for dispute paternity testing is that the biological mother of the child in dispute will be bled together with the child and the putative father. In cases were the biological mother is not available, only the putative father and the child will be bled. In most cases of paternity dispute, the putative fathers are less likely to have a close relationship or to be living in the same household as the child. Therefore, for this study, leftover sera were collected only for mothers and their children. All maternal samples collected for this study were from confirmed biological mothers. The samples were stored at -20˚C.

In total, 2466 sera were available, of which 1287 were from children and 1179 from their mothers. All leftover samples were tested for KSHV and HIV (Figure 3.1). Some of the mothers had more than one child. A larger proportion 82.8%(2044) of the race group were Black, 10.1%(248) were White, 5.4%(136) were from the other race groups [5.2%(129) were Coloured and 0.3%(7) were Asian], while in the remaining 1.5%(38), the race group was unknown (Figure

3.1). The race group distribution of the participants in the study matched the population distribution described for South Africa within the same period (Statistics South Africa, 2004).

The Asian and Coloured populations were grouped together as 'other' as they were only a few in each race group. For some analyses, White, Coloured and Asian populations are grouped together as a non-Black race group to provide larger denominators, especially when comparisons between groups are made. Overall, 384 participants (197 children and 187 mothers) were grouped together as a non-Black population. The population distributions by sex of the child and race group are illustrated in Figure 3.1.

The age of the mothers ranged from as young as 14 years up to 62 years with the mean (± SD) age of 30.0 (± 7.2) and that of the children ranged from 0.08 – 26 years with the mean (± SD) age of 5.5 (±4.9). Only children up to 16 years of age and their mothers were included in this study. The ages of 24 mothers and 35 children were missing; and were therefore excluded when allocating age groups, thus decreasing the total number. The mean age (± SD) of the mothers was 30.0(7.1) years and was 5.5(4.9) for all children (Fig. 3.1). The mean (± SD) of 5.4(4.9) and 5.6(4.9) years was not significantly different between the female and male children, respectively ($p = 0.55$). Black children were older than those in the other race groups and so were their mothers compared to those in the other race groups (Fig 3.1).

3.4 Sample size and power calculations

The following sample size estimations were made when the study was designed based on available KSHV seroprevalence information. Using available literature, it was estimated that about 25% of the mothers would be KSHV seropositive and about 20% HIV seropositive. Assuming a 30% mother to child transmission rate for KSHV (Bourboulia et al 1998), then 240/800 children were expected to be KSHV seropositive. Expecting that some of the mothers will have more than one child it was estimated that about 175/700 mothers would be KSHV

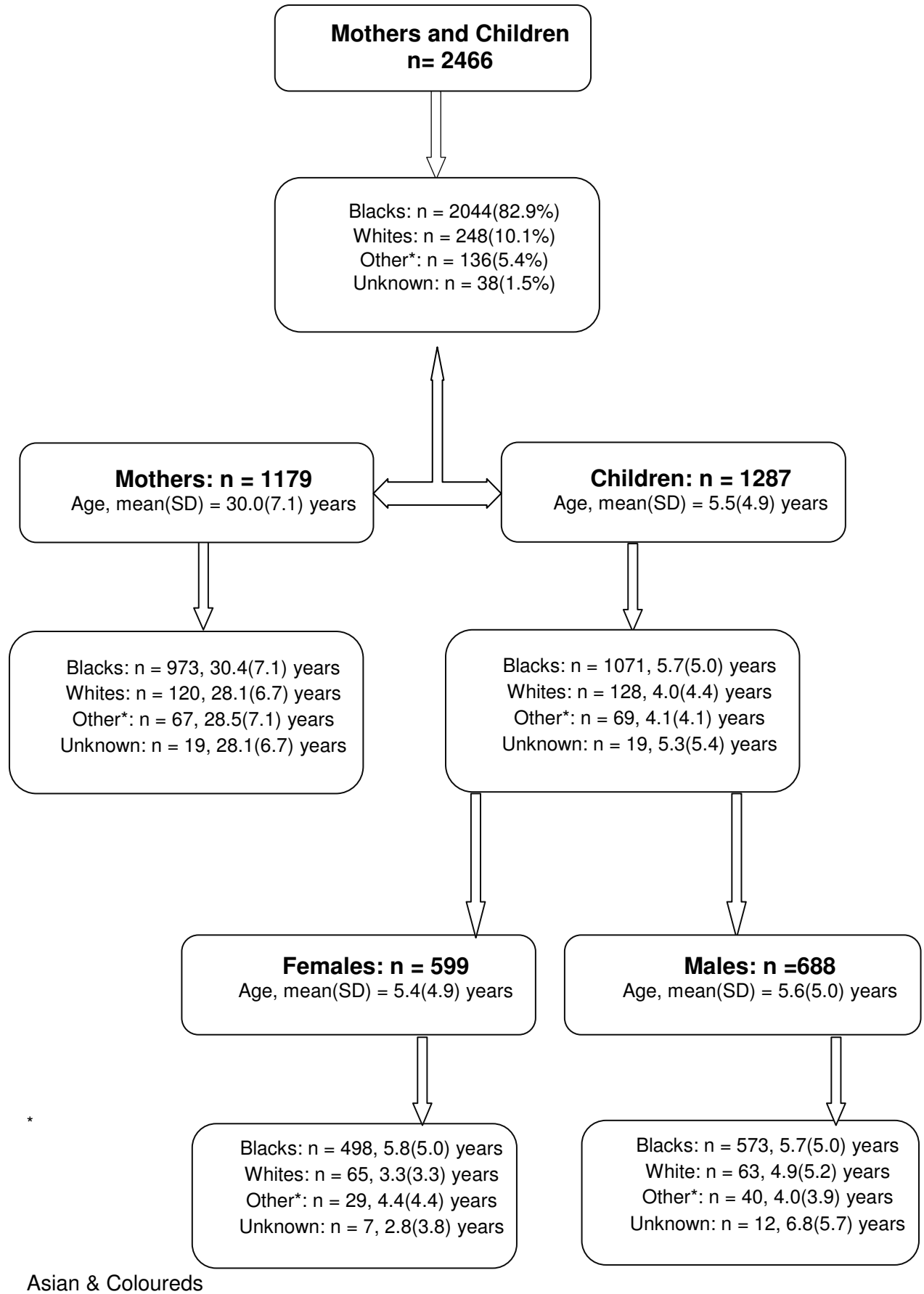


Figure 3-1: Summary of the mothers and their children by race group

seropositive and 140 HIV seropositive. Based on these estimates and if the above assumptions remained true, this study was expected to be able to detect a prevalence odds ratio (POR) of about 4 in HIV seronegative mothers and a POR of 8 in HIV seropositive mothers. The power of detection was likely to increase as the number of samples gathered for the study increased to more than the estimated sample size calculations.

These were pre-study assumptions and therefore it was acknowledged that different outcomes might be produced once the study is completed. The study outcomes will help to refine the power calculations of subsequent local mother to child KSHV transmission and related studies.

3.5 Describing KSHV seroprevalence

It became apparent that not all KSHV infected people express antibody responses to both the lytic K8.1 and latent Orf73 antigens at a given time (Section 2.2.5, Zhu et al, 1999; Simpson; 1996). Thus, to estimate the seroepidemiology of KSHV in populations it is recommended that a criterion that allows inclusion of both the discordant and concordant results for the lytic K8.1 and latent Orf73 assays should be used. This will increases specificity and sensitivity in predicting infection rates. Therefore as explained in section 2.2.5, in this chapter and the next chapters, KSHV seropositivity will be defined to reflect all participants who tested seropositive to both the lytic K8.1 and the latent Orf73 antibodies or either the lytic K8.1 or latent Orf73 antibodies i.e

$$\text{KSHV seropositive} = [K8.1^{+} \text{ only} + Orf73^{+} \text{ only} + (K8.1^{+} + Orf73^{+})]$$

For all the results in this thesis, focus will be on the KSHV status i.e. details for the lytic and latent KSHV will be included in tables but text descriptions will be made only where statistical inferences are significant and/or different to the overall KSHV patterns.

3.6 Considerations for Data Analysis

Statistical analysis was done using SAS 9.2 and SAS enterprise guide 4.2 (SAS Institute INC Cary, NC, USA). Statistical methods used are described in Chapter 2, section 2.5.2. This

section aims only to explain study specific methods, focusing on analysis groups and inclusion and exclusions during analysis.

Studies focusing on HIV infection and transmission in children have indicated that HIV seropositivity in children <18 months of age may be due to the presence of maternal antibodies. Although still not clear, it has been postulated that KSHV seropositivity in very young children might also be a reflection of maternal KSHV antibodies (Minhas et al, 2008; Caterino-de-Araujo & Cibella, 2003). However, vertical transmission of KSHV is thought to be very rare (Sarmati et al, 2004). The time to maternal KSHV antibody clearance is also not clear, however in one study, KSHV maternal antibodies were shown to decrease within 7 months of birth (Lyall et al, 1999). Limited literature exists on this subject, so in this study, it was assumed that as with HIV, the maternal KSHV antibodies will be cleared by 18 months of age. Interpretation of KSHV seropositivity in very young children in this study will be treated cautiously. For this reason, although results for this youngest age group will be shown, some analyses were limited to age groups over 18 months.

The descriptive data for all mothers and their children are shown in Table 3.1. The age-groups used for the children are 0 – 1.5, 1.5 - 3, 3 – 6, 6 – 10 and >10 years. The age categories were based on the following: before 18 months of age children are close to the mother and may also be carrying maternal antibodies. At age 19 – 36 months (1.5 – 3 year group) children are still close to their mothers but start to play with and be exposed more freely to other children. By 3 – 6 years many children attend preschool and are, therefore, exposed to a larger group of children. Children may start attending school (grade R) at 6 years of age and by 7 years almost all children are expected to have started first grade at school. At 10 years and older ages some may begin sexual experimentation. By 16 years of age, some children are sexually active and children at this age and above were, therefore, excluded from any measures of association between mother and child. The youngest mother included in this study was 14 years of age.

For multivariate logistic regression analysis, adjusted odds ratios (ORs) were controlled for possible confounders such as age of the mother (<25, 26–29, 30–34, and >35 years), the age of the child (1.6–3, 4–6, and 7–10 years) and HIV status. SAS 'proc univariate' measures were used to determine the cut-off values for defining the antibody titre levels of the mother, taking into consideration the quartile and interquartile estimates. The antibody titre measures for analysis were calculated following log transformations. Since the distribution of titres of both KSHV lytic K8.1 and latent Orf73 antibodies were positively skewed the data was logarithmically transformed and reported as the geometric mean and one standard deviation range.

3.7 Results

General information about the study population is shown in Figure 3.1 and explained under "Study Participants" in section 3.3. In summary, 2466 participants were included in the study. Of these, there were 1179 mothers and 1287 children. More than 83% of all participants were Black (n = 2044), 10% White (n = 248), 5% either Asian or Coloured and grouped as "Other" (n = 136) and race group was not defined for 2% of the remaining participants (n = 38). As a reminder, the phrases KSHV seroprevalence or KSHV seropositivity are used alternatively to refer to overall seropositivity to either the lytic K8.1, latent Orf73 antibodies or both. If lytic K8.1 and latent Orf73 are used, they refer distinctively to the specific KSHV antibodies.

3.8 Seroprevalence of lytic K8.1 and latent Orf73 in children and mothers

Table 3.1 shows results for seropositivity to the lytic K8.1 and latent Orf73 antibodies and concordance between the two assays in detecting expressed KSHV antibodies. There was a marginal concordance in detection of KSHV antibodies between the two assays in children and in mothers [kappa ***(κ)*** = 0.43, Pearson's Correlation (r) = 0.45, $p<0.0001$]. Overall, 375/2466 (15.2%) of the participants were seropositive to the lytic K8.1 antibodies (Table 3.1A). Of these, 140/1287 (10.9%) were children and 235/1,179 (19.9 %) were mothers (Table 3.1B & C). A total of 379/2466 (15.4%) were also seropositive to latent Orf73. Seven percent of all the participants

tested seropositive to lytic K8.1 only (K8.1$^+$ & Orf73$^-$), another 7.3% were seropositive to latent Orf73 only (K8.1$^-$ & Orf73$^+$), while 8.1% were seropositive to both the lytic K8.1 and latent Orf73 antibodies (K8.1$^+$ & Orf73$^+$). Overall seropositivity to lytic K8.1 and latent Orf73 was similar at 15.2% and 15.4%, respectively (Table 3.1A).

Table 3-1: Seropositivity to lytic K8.1 and latent Orf73 antibodies in children and mothers and concordance between the lytic K8.1 and latent Orf73 assays.

	Latent Orf73-	**Latent Orf73+**	**All**	**Concordance**
A. Children & Mothers				
Lytic K8.1-	1,912(77.5%)	179(7.3%)	**2091(84.8%)**	*κ = 0.43*
Lytic K8.1**+**	175(7.1%)	200(8.1%)	**375(15.2%)**	***r = 0.45,*** *p<0.0001*
All	**2087(84.6%)**	**379(15.4%)**	**2466**	
B. Children				
Lytic K8.1-	1,083(84.1%)	64(5.0%)	**1147(89.1%*)***	*κ = 0.43*
Lytic K8.1+	73(5.7%)	67(5.2%)	**140(10.9%)**	***r = 0.43,*** *p<0.0001*
All	**1156(89.8%)**	**131(10.2%)**	**1287**	
C. Mothers				
Lytic K8.1-	829(70.3%)	115(9.7%)	**944(80.1%)**	κ = *0.43*
Lytic K8.1**+**	102(8.7%)	133(11.3%)	**235(19.9%*)***	**r = 0.43,** p<0.0001
All	**931(7*9*.0%)**	**248(21.0%)**	**1179**	

Antibodies to lytic K8.1 (K8.1$^+$) were detected in 10.9% (140/1287) of the children and were nearly twice as high in mothers at 19.9% (235/1179) (Table 3.1 B & C). Similarly, latent Orf73 antibodies (Orf73$^+$) were detected in 10.2% (131/1287) and 21.0% (248/1179) of the children and mothers, respectively (Table 3.1B & C).

Overall 554/2466 (22.5%) participants tested seropositive to either the lytic K8.1 (175), latent Orf73 (179) or both of the KSHV antibodies (200), (Table 3.1 and Figure 3.2). Excluding those who tested seronegative to any of the KSHV antibodies, the distributions of the expressed antibodies were determined in the 554 participants who had seropositive KSHV status.

Figure 3.2 shows the distribution of the lytic K8.1 and latent Orf73 antibodies in participants who tested seropositive to KSHV. There was no obvious pattern in the percent distribution of the expressed KSHV lytic K8.1 and latent Orf73 antibodies. Although not statistically significant, the majority of the mothers seemed to express both the lytic K8.1 and latent Orf73 antibodies at 38%, with 29% expressing the lytic K8.1 antibodies and the rest (32%) expressing latent Orf73 antibodies (Figure 3.2) (p = 0.12). The percent distribution of the lytic K8.1 and latent Orf73 in children was also not significantly different.

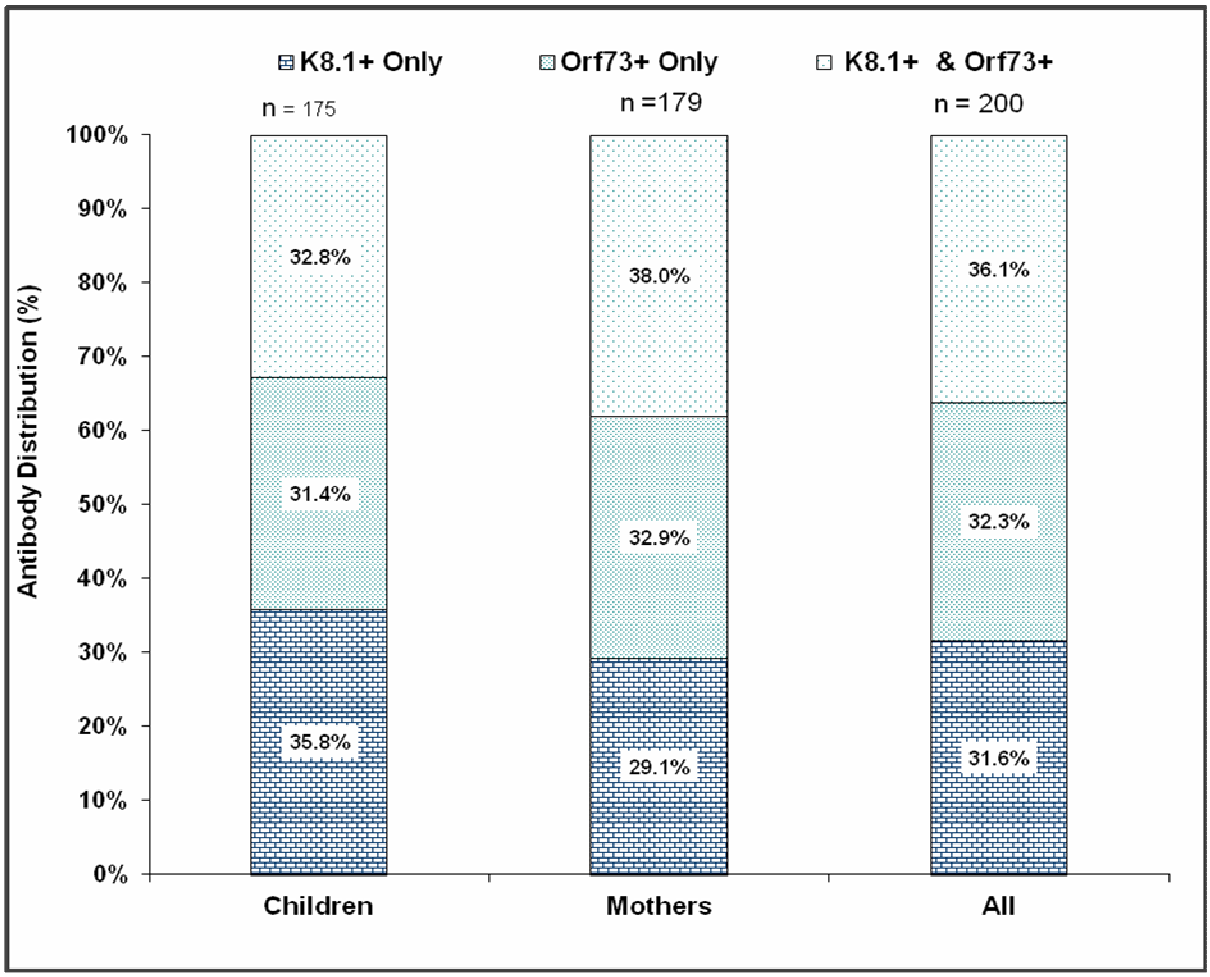


Figure 3-2: Distribution of the lytic K8.1 and latent Orf73 antibodies in participants who tested seropositive to KSHV.

3.9 KSHV seropositivity in children and mothers

Seropositivity to lytic K8.1 and latent Orf73 by age group, race and gender (for children only) is shown inn Table 3.2, together with the overall seroprevalence (based on seropositivity to either lytic K8.1 only or latent Orf73) of KSHV. The overall seroprevalence of KSHV was lower in children at 16% (204/1287) increasing to 30% (350/1179) in their mothers. KSHV seropositivity in children greater than 18 months of age (1.5 years) did not increase significantly with increasing age, ranging from 13% to 18% within the 1.6 years to greater than 10 years age groups ($p = 0.427$).

KSHV seroprevalence was slightly higher in female than male children at 18% and 14%, respectively ($p=0.124$). Although not significantly different, KSHV seroprevalence was slightly higher in children of the Other (Asian & Coloured) race group, followed by black children and lowest in the white children at 17%, 16% and 12%, respectively ($p= 0.432$) (Table 3.2 A).

In mothers KSHV seroprevalence tended to increase with increasing age ($p = 0.03$) (Table 3.2B). Unlike in children, KSHV seroprevalence was significantly different by race group ($p < 0.03$). Black mothers and those from the Other (Asian and Coloured) race group had higher KSHV infections than white mothers. KSHV seroprevalence was more than 2-fold higher in black than white mothers at 32% and 14%, respectively, with 20% of the other mothers expressing KSHV antibodies ($p < 0.03$). Higher KSHV seroprevalences were noted in children and mothers whose race group was unknown (Table 3.2). Overall, this group formed less than 2% of the total group and were not included when race groups are compared, as they seem to misrepresent the overall statistical inferences.

Figure 3.3 shows KSHV seropositivity in all participants by age group. A slight decline in KSHV antibodies from 18.0% in children less than 18 months (1.5 years) to 14.1% in children 1.6 to 3 years was noted. The seroprevalence was stable up to 6 years of age and increasing to 17.7% and reaching a peak of up to 34% in young adults 26 to 29 years of age and remaining stable after a slight drop in the 30 to 34 year olds (Figure 3.3).

Table 3-2: Seropositivity to lytic K8.1, latent Orf73 and KSHV seroprevalence in children and their mothers, by gender (children only), age and race groups.

	Total Number	Lytic K8.1 Seropositivity			Latent ORF73 Seropositivity			KSHV Seropositivity		
		No. Pos (%)	OR(95% CI)	Significance	No. Pos (%)	OR(95% CI)	Significance	No, Pos (%)	OR(95% CI)	Significance
A. Children										
Gender										
Female	599	75(12.5)	1		67(11.2)	1		105(17.5)	1	
Male	688	65(9.5)	0.8(0.7- 1.0)	***p = 0.078***	64(9.3)	0.7(0.8- 1.1)	***p = 0.27***	99(14.4)	0.7(0.5-1.0)	***p= 0.124***
Age, years										
0 – 1.5†	339	44(13.0)	-		41(12.1)	-		61(18.0)	-	
1.6 – 3	283	26(9.2)	1		28(9.9)	1		40(14.1)	1	
4 - 6	218	23(10.6)	1.1(0.6-2.1)		16(7.3)	0.7(0.4-1.4)		29(13.3)	0.9(0.6-1.6)	
7 - 10	220	25(11.4)	1.3(0.7-2.6)		23(10.5)	1.1(0.6-1.9)		39(17.7)	1.3(0.8-2.1)	
>10	227	22(9.7)	1.1(0.5-1.9)	p_{trend} ***= 0.74***	23(10.1)	1.0(0.6-1.8)	p_{trend} ***= 0.51***	35(15.4)	1.1(0.7-1.8)	p_{trend} ***= 0.427***
Race groups**										
Black	1071	111(10.4)	1		113(10.6)	1		170(15.9)	1	
White	128	13(10.2)	1.0(0.5-1.8)		7(5.5)	0.5(0.2-1.1)		15(11.7)	0.7(0.4-1.2)	
Other	69	10(14.5)	1.4(0.7-2.9)	***p= 0.55***	6(8.7)	0.8(0.3-1.9)	***p = 0.19***	12(17.4)	1.1(0.6-2.1)	***p= 0.432***
Unknown††	19	6(31.6)	-		5(26.3)	-		7(36.8)	-	
All	**1287**	**140(10.9)**			**131(10.2)**			**204(15.9)**		
B. Mothers										
Age, years										
≤25	343	54(15.7)	1		60(17.5)	1		83(24.2)	1	
26 – 29	249	57(22.9)	1.6(1.0-2.4)		60(24.1)	1.5(1.0-2.2)		85(34.1)	1.6(1.1-2.3)	
30– 34	278	51(18.4)	1.2(0.8-1.8)		51(18.4)	1.1(0.7-1.6)		77(27.7)	1.2(0.8-1.7)	
>35	309	73(23.6)	1.7(1.1-2.4)	p_{trend} ***= 0.040***	77(24.9)	1.6(1.1-2.3)	p_{trend} ***= 0.078***	105(33.9)	1.6(1.1-2.3)	p_{trend} ***= 0.03***
Race groups										
Black	973	211(21.7)	1		222(22.8)	1		310(31.9)	1	
White	120	9(7.5)	0.3(0.1-0.6)		12(10.0)	0.4(0.2-1.2)		17(14.2)	0.4(0.2-0.6)	
Other	67	8(11.9)	0.5(0.2-1.0)	***p< 0.0006***	10(14.9)	0.6(0.3-1.2)	***p= 0.0023***	14(20.1)	0.7(0.3-1.0)	***p< 0.0002;***
Unknown††	19	7(36.8)	-		4(21.1)	-		9(47.4)	-	
All	**1179**	**235(19.9)**			**248(21.0)**			**350(29.7)**		

† Children < 18 months were excluded from measures of association. ††Children (19) and mothers (19) with unknown race group where excluded from measures of association.

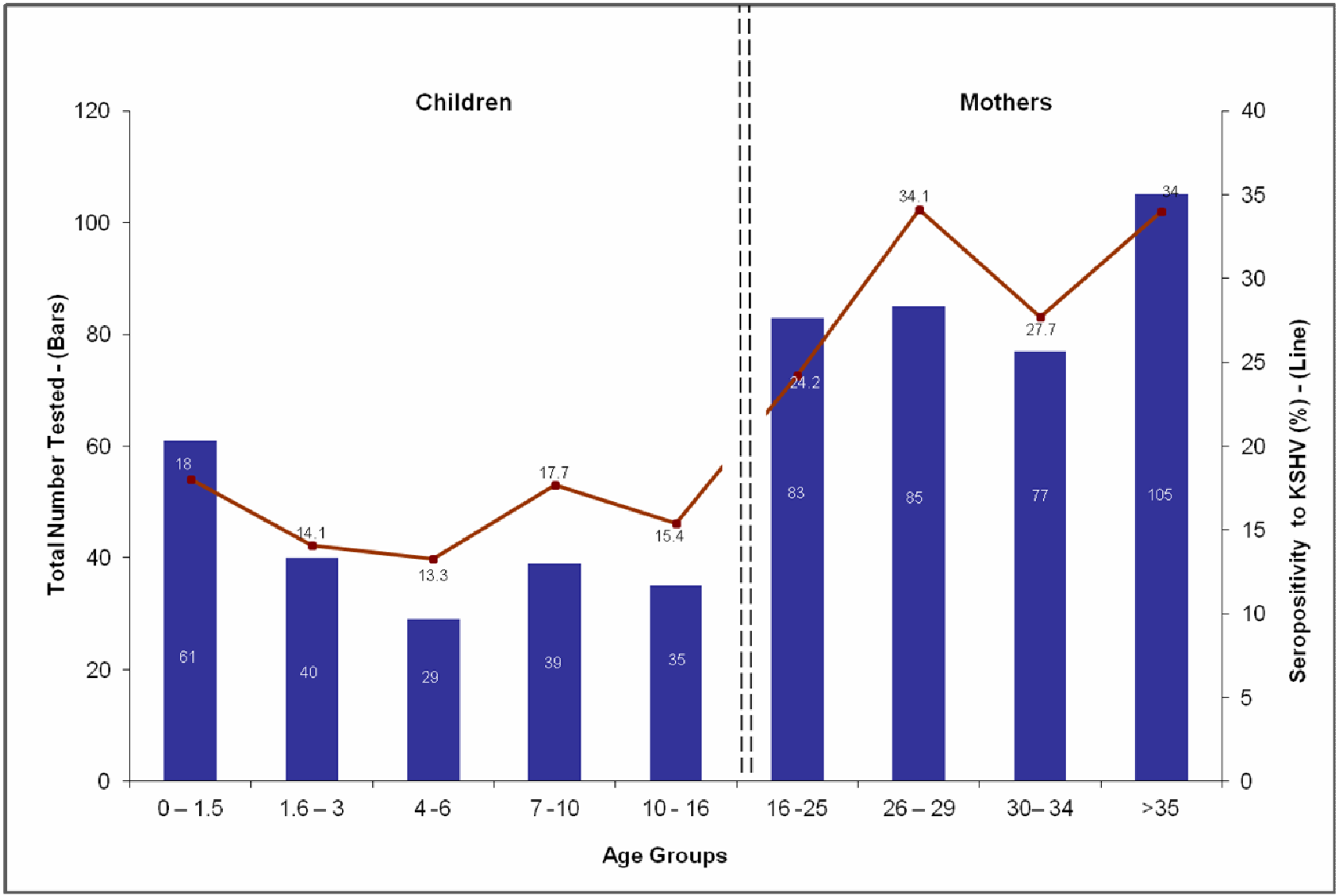


Figure 3-3: KSHV Seroprevalence by age group in children and their mothers.

3.10 KSHV seropositivity in children by KSHV status of the mother

Figure 3.4 & Table 3.3, shows the presence of KSHV antibodies in 1238 children less than 16 years of age measured against the KSHV status of their mother. Children over 16 years of age (n = 49) were excluded. KSHV seropositivity was measured in 376 children born to mothers who were KSHV seropositive and in 862 children born to mothers who were KSHV seronegative.

Overall, children born to KSHV seropositive mothers had higher KSHV seroprevalence than those born to KSHV seronegative mothers. The higher KSHV seroprevalences in children born to KSHV seropositive mothers compared to those of KSHV seropositive mothers are obvious in the different age groups of the children as shown in Figure 3.4. Also in Figure 3.4, it is apparent that the HIV status of the mother plays a significant role in KSHV transmission in children up to 11 years of age. At 12 years of age KSHV seropositivity in children of KSHV infected mothers' drops and after 12 years of age KSHV seropositivity was similar, regardless of the mothers KSHV status (Figure 3.4).

Of the 862 children born to KSHV seronegative mothers, KSHV seropositivity was detected in 13.7% (118) and was significantly lower than KSHV seropositivity in the 376 children born to KSHV seropositive mothers at 21.5% (81), ($p = 0.0005$). In children born to KSHV seropositive mothers, KSHV seropositivity remained the same in children up to 2 years of age and in those between 3 and 6 years of age. There was an unexplained drop to 11.4% in KSHV seropositivity in children aged between 2 to 3 years of age.

When considering the associations between the KSHV status of the mother and child, in addition to the 49 children over 16 years of age, 388 children under 18 months age (<1.5 years) were excluded from some of the analyses, for reasons explained in section 3.6. Table 3.3 in the previous page, shows the risk of the child being KSHV seropositive if they are born to KSHV positive mothers compared to those born to mothers who are

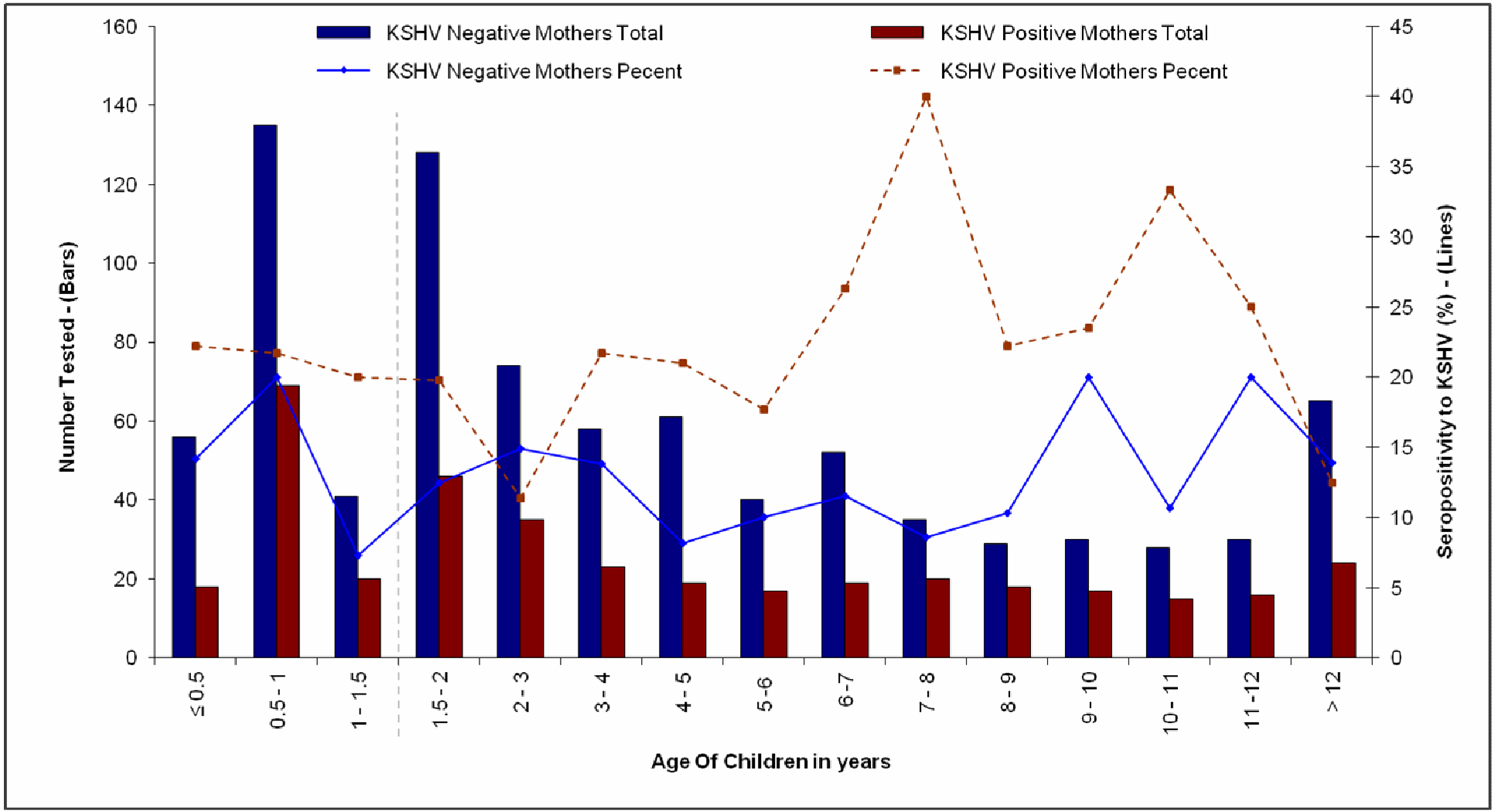


Figure 3-4: KSHV seropositivity in children grouped by the mothers KSHV status (Total n = 1238)

Table 3-3: Association between the KSHV seropositivity[#] in mothers and their children (<16 years of age) by race and age groups of the child.

			Lytic K8.1		Latent Orf73		KSHV	
	KSHV status	**Total tested**	**n(%)**	**OR; 95% CI***	**n (%)**	**OR; 95% CI***	**n (%)**	**OR; 95% CI***
KSHV seropositivity[#] by Age of child								
0 – 16 years	Negative	862	79(9.2)	1	76(8.8)	1	11813.7)	1
	Positive	376	57(15.2)	1.7(1.2 – 2.6)	51(1.3.6)	1.6(1.1 – 2.4)	81(21.5)	1.7(1.3 – 2.4)
1.5 – 16 years[†]	Negative	630	51(8.1)	1	52(8.3)	1	80(12.7)	1
	Positive	269	41(15.2)	2.0(1.3 – 3.2)	34(12.6)	1.6(1.0 – 2.5)	58(21.6)	1.9(1.3 – 2.7)
1.5 - 10 years[†]	Negative	507	39(7.7)	1	41(80)	1	62(12.3)	1
	Positive	214	35(16.4)	2.3(1.4 – 3.8)	26(12.2)	1.6(0.9 – 2.6)	46(21.5)	1.9(1.3 – 2.9)
0 – 1.5 *years*	Negative	232	28(12.1)	1	24(10.3)	1	38(16.4)	1
	Positive	107	16(15.0)	1.3(0.3 – 2.5)	17(15.9)	1.6(0.8 – 3.2)	23(21.5)	1.5(0.8 – 2.7)
1.6 - 3 years	Negative	202	16(7.9)	1	19(9.4)	1	27(13.4)	1
	Positive	81	10(12.3)	1.6(0.7 – 3.8)	9(11.1)	1.2(05 – 2.7)	13(16.1)	1.3(0.6 – 2.7)
4 – 7 years	Negative	211	17(8.1)	1	12(5.7)	1	23(11.5)	1
	Positive	78	15(19.2)	2.7(1.3 – 5.8)	8(10.3)	1.9(0.7 –4.8)	17(21.8)	2.0(0.9 – 4.5)
7 - 10 years	Negative	94	6(6.4)	1	10(10.6)	1	12(12.8)	1
	Positive	55	10(18.2)	3.2(1.1 – 9.5)	9(16.4)	1.6(0.6 – 4.3)	16(29.1)	2.8(1.4 – 5.7)
11- 16 years	Negative	123	12(9.8)	1	11(8.9)	1	18(14.6)	1
	Positive	55	6(10.9)	1.1(0.4 – 3.2)	8(14.6)	1.7(07 – 4.5)	12(21.8)	1.7(0.7 – 4.1)
KSHV seropositivity[#] in the different race groups – children 1.5 to 16								
Black[†]	Negative	528	40(7.6)	1	44(8.3)	1	68(12.9)	1
	Positive	247	35(14.2)	2.0 (1.2 – 3.3)	30(12.2)	1.5(0.9– 2.5)	51(20.7)	1.8 (1.2 – 2.6)
White[†]	Negative	63	4(6.3)	1	3(4.8)	1	4(6.4)	1
	Positive	8	2(25.0)	4.9 (0.7 – 32.7)	1(12.5)	2.9(0.3 – 31.3)	2(25.0)	4.9 (0.7 – 32.7)
Other[†] ***(Asian & Coloured)***	Negative	36	5(13.9)	1	3(8.3)	1	6(16.7)	1
	Positive	7	2(28.6)	2.4 (0.4 – 16.5)	1(14.3)	1.8(0.2 – 20.7)	2(28.6)	2.0 (0.3 – 12.8)

[†]Children under 18 months excluded because of the possible presence of maternal antibodies. [#]KSHV status refers to testing either seropositive to lytic K8.1 or Latent Orf73 antibodies *unadjusted odds ratios, adjusting for age of mother , age of child and or HIV status did not produce significant odds ratio changes

KSHV negative (have no detectable antibodies to either the lytic K8.1 or latent Orf73) in different age groups.

For children 1.5 to 3 years of age, there was no obvious association between maternal and child KSHV seropositivity ($p>0.05$). However, the risk of the child being KSHV seropositive if born to a positive mother was higher in older age groups between 4 and 10 years, disappearing in the oldest age group of 11 and 16 year olds (Table 3.3). When divided by race, the increased risk for KSHV infection in children was apparent for children born to black mothers with KSHV infection rather than those born to uninfected black mothers (OR: 1.8; 95% CI: 1.2 – 2.6). However, the risk was not obvious in the non-black populations and was (OR: 4.9; 95% CI: 0.7 – 32.7 and OR: 2.0 95% CI: 0.3 – 12.8) in whites and the Asian & Coloured (Other), respectively (Table 3.3).

The association between the expression of KSHV lytic K8.1 and latent Orf73 antibodies in children and KSHV status of the mother was also measured (Table 3.3). There was no association between expression of latent Orf73 antibodies and the KSHV status of the mother. However, children were more likely to express lytic K8.1 antibodies if born to KSHV seropositive mothers than to a KSHV seronegative mother. Nevertheless, no clear association between the status of the mother and the child's was noted for the latent Orf73, especially when age and race were considered (Table 3.3) ($p > 0.05$).

3.11 HIV status of children and their mothers

Two thousand two hundred and forty (2240) serum samples from 1075 mothers and 1165 children were tested for HIV infection and the results are shown in Table 3.5 . Overall, HIV prevalence in both children and their mothers was 15.9% (356/2240). In general, HIV seroprevalence was more than two fold higher in all mothers than in children at 23% and 9%, respectively (Table 3.4). In children greater than 1.5 years of age, HIV seroprevalence ranged between 6% and 8% and was not significantly different

(p<0.75). HIV seroprevalence did not differ by the age group of the mother (Table 3.4 & Figure 3.5).

Table 3-4: HIV status of children and mothers, and KSHV seropositivity by HIV status

		HIV Status			KSHV seropositivity by HIV Status				
		All			HIV Negative		HIV Positive		
		Tested	n(%)	P Value	Tested	n(%)	Tested	n(%)	P Value‡
A. Children	**Age groups (years)**								
	0 – 1.5†	301	*44(14.6)*		257	*37 (14.4)*	44	*15 (34.1)*	*0.0014*
	1.6 – 3	260	*21(8.1)*		239	*32(13.4)*	21	*5 (23.8)*	*0.191*
	4 - 6	201	*12(6.0)*	$\chi^2 = 0.84$; *p=0.83*	189	*26(13.8)*	12	*3(25.0)*	*0.284*
	7 - 10	196	*13(6.6)*		183	*31(16.9)*	13	*3 (23.1)*	*0.573*
	>10	207	*15(7.3)*		192	*29(15.1)*	15	*4(26.7)*	*0.240*
	Race groups								
	Black	971	*95(9.8)*		876	127(14.5)	95	*25(26.3)*	*0.0026*
	White	115	*6(5.2)*	$\chi^2 = 2.9$; *p= 0.075*	109	11 (13.1)	6	*3 (50.0)*	*0.0038*
	Other	61	*2(3.3)*		59	11(18.6)	2	*1(50.0)*	*0.573*
	Unknown††	18	*2(11.1)*		16	6 (37.5)	2	*1(50.0)*	*0.739*
	All	**1165**	***105(9.0)***		**1060**	**155 (14.6)**	**105**	***30(28.6)***	***0.0002***
B. Mothers	**Age groups (years)**								
	≤25	317	*74(23.3)*		243	196(19.3)	74	*32 (43.2)*	*<0.0001*
	26 – 29	228	*59(25.9)*	$\chi^2 = 1.4$ *p= 0.7*	169	51(30.2)	59	*24(40.2)*	*0.14*
	30– 34	254	*59(23.2)*		195	50(25.6)	59	*20(33.9)*	*0.2*
	≥ 35	276	*59(21.4)*		217	62 (28.6)	59	*27(45.8)*	*0.01*
	Race groups								
	Black	880	*232(26.4)*		648	*179(27.6)*	232	*96 (41.4)*	*<0.0001*
	White	113	*8(7.1)*	$\chi^2 = 36.8$;	105	*14(13.3)*	8	*1 (12.5)*	*0.947*
	Other	63	*5(7.9)*	*p<0.0001*	58	*12(20.7)*	5	*2(40.0)*	*0.323*
	Unknown††	19	*6(31.6)*		13	*5(38.5)*	6	*4(66.7)*	*0.265*
	All	**1,075**	***251(23.4)***		***824***	**210(25.5)**	***251***	**103(41.0)**	***<0.0001***

†Children less than 18 months excluded from trend analysis because of the possible presence of maternal antibodies.

Although not significant, differences were noted in children by race group, detection of HIV infection was highest in Black followed by White then Coloured and Asian children at 10%, 5% and 3%, respectively (p = 0.075). In mothers the overall HIV seroprevalence was 23% and was not significantly different amongst the age groups (p=0.7). However,

HIV seroprevalence was significantly different amongst the different racial groups (p< 0.0001). The overall HIV prevalence was more than threefold higher in the Black than in the White and Other (Coloured and Asian) participants at 26.%, 7% and 8%, respectively (p< 0.0001) (Table 3.4).

3.12 KSHV seropositivity and HIV status

Figure 3.5 summarises the seropositivity to antibodies to the lytic KSHV K8.1 and latent Orf73, including KSHV seroprevalence in children and mothers according to their HIV status. In both children and mothers the KSHV seropositivity was significantly higher in HIV positive than negative subjects. In children, both lytic and latent KSHV seropositivity was 2 fold higher in HIV positive than negative children.

The overall lytic KSHV seropositivity was 9% in HIV negative and 20% in HIV positive children and similar distributions were noted for the latent KSHV seropositivity. The overall KSHV seroprevalence in HIV negative mothers was 17% for both the lytic and the latent Orf73, while in HIV positive mothers, it was 32% and 30% for the lytic and latent Orf73 respectively (Table 3.4).

Table 3.5 shows the seroprevalence of KSHV (lytic KSHV K8.1 or latent Orf73 seropositive) in children who were either HIV seronegative or seropositive and also in mothers who were HIV seronegative or seropositive. KSHV seroprevalence was 15% in all HIV seronegative children and 29% all HIV seropositive children ($p < 0.001$). In mothers, the KSHV seroprevalence was 26% and 41% in those who were HIV seronegative and those who were HIV seropositive respectively ($p < 0.001$).

In general, HIV seropositive children have higher KSHV seroprevalence than HIV seropositive subjects (Table 3.5). The only trend for KSHV by age is noted in HIV seronegative mothers ($x2 = 7.7$; $p = 0.005$).

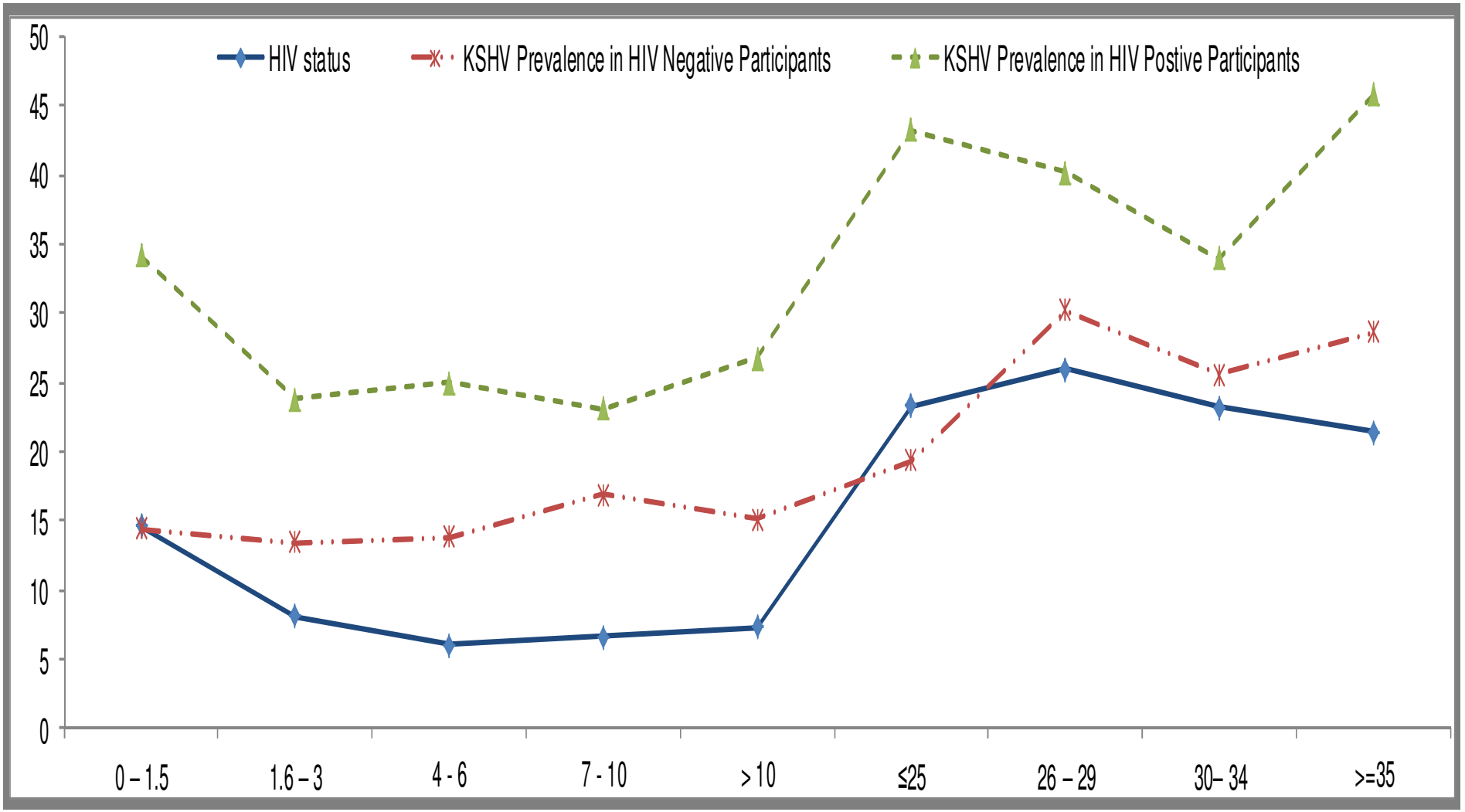


Figure 3-5: HIV seroprevalence and KSHV seroprevalence by HIV status in children and mothers

3.13 KSHV seropositivity in children by maternal HIV status

Table 3.5 shows the odds of being KSHV seropositive in children (above 18 months) born to HIV seropositive mothers and those born to HIV seronegative mothers, regardless of the KSHV status of the mother. In general, children born to HIV positive mothers are more likely to be KSHV seropositive than children born to HIV negative mothers (OR: 1.7; 95%CI: 1.3 – 2.4; p = 0.005). The risk is the same for female and male children (p = 0.005). The risk for being KSHV seropositive is 1.6 fold (OR 1.6; 95%CI: 1.2 – 2.3) for Black children and higher at 2.7 fold (OR: 2.7; 95%CI: 1.0 – 6.8) for non-Black children born to HIV positive mothers than if the mother is HIV seronegative.

Table 3-5: KSHV seropositivity in children in relation to the maternal HIV status

		KSHV Seroprevalence in children			
		Children of HIV Negative mothers (%)	**Children of HIV Positive Mothers (%)**	**P value**	**OR; 95CI‡**
All		118/862 (13.7)	81/376 (21.5)	0.0005‡	1.7 (1.3 – 2.4)
Gender					
	Female	61/404 (15.1)	41/171 (24.0)	0.01‡	1.7 (1.1 – 2.7)
	Male	57/458 (12.5)	40/205 (19.5)	0.02‡	1.7 (1.1 – 2.8)
Race					
	Black	199/164 (11.6)	8/31 (25.8)	0.035‡	1.6 (1.2 – 2.3)
	Non-Black	57/458 (12.5)	40/205 (19.5)	0.02‡	2.7 (1.0 – 6.8)
Age					
	1.5 – 3	38/232 (16.4)	23/107 (21.0)	0.25‡	1.4 (0.8 – 2.5)
	3 – 5	35/260 (13.5)	18/104 (17.3)	0.35‡	1.3 (0.7 – 2.5)
	6 - 9	18/188 (9.6)	20/75 (26.7)	0.0004‡	3.6 (1.7 – 7.4)
	≥10	27/182 (14.8)	20/90 (22.2)	0.13	1.6 (0.8 – 3.1)

‡ The odds of children being KSHV seropositive if born to HIV positive mothers compared to if mother is HIV negative.

When children were divided into age groups and associations made with each age band, no significant associations were noted between KSHV status of children by HIV status of the mother (p > 0.1), except in children aged 6 – 9 years of age where the risk was 3.6 fold higher ($\chi 2$ =; p < 0.0004) (Table 3.5).

3.14 KSHV seropositivity in children by maternal KSHV and HIV status

KSHV seropositivity in the child was also related to the KSHV and HIV status of the mother (Table 3.6). The child was 2.1 fold more likely to be KSHV seropositive if the mother was HIV negative and KSHV seropositive (HIV^{-} & $KSHV^{+}$) compared to when the mother was seronegative to both HIV and KSHV (HIV^{-} & $KSHV^{-}$). However, no increased risk of being KSHV seropositive was noted for children if the mother was HIV seropositive but KSHV seronegative (HIV^{+} & $KSHV^{-}$) compared to if the mother was both HIV and KSHV seropositive (HIV^{+} & KSHV+).

Table 3-6: Percentages and odds ratios of KSHV seropositive children in relation to maternal HIV and KSHV serostatus.

Mothers status	**KSHV seropositive children†**)	Percentage (%)	Unadjusted OR (95% CI)*	*Adjusted OR (95% CI)*
HIV Negative Mothers				
KSHV-	526	12.9	1	1
KSHV+	91	21.3	2.3(1.5 – 3.7)	2.1 (1.3 –3.4)
HIV positive Mothers				
KSHV-	167	17.2	1	1
KSHV+	37	25.3	1.6 (0.9 – 3.0)	1.4 (0.7– 3.0)

†Children up to 18 months are not included.* Ratios adjusted for age of mother and age of child

The risk for KSHV seropositivity in children in relation to the KSHV and HIV status of the mother was further computed according to her status within the different age groups, as shown in Table 3.7. KSHV seropositivity was compared in children born to mothers who were seronegative to both HIV and KSHV, to those born to mothers who were seropositive to either HIV or KSHV and those who were seropositive to both. The risk for KSHV was obvious and highest in children born to mothers who were co-infected by both HIV and KSHV, up to 10 years of age. This association was not obvious in children above 10years of age. In the youngest children up to 3 years of age, the risk for KSHV infection was similar in children of mothers who were HIV negative divided by the KSHV

statuses of the mother. However, the association between KSHV seropositivity in children of HIV negative mothers was strongest in the children over 3 years, with children of KSHV positive mothers at least 3 times more likely to be KSHV seropositive than if the mother was not infected by KSHV.

Table 3-7: Percentages and odds ratios of KSHV seropositive children in relation to maternal HIV and KSHV status by age groups

		Children's KSHV serostatus		
	Mothers KSHV serostatus	**KSHV seropositive children†**	**Percentage (%)**	**OR (95% CI)***
0 – 1.5 years	HIV– & KSHV-	29/184	15.8	1
	HIV– & KSHV+	11/68	16.2	1.1(0.5 – 2.3)
	HIV+ & KSHV-	7/31	22.6	1.6 (0.6 – 4.2)
	HIV+ & KSHV+	10/27	37.0	3.4 (1.4-8.3)
1.6 – 3 years	HIV– & KSHV-	18/147	12.2	1
	HIV– & KSHV+	3/40	7.5	0.6 (0.2 – 2.3)
	HIV+ & KSHV-	5/35	14.3	1.2 (0.4 – 3.7)
	HIV+ & KSHV+	8/29	27.6	3.0 (1.1 – 8.0)
4 - 6 years	HIV– & KSHV-	11/118	9.3	1
	HIV– & KSHV+	11/39	28.2	3.5 (1.3 – 9.1)
	HIV+ & KSHV-	6/30	20.0	2.6(0.9 –8.1)
	HIV+ & KSHV+	0/16	0	-
7- 10 years	HIV– & KSHV-	12/104	11.5	1
	HIV– & KSHV+	13/49	26.5	2.9 (1.2 – 7.0)
	HIV+ & KSHV-	5/29	17.2	1.7 (0.5 –5.4)
	HIV+ & KSHV+	8/20	40.0	5.2 (1.7– 15.4)
> 10 years	HIV– & KSHV-	13/91	14.3	1
	HIV– & KSHV+	10/29	34.5	3.5 (1.3 – 9.4)
	HIV+ & KSHV-	3/26	11.5	0.7 (0.2 –2.6)
	HIV+ & KSHV+	2/19	10.5	0.7 (0.1– 3.6)

3.14.1 KSHV seropositivity in children in relation to maternal KSHV antibody titre levels

The KSHV seropositivity of the child was measured against the maternal KSHV antibody titre levels indicated in Table 3.8. The antibody titre levels of the mother were divided into the following 3 groups: low, medium and high antibody titre levels. As described in

chapter 2 (section 2.2.5), plate to plate and day to day variations of the assays were taken into consideration when the cut-off values for the antibody titre levels were made. The lytic K8.1 and latent Orf73 antibody titres of all mothers who were seropositive to KSHV antibodies were divided in two groups' assigned medium and high antibody titre levels and all seronegative antibodies were grouped as low antibody titre levels.

Table 3.8 shows the risks for KSHV seropositivity in children aged 1.6 to 10 years for the latent KSHV ORF73 and lytic KSHV K8.1 assays in relation to maternal KSHV antibody levels and HIV status. Overall there was no increased risk of seropositivity in children observed with increasing maternal levels of antibody to ORF 73 (p_{trend} = 0.12). Seropositivity to latent Orf73 seemed to be marginally associated with medium maternal latent Orf7 but this association was not clear (OR = 4.0, 95% CI: 1.0 – 9.1: P Trend, 0.12).

The risk of KSHV K8.1 seropositivity in children was higher with increasing maternal levels of antibody to KSHV K8.1 (OR = 3.2, 95% CI: 1.6 to 6.1; P trend, 0.0001), regardless of the HIV status of the mother. When divided by HIV status, the increased risk with increasing maternal lytic K8.1 antibody level was higher if the mother was HIV-negative (p_{trend} = 0.0002). This association was not apparent in HIV positive mothers (p_{trend} = 0.09) (Table 3.8C).

Table 3-8: Risks for KSHV seropositivity in children aged 1.6-10 years in relation to maternal KSHV antibody levels and HIV status

	All Mothers		HIV Negative mothers			HIV Positive mothers	
Mothers' KSHV Antibody Levels	**KSHV seropositive Children n/total (%)**	**OR[#](95% CI)**	**KSHV seropositive Children n/total (%)**	**OR[#](95% CI)**		**KSHV seropositive Children n/total (%)**	**OR[#](95% CI)**
A. KSHV seroprevalence[†]							
Negative	62/507 (12.2)	1	41/369 (11.1)	1		16/94(17.0)	1
Positive	46/214 (21.5)	1.9 (1.3 – 3.0)	27/128 (21.1)	2.0 (1.2 – 3.5)		16/65 (24.6)	1.6 (0.7 –3.6)
All	108/721 (15.0)		68/497 (13.7)			32/159 (20.1)	
					1.6 (1.0-2.6)[§]		
B. Seropositivity to Latent KSHV ORF73							
Low (Negative)	47/567 (8.3)	1	34/409 (8.3)	1		8/111 (7.2)	1
Medium	11/80 (13.8)	1.8 (0.9 – 3.6)	4/44 (9.1)	1.0 (0.3 – 3.0)		6/25 (24.0)	4.0(1.0 – 9.1)
High	9/74(12.2)	1.5 (0.7 – 3.3)	5/44 (11.4)	1.3 (0.5 – 3.6)		3/23 (13.0)	2.0 (0.9 – 9.3)
All	67/721 (9.3)	*p_{trend}*=0.12*	43/497 (8.7)	p_{trend}*= 0.50		17/159 (10.7)	*p_{trend}*=0.12*
					1.4 (0.7-2.6)[§]		
C. Seropositivity to Lytic KSHV K8.1							
Low (Negative)	44/571 (7.7)	1	30/ 409 (7.3)	1		12/107 (11.2)	1
Medium	15/79 (19.0)	2.8 (1.5 – 5.3)	11/49 (22.4)	3.6 (1.7 – 7.8)		4/28 (14.3)	1.3 (0.4– 4.5)
High	15/71 (21.1)	3.2 (1.6 – 6.1)	8/39 (20.5)	3.2 (1.3– 7.6)		6/24 (25.0)	2.6 (0.9– 7.9)
All	74/721 (10.3)	*p_{trend}*<0.0001*	49/497 (9.9)	*p_{trend}*=0.0002*		22/159 (13.8)	*p_{trend}*=0.09*
					1.3 (0.7-2.3)[§]		

[#] Adjusted for age of mother and age of child. [§] Risk (OR, 95%CI) of KSHV infection in children in HIV positive compared with HIV negative mothers. Adjusted for age of mother and HIV seropositivity in child. * p^{trend} refers to values based on $\chi 2$ test for trend

3.15 Discussion

3.15.1 KSHV seroprevalence

In children, KSHV seropositivity was not associated with an increase in age ($p = 0.42$), unlike in mothers where KSHV seropositivity increased with increasing age ($p = 0.03$). However, the overall KSHV seropositivity increased from 16% in children to 30% in mothers ($p < 0.001$). This confirms findings of other African studies, in South Africa, Uganda, Cameroon, and Tanzania, which have consistently indicated an increase in KSHV seropositivity with increase in age from childhood, stabilising in puberty and reaching peak prevalence in adulthood (Dedicoat et al, 2004; Stein et al, 2004; Wojcicki et al, 2004, Mayama, 1998, de The, 1999, Gessain, 1999, Mbulaiteye, 2003). Several studies in France, Italy and Brazil have also reported an increase in KSHV seroprevalence with an increase in age (Plancouline, 2002; Freitas, 2002).

Maternal KSHV seroprevalence in this study, as indicated by seropositivity to either the lytic K8.1 or latent Orf73 antibodies, was lower than that reported in another study of South African mothers using the same classification criteria (30% *vs* 46%) (Dedicoat , 2004). However, KSHV seroprevalence of children in both this study and the abovementioned South African study was within close range (15% vs. 18%). KSHV seroprevalence reported in South African children is lower compared to that reported in other African countries. In Uganda, seroprevalence for children < 5 and those 5-9 years was as high as 37% - 58% (Mayama, 1998). In Cameroon increases of KSHV seropositivity to 48% in children up to 15 years (Gessain, 1999), have been reported and this trend matches that of Egyptian children (Andreoni, 1999). The prevalence of KSHV reported in a study involving Jewish children was comparable to that of children in this study (Davidovici, 2001). However, low KSHV prevalence has been reported in healthy children in Brazil (Souza, 2004, Machado, 2005).

3.15.2 Mother to child transmission

The high prevalence of KSHV antibodies found in children in this study and all other settings suggests that KSHV transmission in Africa and in countries where KSHV is common is unlikely to be sexual. In countries where KSHV is common amongst young children, non-sexual transmission routes may exist and infection from the mother seems to play an important role (Bourboulia et al, 1998, Dedicoat et al, 2004, Mbulaiteye et al, 2004). Several studies have excluded vertical transmission of the virus from mother to child. However, in certain studies in Africa and other studies of the Jewish population in Israel, evidence for horizontal transmission has been reported and the suspected routes are intra- and extra- familial (Mbulaiteye, 2005, Davidovici, 2001, Plancouline, 2000).

This study adds to the available information that children born to mothers who are KSHV seropositive are at a higher risk of being KSHV seropositive than children born to KSHV seronegative mothers. However, most studies on mother to child KSHV transmission in Africa have shown the association in the black population and none have included an association in non-black mothers and their children. In this study African children over 18 months of age born to KSHV infected mothers were 1.7 fold more likely to be KSHV positive than children born to KSHV uninfected mothers. Unexpectedly the risk was even higher in children born to mothers in other racial groups if the mother was KSHV seropositive than if they were born to KSHV seronegative mothers. However, the numbers were too small to draw a compelling inference. When divided into age groups, the risk of KSHV seropositivity if the mother was KSHV seropositive was only significantly higher in the 6 to 9 year olds. Nine percent (92/986) of children in this study, who were KSHV seropositive, were born to mothers who are KSHV seronegative, suggesting other possible transmission routes.

KSHV was only recently discovered and available information on associations of KSHV and probable routes of mother to child transmission is not consistent for all studies. In a

South African pilot study in 1999 that looked at the effect of maternal antibody titres to KSHV on mother to child transmission, Sitas and co-workers showed that the proportion of children who were seropositive for KSHV increased in relation to the maternal KSHV antibody titre. While in a study conducted on Zambian women and their children, He and coworkers, showed that all children with Kaposi's sarcoma had mothers who were KSHV seropositive, while not all children whose mothers had Kaposi's sarcoma were KSHV seropositive. In a study conducted in children born to KSHV seropositive Haitian and American mothers, all 9 children were not infected with KSHV.

In a study of KSHV K1/V1 phylogenetic comparisons in 2002, Cook and co-workers observed the existence of identical and non-identical DNA sequences between family members, suggesting that KSHV transmission can be both intra- and extra- familial. This adds to the theory that patterns of KSHV transmission in endemic regions may be more complicated than merely from mother to child. The presence of KSHV antibodies in children of KSHV seronegative mothers suggests that children can also acquire KSHV horizontally from sources other than the mother. In 2000, Plancouline and co-workers demonstrate no significant KSHV association between father-mother and father-child pairs ($p > 0.05$). However a significant association was noted between siblings and between mothers and their children (OR: 6.5; $p < 0.0001$), respectively. A stronger association between mother to child pairs was noted if the child was <10 years old (OR = 6.2, $p = 0.006$) than if the child was older (OR: 3.5; $p < 0.001$) (Plancouline et al. 2000). They suggested that, in an endemic area, KSHV is mainly transmitted before the age of 15 years. Of great importance is the lack of association they reported in father and mother pairs.

The most probable and supported route for mother to children transmission of KSHV, involves salivary contact, which also may explain viral acquisitions between siblings in crowded conditions. The role of saliva as a reservoir for KSHV has been explored in several studies but results are contradictory. For example, in a study conducted by

Dedicoat and colleagues in 2004, an association was only apparent with a very high salivary KSHV DNA titre, and the DNA could not be isolated in all subjects who were KSHV seropositive. While in another study, KSHV DNA could not be isolated in saliva of KSHV seropositive subjects. In has also been postulated that, if a great source of KSHV transmission could be through the saliva or close interpersonal contact, father-mother association would be expected to be high. The study could not show any strong associations between these partners if one was infected.

3.15.3 HIV status

In this study, the overall HIV prevalence increased from 9% in children to 23% in mothers. The seroprevalence of HIV decreased with increase in age of the child up to 10 years ($p < 0.001$) but was the same across all age groups of mothers ($p > 0.7$). As expected, the seroprevalence of HIV was highest in black mothers and their children than in other racial groups. The seroprevalence of HIV for mothers in this study is in agreement with the national HIV prevalence reported for South African females attending antenatal clinics (South African Department of Health, 2004) and the general seroprevalence for children as reported by Statistics South Africa in 2004. Children in this study have a lower HIV seroprevalence than children in another mother child study conducted in South African children in KwaZulu Natal province (Dedicoat et al, 2004). However, this is also consistent with other HIV studies in the country were KwaZulu Natal is shown to have the highest HIV-AIDS prevalence than in the other South African provinces (Statistics South Africa, 2004). The high HIV infection in children reflects an increased rate of mother to child transmission and during this period, the use of antiretroviral therapy to prevent MTCT was very limited. However, a proportion of children in the study were born to HIV negative mothers, suggesting different modes of acquisition. All samples, which were discordant for mother and child pairs, were retested to confirm the findings. Nevertheless, this subject was not explored further as it was not

the purpose of this thesis and similar finding on mother child HIV discordant statuses has been reported in other South African studies (Shisana et al, 2005)

3.15.4 KSHV and HIV

The epidemiological association between KSHV transmission and HIV infection remains unclear. In this study, the seroprevalence of KSHV was twofold higher if the child was HIV seropositive than if the child was HIV seronegative ($p< 0.0002$). Similarly, KSHV seropositivity was higher in HIV seronegative mothers than HIV seropositive mothers ($p < 0.0001$). This observed association of KSHV seropositivity and HIV status has been shown in other local studies (Sitas et al, 1999, Dedicoat, 2004). In addition, this study shows that the seroprevalence of KSHV in children born to HIV positive mothers is significantly higher than that of children born to HIV negative mothers (14% vs. 22%). The prevalence is similar between male and female children ($p > 0. 6$). A similar pattern of higher KSHV seroprevalence in HIV seropositive than HIV seronegative mothers was noted for all racial groups ($p < 0.04$). Our study also shows a greater risk for KSHV in children up to 10 years of age born to mothers who are co-infected by both HIV and KSHV, than if the mother is infected by KSHV alone. No risk was noted if the mother was HIV positive only but KSHV seropositive compared to when the mother tested negative to both viruses.

This association between KSHV seropositivity and HIV infection has been confirmed in other studies. In a local study by Dedicoat and colleagues, 2004, maternal HIV infection alone was not associated with increased risk of KSHV in the children of KSHV seropositive mothers but increased if the mother was both HIV and KSHV positive. While in a study by Gambus and colleagues in 2001, the seroprevalence of KSHV was more than three fold higher in HIV infected than uninfected participants.

However, there are studies that report no association between HIV and KSHV infections. In one study conducted in a population with lower HIV prevalence, it was concluded that

HIV was unlikely to play a role in the high risk for mother to child transmission of KSHV (Plancouline et al, 2000). Similarly, in another study conducted in Malawian hospitalised patients, De Santis and colleagues, 2000, suggested that KSHV seroprevalence was not associated with HIV status. Also, in a Zambian study conducted amongst pregnant women without Kaposi's sarcoma, women with Kaposi's sarcoma and children with Kaposi's sarcoma, the prevalence of KSHV was not significantly different between the HIV seronegative (47%) and HIV seropositive (51%) subjects.

3.15.5 KSHV Seropositivity in Children in Relation to Maternal Kaposi Sarcoma–Associated Herpesvirus Antibody Levels

The study could not show a clear association between the maternal latent Orf73 antibody titres to the KSHV status of the child. High maternal latent Orf73 antibody levels were not associated with increased risk. However, a significant association was observed between the maternal increasing lytic KSHV K8.1 antibody levels and the risk for KSHV seropositivity in the children. This association was apparent in HIV negative mothers and was not clear in HIV infected mothers. However, a study in South Africa has shown a greater association between increasing KSHV antibody titre in HIV-seropositive compared with HIV-seronegative individuals (Bourboulia, 1998).

Expressions of lytic K8.1 antibodies suggest an active phase of the KS virus, while latent antibodies suggest a dormant phase of the virus. Therefore, these findings may suggest that mothers with well-controlled infection characterized by high latent but low lytic antibody levels are less likely to be a source of KSHV infection for their children than mothers who are undergoing frequent reactivation of virus and viral shedding, as characterized by high levels of lytic but not latent antibodies.

3.15.6 Conclusion

In this study, the risk of acquisition of KSHV was higher among children of KSHV-seropositive mothers, which confirms that KSHV-infected mothers are a major source of

KSHV infection in children. Furthermore, KSHV infection is considerably higher in HIV-infected subjects in South Africa, a finding that has been shown to be true by other South African studies. Although KSHV seroprevalence was significantly higher in children and mothers who were infected with HIV, the HIV status of the mother was only marginally associated with an increased risk of KSHV seropositivity in the child.

However, there is significant literature that reports contrasting information on HIV status and KSHV infection indicating that KSHV infection does not vary by HIV infection status. This subject will therefore require further exploration by conducting studies, which will be able to show the direct association between KSHV acquisition and HIV infection.

In South Africa and other African countries where HIV infection has been associated to increased KSHV seropositivity, the HIV epidemic may change the pattern of KSHV infection. The incidence of KS in the HIV-positive and HIV-negative populations in South Africa over the next few decades may be altered significantly. Thus, longitudinal studies would provide the most useful data to examine the effects of HIV on the transmission of KSHV in this population.

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4 Seroepidemiology of KSHV in South African Females Attending Antenatal Clinics

In African and Mediterranean countries where KSHV infection is very common in the general adult populations, defining the modes of transmission remains complex. Unlike in the United States and Northern Europe, where KSHV is common mostly in men who have sex with men (MSM), in these endemic regions KS and KSHV affect the general population and it is increasingly apparent that non-sexual modes of transmission play a significant role in the maintenance and spread of KSHV (Dedicoat et al, 2004, Mbulateiye et al, 2004). Heterosexual transmission routes have been described as one of the most probable sexual modes in general populations. KSHV infection is common in the South African adult population (Chapter 3, Sections 3.3 and 3.4). However, as with other African countries, associations between KSHV infection and other sexually transmitted infections (STIs), is not clear.

There are several studies from African and Mediterranean countries that show strong associations between KSHV, HIV and other STI's, such as syphilis, herpes simplex virus 2, gonococcal infections, etc. (Wilkinsons et al, 1999). On the other hand, contradicting studies within the same countries have been reported. In the United States, Europe, and Australia, KSHV is confined to the homosexual/bisexual populations and KSHV is thought to be largely transmitted through sexual routes. This is mainly because men who have sex with other men (MSM) are largely shown to be high-risk sexual groups practising high-risk sexual behaviours (Chapter 1, section 1.8).

Still, the lack of association between KSHV and STIs has also been reported in few studies within the United States. Of importance to this pattern is the finding of non-significant associations between KSHV infections between heterosexual partners in a study conducted in the United States. Evidently, there is increased demand for studies worldwide that will help disentangle the epidemiological conflicts related to the transmission of this virus.

HIV is indisputably associated with the development of KS and its incidence and therefore overall manifestation in epidemic countries and in high-risk groups has been exacerbated. In keeping with the HIV epidemic, there have been substantial increases in reported cases of KS by cancer registries and studies around Africa. In South Africa, KSHV and HIV co-infection is associated with up to a 50-fold increase in risk for developing KS. However, the role of HIV as a risk factor for KSHV infection in African countries, including South Africa remains unclear. Some reports show a strong association whereas others show none [Malope et al, 2007 & 2010, Dedicoat et al, 2004]. Several studies that show a strong association between HIV and KSHV infection fail to show a similar strong association with other sexually transmitted infections that are clearly associated with HIV infection (Campbell et al, 2009, Crum et al, 2003). Evidence against sexual transmission of KSHV in heterosexual populations continues to emerge.

Although it seems complex to distinguish the exact mode of KSHV acquisition in sexually active populations, emerging studies continue to support the existence of non-sexual transmission routes amongst heterosexual populations (chapter1, section 1.8.2). In Africa, nonsexual KSHV transmission has been associated with familial and other person-to-person interactions as well as environmental factors (Mbulaiteye et al, 2005 & 2006). KSHV infection has also been associated with sources of drinking water and with living in close proximity to rivers or streams. However, the role of vectors and environmental factors in KSHV endemic countries is a topic of on-going study.

In South Africa, studies are commonly conducted in pregnant women attending antenatal clinics, to measure HIV prevalence and the magnitude of other sexually transmitted infections (Rice et al, 2007; Shaikh et al 2006). These studies are used to estimate incidence, prevalence and the overall risk for HIV and STIs in the general population. Thus, understanding KSHV infection patterns in this group of women will provide a reasonable and comparable estimate of its impact in the same communities.

The current study aims to examine the seroprevalence of KSHV in pregnant women attending antenatal clinics and to identify the risk for KSHV infection in relation to already collected information on socio-demographic and geographical factors, HIV and syphilis serology.

4.1 Objectives

- To measure the overall seroprevalence of KSHV in South African females attending antenatal clinics
- To measure the seroprevalence of KSHV in South African females attending antenatal clinics in the different regions within the Gauteng province
- To measure the association between KSHV seropositivity and education and parity
- To measure the association between KSHV seropositivity and the two identified main river catchments
- To measure the association between syphilis infection and KSHV seropositivity
- To measure the association between HIV infection and KSHV seropositivity

4.2 Study Design

This was a cross sectional study that involved secondary data obtained from the recruitment of pregnant women attending public sector antenatal clinics in the Gauteng province of South Africa. The women formed part of a national HIV and sexually transmitted infections (STI) study conducted by the National Department of Health in 2001. A total of 38 clinics within the Gauteng Province took part in this study.

Women were recruited for the study at their first visit to the clinic during their current pregnancy. The 38 clinics were distributed throughout five municipal regions within the Gauteng Province. The municipal regions included were - Ekurhuleni (East Rand), Emfuleni (Vaal Triangle), Soweto, Tshwane (Pretoria) and West Rand (Figure 4.1). About 98% of the clinics

forming part of this study are based in the Township areas and are thus representative of the South African black population in the Gauteng Province, with only a few pregnant women recruited from clinics in town.

Gauteng province is the smallest, but second most populated, province in South Africa, occupying a total area of 17,010 km^2. It is mostly urbanized and is home to over 9.6 million people, over a fifth of the national population. The East and West Rand regions are dominated by mining (Figure 4.2), while the Vaal Triangle contains mainly manufacturing sectors with a mix of agriculture, heavy and petrochemical industries. Tshwane contains light industrial and residential areas, while Soweto is largely residential with residents working mainly in Central Johannesburg and the West Rand.

Information on age, education level, parity, gravidity and syphilis and HIV infection was collected as part of the original study. Permission to use the information and analyse the available serum samples for KSHV antibodies was obtained from the National Department of Health, Pretoria, South Africa. Ethics approval was granted by the University of the Witwatersrand Committee for Research on Human Subjects (Medical). (Protocol Number - M 961024) (Appendix B).

4.1 Study Participants

Originally, data were available from the Department of Health, Pretoria, South Africa, for approximately 4,354 females attending antenatal clinics from Mpumalanga (1,748) and Gauteng provinces (2,606). Data provided from the Mpumalanga province included information on the age and the HIV status of the subjects. Data provided for the Gauteng province included information on the name of the clinic, municipal region, age, race, highest education level, gravidity, parity, and RPR and HIV status of the women. Permission to use the leftover sera for

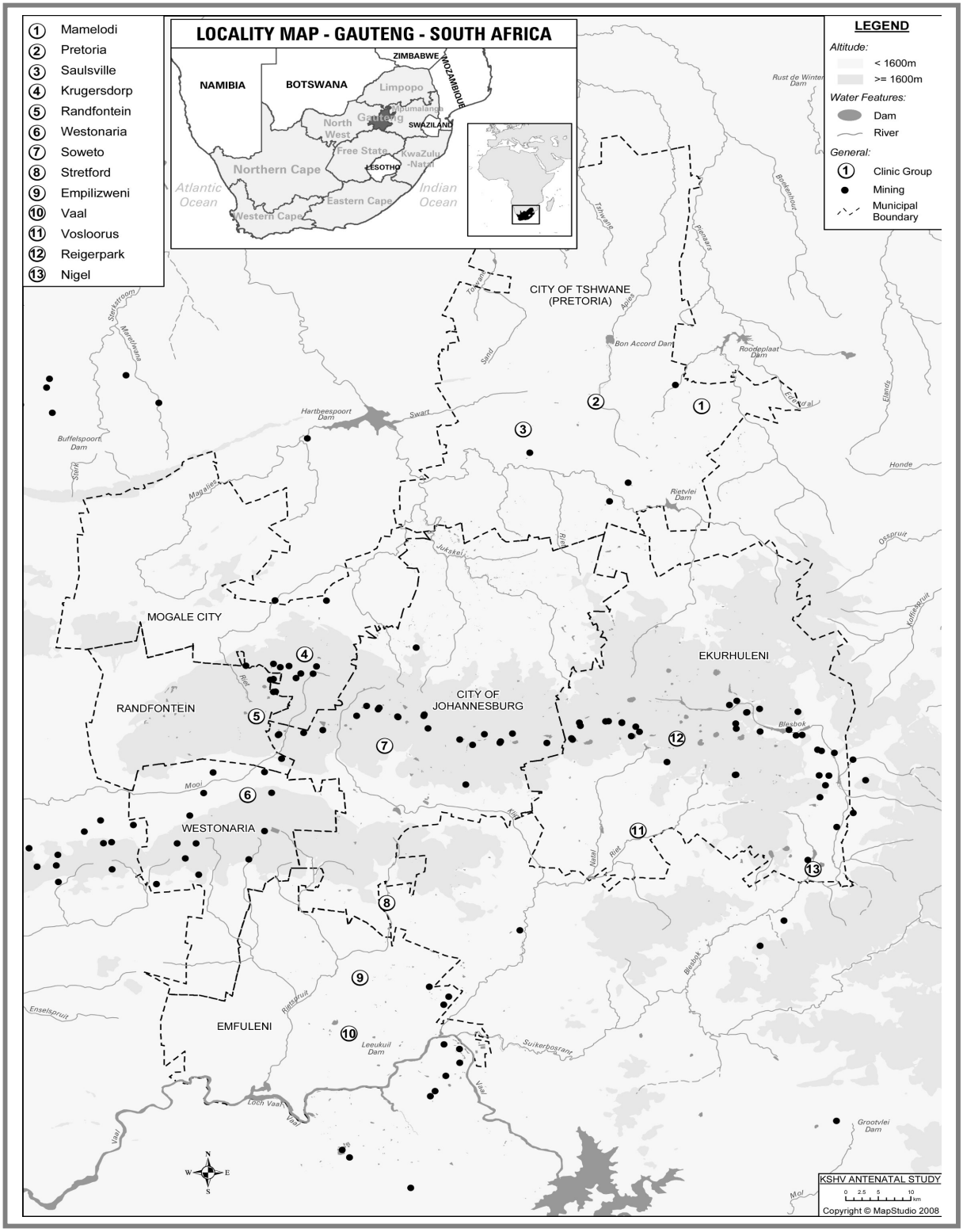


Figure 4-1: Map of Gauteng province showing the locations of the ante-natal clinics. Locations of ante-natal clinics from which study participants were recruited are shown according to the embedded legend. Altitude, water features and municipal boundaries are also shown according to the embedded legend.

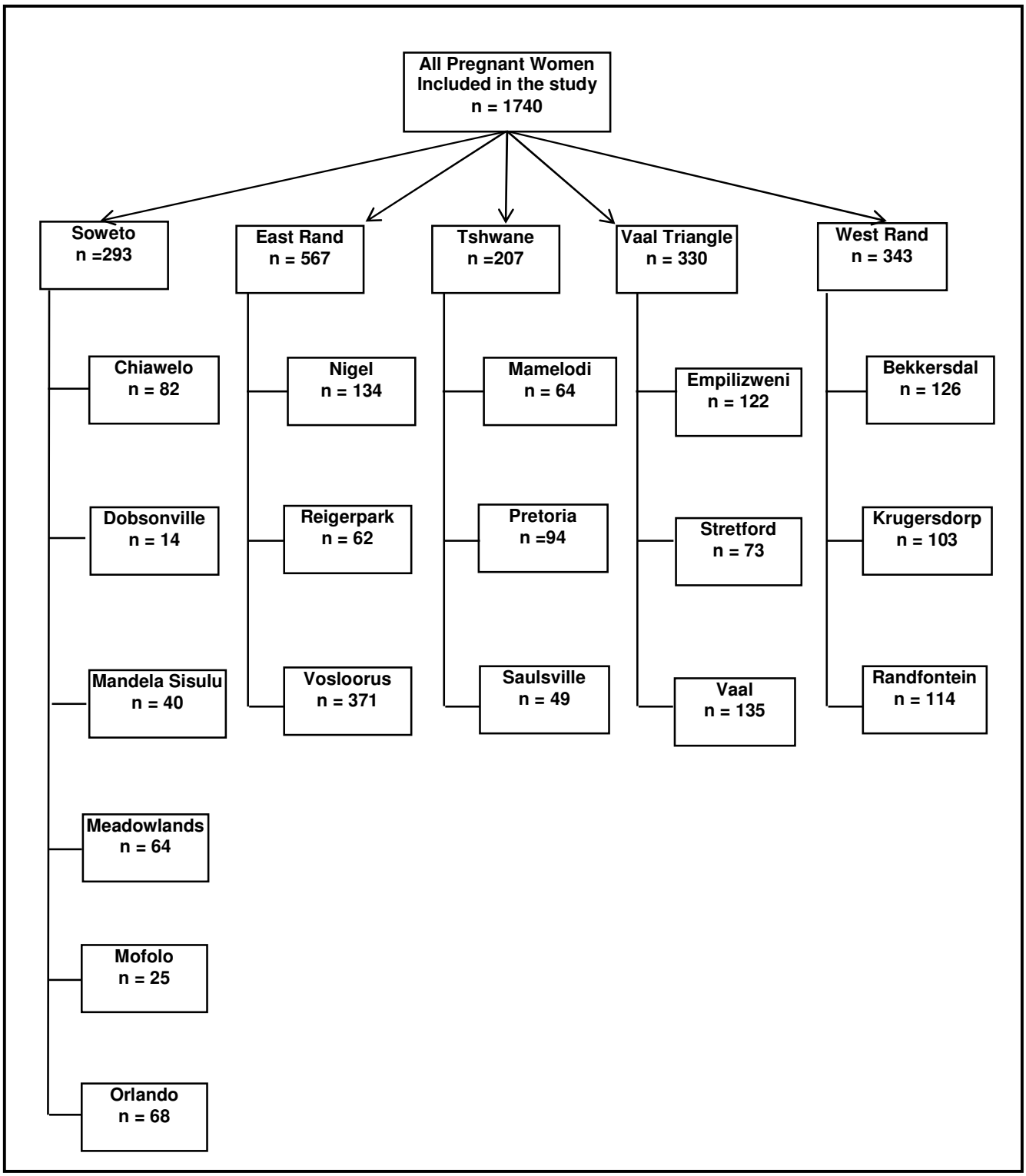


Figure 4-2: Municipal areas and grouping of clinics that the pregnant women included in the KSHV study were originally recruited from.

testing for KSHV antibodies was also sought and available sera were handed to the Cancer Epidemiology and Research Group (CERG).

Initially, a list for all the available leftover serum samples was compiled using de-identified study identity numbers recorded on the tubes in which the samples were stored. The list was then sent to Dr Jonathan Levin, then a biostatistician at the Medical Research Council, Pretoria, South Africa, to link the available results with the de-identified sample numbers. During the linking process, it was noted that only the de-identified Gauteng samples were linkable to the available results and it was not possible to link the Mpumalanga samples. The Mpumalanga information and unmatched samples were therefore not used for this study. The inclusion and exclusion process is illustrated on Figure 4.2 on the next page.

Only de-identified data with information linkable by unique participant identification numbers to the available stored serum samples was returned to the Cancer Epidemiology Research Group and was used for laboratory testing and data analysis. All 2324 stored serum samples were sufficient for KSHV testing. Of these, 302 had no other matching information apart from study identity and they were not tested for KSHV and were therefore excluded from the study.

Overall 2,004 pregnant women from Gauteng province were tested for KSHV. The majority (96%) of the women were from the black population (1919) and the other 4% (85) was made of 2 Asian, 62 coloured and 21 white participants. For this study, data was included for analysis if there was some demographic information and HIV results available. One hundred and seventy two participants were excluded from the study, as they did not meet the above inclusion criteria In addition, clinic information for 13 participants (12 black and 1 white) women was not consistent to the allocated area and therefore also excluded from any analysis as they could not be grouped into any of the areas. Overall, 1819 participants had complete information, of which 1740 where black and only 79 were from other population groups. Analysis for this chapter will

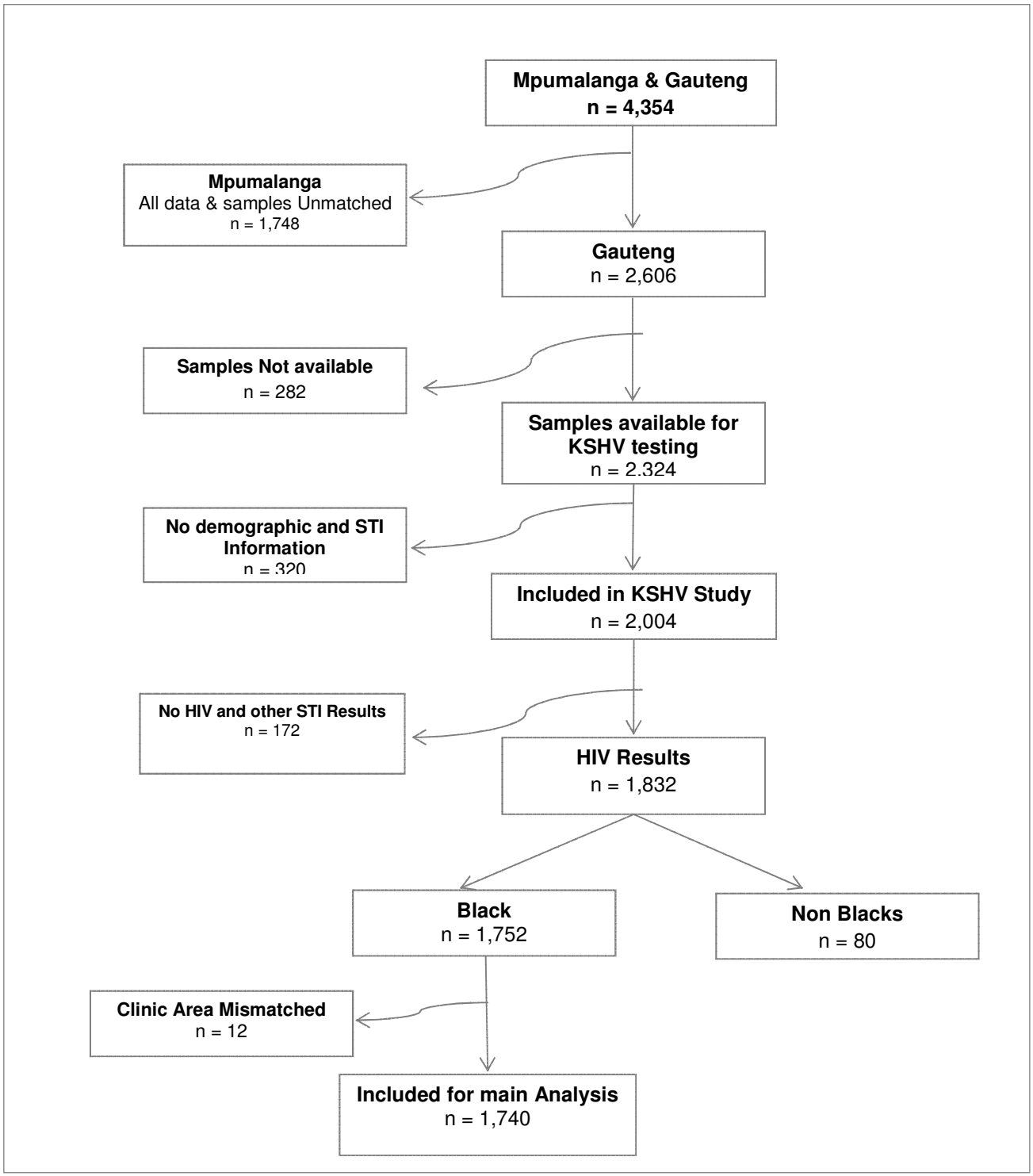


Figure 4-3: Inclusion criteria for the total number of pregnant women included in the KSHV study and the journal article publication (Appendix).

focus on the black population as there is sufficient sample size to make sound statistical inferences. The other racial group will be analysed separately, and no measures of association will be made. This is because no reasonable conclusion can be made based on the smaller sample sizes.

4.2 Laboratory Analysis

Laboratory diagnoses of HIV and syphilis infections were done by the National Health Laboratory Services (NHLS), South Africa. HIV antibodies were determined using an Abbot Axysm System for HIV-1/HIV-2 ELISA assays (Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois) and syphilis antibodies were determined using a nontreponemal carbon antigen test. All the above tests were conducted by the Department of Health, South Africa for the original study looking and HIV and STI in pregnant women.

For this study, leftover sera were stored at -20℃ before being shipped to the USA for determination of antibodies to lytic K8.1 and latent open reading frame (Orf) 73 KSHV antigens (Chapter 2). In-house assays for detection of antibodies to lytic K8.1 and latent Orf73 KSHV antigens were used as detailed in materials and methods (Chapter 2, section 2). Seropositivity to KSHV was further defined when subjects tested seropositive to either lytic KSHV K8.1 or latent KSHV Orf73 antibodies. Available information on age, race, education level, parity, gravidity, laboratory HIV and syphilis results was anonymously linked to the KSHV data using unique participant identification numbers.

4.3 Sample size and power calculations

Sample size calculations were done using Epi-Info statistical software. A confidence of 95% and a power of 80% were projected. It was then deduced that if about 2,000 serum samples were collected from pregnant women attending antenatal clinics, the sample size would be sufficient

to detect a prevalence odds ratio of 1.5 between syphilis prevalence (RPR result) and KSHV. This was based on the reported syphilis prevalence of 15% noted in the (South African Department of Health, Epidemiological Comments, 1999). Data for KSHV serology for South African populations was scarce so a seroprevalence of 15% was also assumed for this age group (Sitas et al, 1999a). Similar assumptions were made for associations between KSHV and HIV. Therefore, the 1740 serum samples obtained from black women in the clinics throughout the Gauteng province were lower than the anticipated sample size; however we thought it may be sufficient to provide significant statistical inferences for KSHV sero-epidemiology in these South African women. The additional 85 non-black women will be analysed separately to only indicate the KSHV seroprevalence in the group. However, results from this will be interpreted carefully as the sample size per population group was small and also they are likely to be less representative of the current non-black South African population.

4.4 Results

The mean age (±SD) for all the 1740 black pregnant women included in this study was 26.0(6.2), years (Table 4.1). The youngest pregnant woman included in the study was 13 years and the oldest was 46 years of age. Age was similar amongst the following four municipal regions: Ekurhuleni (26.5 (6.3)), Tshwane (25.8 (6.1)), West Rand (26.4 (6.1)) and Emfuleni (25.9 (6.3)) (Table 1). However, pregnant women attending clinics in Soweto (24.9 (5.8)), were significantly younger than those from the West Rand and Ekurhuleni municipalities ($p = 0.043$). (Table 4.1).

The participants were also divided by education level; information on the level of education completed was not available for 32/1,740 (1.8%) women. Of the 1,708 black women with a known education level, 117(6.9%) had less than 3 years of schooling (0 – 2 years), 513(30.0%) had 3 to 5 years, 119(7.0%) had 6 – 12 years of schooling and 959 (56.1%) had matriculated.

Table 4-1: Lytic and latent KSHV antibody titres and Odds Ratios (OR's) for seropositivity by municipal region, HIV status, syphilis status and level of education

			A. Lytic K8.1			B. Latent Orf73		
	Total (n)	Age Mean(±SD)	Antibody Titre μ_g(SD range)[#]	Total Positive (n)(% Positive)	Adjusted OR[^#] (95%CI)	Antibody Titre μ_g(SD range)*	Total Positive (n) (% Positive)	Adjusted OR (95%CI)
All Subjects	1,740	26.0(6.2)	0.77(0.31 -1.38)	568(32.6)	-	0.40(0.11-0.76)	568(32.6)	-
Municipal Region								
Soweto	293	24.9(5.8)[ab]	0.71(0.27-1.29)[b]	76(25.9)	1	0.37(0.09-0.73)	79(27.0)	1
Ekurhuleni	567	26.5(6.3)[a]	0.79(0.30 – 1.46)	204(36.0)	1.7(1.2 – 2.3)*	0.40(0.11-0.77)	195(34.4)	1.5(1.0 – 2.1)
Emfuleni	330	25.9(6.3)	0.71(0.32-1.21)	107(32.4)	1.5(1.0 – 2.1)*	0.36(0.10-0.68)	118(35.8)	1.7(1.2 – 2.4)*
Tshwane	207	25.8(6.1)	0.75(0.32 -1.32)[a]	53(25.6)	1.1(1.0 – 1.7)	0.40(0.13-0.74)	52(25.1)	1.0(0.7 – 1.6)
West Rand	343	26.4 (6.1)[b]	0.84(0.37-1.49)[ab]	128(37.3)	1.6(1.1 – 2.3)*	0.43(0.13-0.82)	124(36.2)	1.5(1.0 – 2.2)*
P_{4df}		*0.0063*	*0.0069*		*0.0233*	*0.0675*		*0.0185*
Education Level^								
< 2 years	117	28.5 (7.9)[a]	0.90(1.67-0.35)[a]	46(39.3)	1	0.45(0.12-0.90)	47(40.2)	1
2 - 5 years	513	26.5 (6.8)[b]	0.82(0.32-1.48)[b]	193(37.6)	0.8 (0.5 – 1.2)	0.40(0.11-0.85)	176(34.3)	0.6(0.4 – 1.0)
6 - 12 years	119	26.4 (5.6)	0.75(0.30-1.36)	35(29.4)	0.4(0.3 – 0.8)*	0.42(0.14-0.80)	37(31.1)	0.5(0.3 – 1.9)*
Post Matric	959	25.4 (5.5)[ab]	0.73(0.31-1.30)[ab]	286(29.8)	0.6(0.4 – 0.8)*	0.42(0.11-0.72)	300(31.3)	0.6(0.4 – 0.9)*
P_{trend}		*0.042*	*0.0019*		*0.002*	*0.0874*		*0.0426*
HIV Infection^								
Negative	1,338	26.2(6.4)	0.70(0.28-1.27)	345(25.8)	1	0.36(0.09-0.68)	341(25.5)	1
Positive	402	25.3 (5.4)	1.00(0.50-1.69)	223(55.5)	3.7(2.9 – 4.7)*	0.54(0.21-0.98)	227(56.5)	3. 8(3.0 – 4.8)*
P_{1df}		*0.011*	*< 0.0001*		*< 0.0001*	*<0.0001*		*< 0.0001*
Syphilis Infection								
Negative	1,442	25.9 (6.2)	0.77(0.31-1.38)	461(32.0)	1	0.39(0.10-0.75)	454(31.5)	1
Positive	298	26.4 (5.8)	0.79(0.34-1.38)	107(35.9)	1.0 (0.7 – 1.3)	0.43(0.15-0.81)	114(38.3)	1.2 (0.9 – 1.6)
P_{1df}		*0.24*	*0.55*		*0.8924*	*0.087*		*0.2395*

^ Education level and HIV status not recorded for 53 and 172 participants, respectively [#]μ_g(SD range) geometric mean and standard deviation range. Means with the same letter are significantly different (p, 0.05). [^#]Odds ratios adjusted for region, education level HIV and syphilis infections.* statistically significant Odds Ratios.

Those with 0 – 2 years of schooling were significantly older than all the other women at 28.5(7.9) years of age, with mean age (±SD) of 26.5(6.8) years for those with 2 - 5 years of education, 26.4(5.6) years for those with 6 – 12 years of education and 25.4(5.5) those who had matriculated and/or had higher level of education, p = 0.042 (Table 4.1).

The mean age (±SD) of the 1,740 black pregnant women was 26.0(6.2) years and was not significantly different from that of women in the other race groups at 24.2(6.3) years (p > 0.84). Overall, 72% (61) of the 85 women from the non-black population had matriculated compared to 56% (919) of the 1708 black women attending the antenatal clinics who had information on education (Table 4.1).

4.5 Lytic K8.1 and latent Orf73 Serology in the antenatal women

4.5.1 Lytic K8.1 and latent Orf73 Antibody titers

Antibody titre levels for lytic K8.1 and latent Orf73 are expressed as optical densities, and are shown in table 4.1. The geometric mean (µg) of the optical density for lytic K8.1 was 0.77(sg 0.31 - 1.38). The mean lytic K8.1 antibody level, was significantly heterogeneous between municipal regions (p_{4df} = 0.0069) and education levels (p_{4df} = 0.0019) (Table 4.1). Women in Tshwane and Soweto had lower lytic K8.1 optical densities than those from the West Rand (µg (sg):0.71(0.32 - 1.21) and .71(0.27 - 1.29), *vs.* 0.84(0.37 - 1.49), respectively (P4df = 0.0069)]. The µg (sg) for latent orf73 was 0.40 (0.11 - 0.76) and did not differ significantly between regions (p_{4df} = 0.0675) or education levels (p_{3df} = 0.0874). HIV positive women had significantly higher lytic and latent KSHV antibody levels than HIV negative women did (µg (sg): 1.0(0.50 - 1.69) *vs.* 0.70(0.28 - 1.27), (p_{1df} < 0.0001)) and 0.54(0.21 - 0.98), *vs.* 0.36(0.09 - 0.68), respectively) (p_{1df} < 0.0001). However, antibody titre levels for both the lytic K8.1 and latent Orf73 were not significantly different between women with and without syphilis infection (Table 4.1).

4.5.2 Concordance between Lytic K8.1 and latent Orf73 Assays

A total of 964 black women tested seronegative to both the lytic K8.1 and latent Orf73 antibodies. Not all pregnant women who were seropositive to lytic K8.1 were also seropositive to latent Orf73 and *vice versa*. Overall, 568(32.6%) participants were seropositive to lytic K8.1 and an overlapping 568(32.6%) were seropositive to latent Orf73. Overall, 776(44.6%) of the 1,740 participants were seropositive to either the lytic K8.1 or latent Orf73 antibodies (Tables 4.1 and 4.2) and were considered to be KSHV seropositive.

Table 4-2: Seropositivity to lytic K8.1 and latent Orf73 antibodies in black children and mothers and concordance between the lytic K8.1 and latent Orf73 assays.

	Latent Orf73-	**Latent Orf73+**	**All**	**Concordance**
Lytic K8.1-	964(54.4%)	208(12.0%)	**1,172(67.4%)**	*κ = 0.46*
Lytic K8.1**+**	208(12.0%)	360(20.7%)	**568(32.6%)**	*r = 0.46, p<0.0001*
All	**1,172(67.4%)**	**568(32.6%)**	**1740**	

Of the 881 participant who were KSHV seropositive, 232(26.3) had antibodies to lytic K8.1 only, 247 (28.0) had antibodies to latent Orf73 only and 402 (45.6%) had antibodies to both the lytic K8.1 and latent Orf73 antibodies. There was moderate concordance in seropositivity between the two (K8.1 and Orf73) assays (κ = 0.46 95% CI: 0.41 - 0.49). This is consistent with the previous study (Chapter 3). Table 4.1 also shows lytic K8.1 and latent Orf73 seropositivity by municipal region, education level, HIV and syphilis status. Both lytic K8.1 and latent Orf73 infections were associated with all the above mentioned factors, including HIV, but was not associated with the presence of syphilis.

4.6 KSHV Seropositivity

Table 4.3 indicates KSHV seropositivity and factors associated with KSHV seropositivity in black pregnant women. Seroprevalence to KSHV was 44.6% (776/1740) in all pregnant women. KSHV seroprevalence ranged from 42.4% in the youngest women less than 20 years of age and although not statistically significant was highest in the women from 21 – 25 years of age, increasing to 46.2% (p_{3df} = 0.2635). Those with the highest level of education had the lowest KSHV seroprevalences at 41.7% and those with the lowest years of formal education had the highest KSHV seroprevalences at 53.0% (p_{3df} = 0.0039). KSHV seroprevalence in the 85 non-black pregnant women was significantly lower than in the black population (p<0.04).

4.6.1 Socio-demographic Risk Factors for KSHV

4.6.1.1 Age, Education and selected characteristics

Seropositivity to KSHV in these women in their reproductive years was not associated with age (p_{trend} = 0.5988) (Table 4.3). An inverse trend for KSHV was noted with higher levels of education (p_{trend} = 0.0080). Those with higher education levels, at least 6 years of education and higher, were protected against KSHV infection. More than half (53.0%) of the 117 women with no formal education (< 2 years) were seropositive to KSHV decreasing to 41.7% in the 959 women with the highest high school matriculation certificate (p_{3df} = 0.0080). The number of pregnancies and live births had no effect on KSHV seropositivity, (p >0.28) (Table 4.3).

4.6.2 Geographic Risk Factors for KSHV

Women attending clinics within the Soweto and Tshwane municipal regions had the lowest KSHV seroprevalences at 35.4% and 37.2%, respectively. While those at the Ekurhuleni and West Rand Regions had higher KSHV seroprevalences, at about 48% and 49%, respectively (p4df = 0.0008) (Table 4.3). In the univariate model, KSHV was associated with the municipal region where the clinics are situated (p_{4df} 0.0030). Municipal region remained strongly associa-

Table 4-3: Risk factors (Odds ratios (OR)) for KSHV in pregnant women attending antenatal public clinics in the Gauteng province

			KSHV Seropositivity				HIV Seropositivity	
		Total Tested (n)	**Prevalence Total Positive n (%)**	**Unadjusted OR[#] (95%CI)**	**Adjusted OR (95%CI)**	**Prevalence Total Positive n (%)**	**Unadjusted OR (95%CI)**	**Adjusted OR[#] (95%CI)**
All Subjects		**1,740**	**776(44.6%)**	**-**	**-**	402(23.1%)	-	-
Age Group	≤ 20	**362**	154 (42.4)	1	1	87(24.0%)	1	1
	21 – 25	**533**	246 (46.2)	1.2 (0.9 – 1.5)	1.2 (0.9 – 1.7)	136(25.5%)	1. 1 (0.8 – 1.5)	1. 1 (0.7 – 1.5)
	26 – 30	**438**	192 (43.8)	1.1 (0.8 – 1.4)	1.1 (0.8 – 1.5)	108(24.7%)	1.0 (0.7 – 1.3)	1.0 (0.7 – 1.5)
	≥ 31	**407**	184 (45.2)	1. 1 (0.8– 1.5)	1.2 (0.9 – 1.6)	71(17.4%)	0.7 (0.4 – 0.9)*	0.7 (0.5 – 1.0)*
	P_{3df}			***0.6240***	***0.4830***		***0.0286***	***0.0373***
Municipal Region	Soweto	**293**	105(35.4)	1	1	72(24.6%)	1	1
	Ekurhuleni	**567**	274(48.3)	1.6(1.3 – 2.2)*	1.8 (1.3 – 2.4)*	147(25.9%)	1.0 (0.7 – 1.5)	1.1(0.7 – 1.6)
	Emfuleni	**330**	152(46.1)	1.5 (1.1 – 2.1)*	1.8 (1.1 – 2.6)*	63(19.1%)	0.8 (0.5 – 1`.1)	0.7 (0.5 – 1.1)
	Tshwane	**207**	77(37.2)	1.1 (0.7 – 2.5)	1.3(0.5 – 1.9)	29(14.0%)	0.5 (0.3 – 0.8)*	0.5(0.3 –0.8)*
	West Rand	**343**	168 (49.0)	1.7 (1.2 – 2.4)*	1.7(1.1 – 2.5)*	91(22.6%)	1.1 (0.8 – 1.6)	1.1 (0.7 – 1.7)
	P_{4df}			***0.0015***	***0.0095***		***0.0017***	***0.0013***
Education Level	< 2 years	**117**	69 (53.0)	1	1	17(14.5%)	1	1
	2- 5 years	**513**	253 (49.3)	0.9 (0.6 – 1.3)	0.7 (0.5 – 1.1)	119(23.2%)	1. 3(0.8 – 2.1)	1.4 (0.8 – 2.6)
	6 – 12 years	**119**	51 (42.9)	0.7 (0.4 – 1.1)	0.5 (0.3 – 0.8)	31(26.0%)	1.6(0.8 – 3.1)	1.4 (0.7– 2.8)
	Post Matric	**959**	400 (41.7)	0.6(0.4 – 0.9)*	0.6(0.4 – 0.8)*	228(23.8%)	1.4(0.9 – 2.1)	1.6(0.9 – 2.8)
	P_{3df}			***0.0080***	***0.0034***		***0.1645***	***0.3437***
Gravidity Group	1	**613**	269 (42.6)	1	-	144(22.8%)	1	-
	2	**481**	223 (46.4)	1.2 (0.9 – 1.5)	-	129(26.8%)	1.2(1.0 – 1.6)*	-
	≥2	**532**	246 (46.2)	1.2 (0.9 – 1.5)	-	106(28.0%)	0.8 (0.6 – 1.1)	-
	P_{2df}			***0.2863***			***0.0284***	
Parity group	0	**649**	279(43.0)	1	-	149(23.0%)	1	-
	1	**487**	223(45.8)	1.1 (0.9 – 1.5)	-	129(26.8%)	1.2(0.9 – 1.6)	-
	>2	**506**	235(46.4)	1.2 (0.9 – 1.5)	-	56(20.1%)	0.9(0.6 – 1.1)	-
	P_{2df}			***0.3594***			***0.0263***	
River Catchment	North	**279**	109 (39.1)	1	-	48(17.2%)	1	-
	South	**1461**	667 (45.6)	0.8 (0.4 – 1.5)	-	354(24.2%)	0.6 (0.4 – 0.9)*	-
	P_{1df}			***0.4312***			***0.0075***	
Syphilis	Non-Reactive	**1,440**	621 (43.1)	1	1	303(21.0%)	1	1
	Reactive	**298**	154 (51.7)	1.4 (1.1 – 1.8)*	1.2 (0.9 – 1.6)	97(32.5%)	1.8 (1.4 – 2.4)*	1.9 (1.4 – 2.5)*
	P_{1df}			***0.0028***	***0.1168***		***<0.0001***	***< 0.001***
HIV infection	Negative	**1,338**	496 (37.1)	1	1			
	Positive	**402**	280 (69.7)	3.9 (3.1 – 5.0)*	4.1 (3.1 – 5.2)			
	P_{1df}			***< 0.0001***	***< 0.0001***			

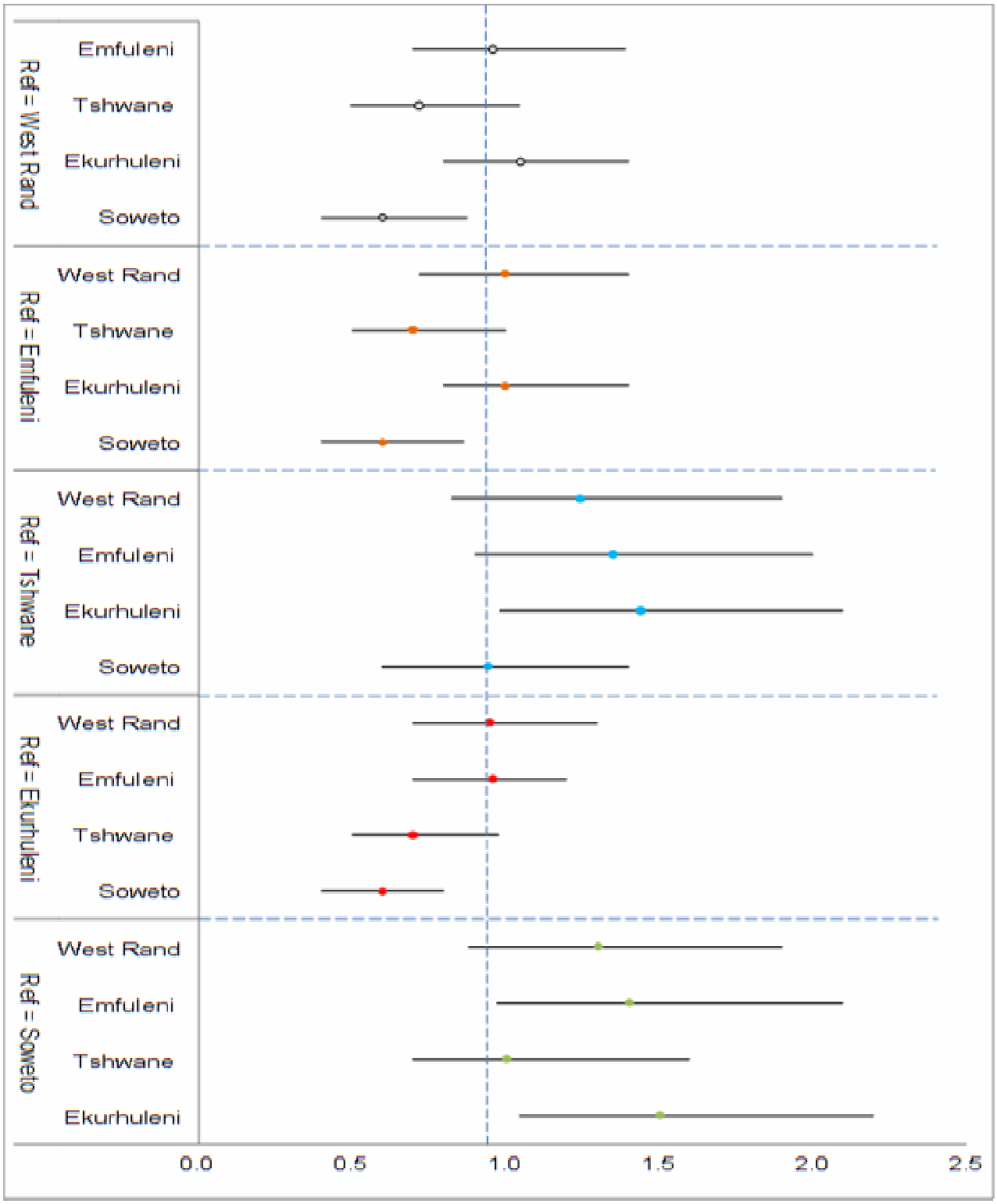


Figure 4-4: Association between KSHV and municipal regions indicating comparisons for each of the regions with all other municipalities

ted with KSHV infection in the adjusted model. Table 4.3 indicates the risk for KSHV in the other regions in comparison to Soweto. Risks of KSHV infection was higher in all pregnant women attending clinics in Ekurhuleni and Emfuleni and the West Rand regions, compared to women attending clinics in Soweto (AOR 1.8 95% CI:1.3 - 2.4; 1.8 95% CI: 1.1 - 2.6; and 1.7 95% CI: 1.1 - 2.5) (Table 2). While the risk for KSHV was similar between Soweto and Tshwane, women in the Tshwane region seem to be at a low risk for HIV infection compared to those from Soweto (AOR 0.5 95%CI: 0.3 - 0.8 (Figure 4.4).

To further explore this geographic variation in KSHV serology within this region, we looked at the river catchments within the different regions. It was clear that there were two main river catchment systems draining south and north (Figure 4.1). KSHV seroprevalence was higher in the areas with the southern river drainage (45.6%) than those to the north (39.0%), however this difference was not significant (p1df = 0.4, Table 4.3).

4.7 KSHV Seropositivity and Sexually Transmitted Infections

4.7.1 Seropositivity to HIV and syphilis infection

HIV status was available for all 1740 black pregnant women tested for KSHV and two were not tested for syphilis. The overall seroprevalence of HIV and syphilis, in pregnant women across all the clinics was 23.1% (402/1740) and 17.1% (278/1738), respectively. Overall, 34.6% of all the women had either HIV or syphilis but only 6% were infected with both (Table 4.3). HIV prevalence was highest in women aged 21 to 25 years and was lowest in the older women from 31 years of age and above, ranging from 17.4% to 25.5% (Table 4.3). Women attending clinics within the Tshwane municipal region had the lowest HIV prevalence at 14.0%, followed by those in the Emfuleni region at 19.1% and was highest in the Ekhurhuleni at 25.9% (P4df = 0.0017) (Table 4.3). Women with less than 2 years of formal education had the lowest HIV prevalence at

14.5% and those with 6 to 12 years of formal education had the highest HIV prevalence at 26.0% (p = 0.1645). HIV infection was marginally associated with total number of pregnancies. HIV infection was highest in women with syphilis infection at 32.5% and was 21.0% in the 1440 women who had no syphilis infection (AOR: 1.9; 95%CI: 1.4 - 2.5) (p < 0.0001).

4.7.2 Risk factors for HIV

HIV infection was associated with age of the women and municipality in which these clinics are situated. The older women aged 31 years and above were protected from HIV infection compared to the younger women under 20 years of age (A*OR: 0.7: 95%CI 0.5 – 1.0*). Also women of reproductive age attending clinics within Tshwane regions were protected against HIV infection compared to women from clinics within the Soweto region *(AOR: 0.5: 95%CI 0.3 – 0.8*). HIV infection was not associated with education level (p = 0.1645). HIV infection was also not associated with the number of live births and total number of pregnancies (p > 0.26) (Table 4.4). A 1.9 fold increased risk for HIV was noted in women with syphilis infection compared to those who were not infected (A*OR: 1.9: 95%CI 1.4 – 2.5*).

4.7.3 KSHV associated with HIV but not Syphilis infection

Seropositivity to KSHV was 69.7% (496/1338) in HIV infected black women and 37.1% (280/402) in uninfected women (p < 0.0001) (Table 4.3). Black pregnant women with syphilis also had higher KSHV seroprevalence than those without [51.7 %(154/298) vs 43.1 % (621/1440) respectively; p = 0.0028] (Table 4.3). The prevalence of HIV and syphilis were 12.7% and 14.9% in pregnant women without KSHV, and 36.1 and 19.9% in those with positive KSHV serology. The risk for KSHV was 4.1 fold higher in those with HIV infection (AOR: 4.1 95% CI: 3.1 - 5.2). In the unadjusted model, risk for KSHV seemed higher in those with syphilis infection than those without, but the association was lost after adjusting for HIV and the above factors (AOR 1.2 95% CI: 0.9 - 1.6) (Table 4.3). Also, KSHV antibody levels were higher in women with HIV but not in women with syphilis (Table 4.1)

4.7.4 KSHV seropositivity in HIV positive and HIV negative participants

Table 4.4 shows KSHV seropositivity in HIV negative and HIV positive women independently. More than a third (69.7%) of HIV positive women were infected with KSHV compared to 37.1% in the HIV negative group. In HIV negative women, KSHV was associated with the level of education (p3df = 0.0374), an association not noted in HIV positive women (p = 0.0574). HIV negative women with a highest level of education were protected from KSHV infection compared to those with no, or less than 2 years of, formal education (AOR:0.5; 0.4 – 0.8) (Table 4.4). The association between KSHV and municipal region in HIV negative women was not clear, with women in Ekurhuleni being at a 1.4 fold risk (*OR 1. 5: 95%CI : 1.0 – 2.*2) of KSHV infection compared to those in Soweto, however KSHV risk was similar in all the other regions when compared to Soweto.

In HIV positive women, KSHV seropositivity was highest (76.9%) in women aged 26 – 30 years and the risk for KSHV infection in this age group was 1.9 fold (AOR 1.9: 95%CI :1.0 – 3.7) compared to the youngest women aged < 20 years. KSHV in HIV infected women was strongly associated with municipal region, with women at Ekurhuleni, Emfuleni and West Rand Region at the highest risk for KSHV infection compared to those from Soweto (P4df = 0.0153). In both HIV negative and positive women, KSHV was not associated with syphilis infection, gravidity or parity.

Table 4-4: KSHV seropositivity in pregnant women attending antenatal public clinics in the Gauteng province by HIV status.

	A. HIV Negative				B. HIV Positive			
	Total (n)	**Prevalence Total n(%)**	**Unadjusted OR[#] (95%CI)**	**Adjusted OR (95%CI)**	**Total (n)**	**Prevalence Total n(%)**	**Unadjusted OR (95%CI)**	**Adjusted OR[#] (95%CI)**
All Subjects	**1338**	496(37.1%)	**-**	**-**	**402**	280(69.7%)	-	-
Age Group								
≤ 20	**302**	101 (36.7)	1	1	**87**	53(60.9 %)	1	1
21 – 25	**422**	153 (38.5)	1.1 (0.8 – 1.5)	1.1 (0.8 – 1.5)	**136**	93(69.1%)	1.4 (0.8 – 2.4)	1.6 (0.9 – 2.8)
26 – 30	**347**	109 (33.0)	0.9 (0.6 – 1.2)	0.9 (0.6 – 1.2)	**108**	83(76.9%)	2.1(1.1 – 4.0) *	1.9 (1.0 – .3.7) *
≥ 31	**352**	133 (39.7)	1. 1 (0.9 – 1.6)	1. 0 (0.7 – 1.5)	**71**	51(71.8%)	1.7 (0.8 – 3.2)	1.7 (0.8 – 3.4)
P_{3df}			*0.2131*	*0.2131*			*0.1134*	*0.1803*
Municipal Region								
Soweto	**221**	66 (29.9)	1	1	**72**	39(54.2%)	1	1
Ekurhuleni	**464**	172 (41.0)	1.6 (1.2 – 2.3)*	1. 5 (1.1 – 2.2)*	**147**	1082(70.2%)	1.9 (1.1 – 3.4)*	2.3 (1.2 – 4.4)*
Emfuleni	**204**	105 (39.3)	1.5 (1.0 – 2.2)*	1.4 (1.0 – 2.2)	**63**	47(74.6%)	2.4 (1.1 – 5.2)	2.4 (1.1 – 5.2)*
Tshwane	**274**	58 (32.6)	1.1 (0.7 –1.7)	1.1 (0.7 – 1.6)	**29**	19(65.5%)	1.6 (0.7 – 3.9*	1.5 (0.6 – 3.9)
West Rand	**260**	95 (37.7)	1.4 (1.0 – 2.1)*	1. 3 (0.9 – 2.0)	**91**	73(80.2%)	3.4 (1.7 – 6.9)*	3.2 (1.6 – 6.7)*
P_{4df}			*0.0180*	*0.0180*			*0.0093*	*0.0153*
Education Level								
< 2 years	**104**	50 (50.0)	1	1	**17**	12(70.5 %)	1	1
2- 5 years	**411**	159 (40.4)	0.7 (0.4 – 1.1)	0.7 (0.4 – 1.0)	**119**	94(79.0%)	1.6 (0.5 – 4.7)	1.5 (0.5 – 4.8)
6 – 12 years	**93**	35 (39,8)	0.7 (0.4 – 1.2)	0.6 (0.4 – 1.0)	**31**	16(51.6%)	0.4 (0.1 – 1.6)	0.4 (0.1 – 1.5)
Post Matric	**790**	247 (33.4)	0. 5(0.3 – 0.8)*	0.5 (0.4 – 0.8)*	**228**	153(67.1%)	0.9 (0.3 – 2.5)	0.9 (0.3 – 2.9)
P_{3df}			*0.0374*	*0.0374*			*0.0184*	*0.0574*
Gravidity Group								
1	**520**	172 (35.3)	1	-	**144**	97(67.4%)	1	-
2	**369**	134(38.1)	1. 1 (0.8 – 1.5)	-	**129**	89(68.9%)	1.1 (0.6 – 1.8)	-
≥2	**459**	165 (38.7)	1.2 (0.9 – 1.5)	-	**106**	81(76.4%)	1.6 (0.9 – 2.8)	-
P_{2df}			*0.4225*				*0.3931*	
Parity group								
0	**536**	178 (35.6)	1	-	**149**	101(67.8%)	1	-
1	**377**	133(37.2)	1.0 (0.7 – 1.4)	-	**129**	92(69.8%)	1.1 (0.7 – 1.8)	-
>2	**436**	160(39.3)	1.2 (0.9 – 1.5)	-	**99**	76(75.8%)	1.5 (0.8 – 2.6)	-
P_{2df}			*0.2937*				*0.2750*	
River Catchment								
North	**279**	444 (36.5)	1	-	**279**	215(69.1%)	1	-
South	**1461**	86(41.8)	0.8 (0.4 – 1.5)	-	**1461**	71(72.5%)	0.6 (0.4 – 0.9)*	-
P_{1df}			*0.4312*				*0.0075*	
Syphilis								
Non-reactive	**1217**	413 (36.3)	1	1	**305**	215(68.5%)	1	1
Reactive	**206**	83 (41.3)	1.2 (0.9 – 1.7)	1.2 (0.9 – 1.7)	**97**	71(73.2%)	1.2 (0.8 – 2.1)	1.2 (0.7 – 2.1)
P_{1df}			0.1489	0.5608			*0.3840*	*< 0.7848*

[#] Adjusted for age group, education levels, municipal region, syphilis seropositivity and/or HIV status.*Statistically significant Odds Ratios.

4.8 Discussion

Although HIV prevalence in South African women attending public sector antenatal clinics is well described (Rice et al, 2007) little is known about their KSHV status. As indicated in Chapter 3 of this thesis and by other studies, in the sub-Saharan African setting it is now apparent that KSHV is an endemic infection affecting both children and adults (Malope et al, 2007; Minhas et al, 2008; Dedicoat et al, 2004; Mbulateiye et al, 2004). It is also clear that HIV is a significant co-factor in the pathogenesis of Kaposi's sarcoma (Whitby et al, 1995; Sitas et al, 2000, Newton et al, 2001). In addition to studies suggesting non-sexual transmission of KSHV (Dedicoat et al, 2004) and among HIV negative individuals (Wojcicki et al, 2004), other studies on the epidemiology of KSHV from endemic African and Mediterranean countries have also established that the virus is transmitted via non-sexual routes (Malope et al, 2007 & 2008; Minhas et al, 2008; Whitby et al, 2000).

This study provides further evidence for non-sexual horizontal transmission of KSHV, and as postulated in other studies this is likely to be via saliva. However, risk factors for KSHV infection, the exact mode of KSHV transmission and other epidemiological cofactors that may promote KSHV infection need further elucidation.

4.8.1 KSHV infection is common in South African women

In this chapter, KSHV seroprevalence in pregnant women attending antenatal clinics in the Gauteng province of South Africa was very high (45%) and nearly double that of HIV infection (23%). This further confirms that KSHV infection is very common in Southern African women consistent with previous reports where reported prevalence ranged from 30% - 46% (Malope et al; Dedicoat et al, 2004). Recent studies in Uganda report KSHV seroprevalences of up to 55% in adult female and male populations (Butler et al, 2011, Biryahwaho et al, 2010). Based on these studies, together with other studies in the sub-Saharan African setting, it is now apparent that in the sub-Saharan setting KSHV is an

endemic infection (Chapter 1, section; 1.6.1.1). It is also clear that, on the African continent, KSHV infection is not only common in adults but also affects children of all ages.

Not everyone who was KSHV seropositive expressed antibodies to lytic K8.1 and latent Orf73. Only 32.6% (568/1740) of those tested had antibodies to both lytic K8.1 and latent Orf73 antigens, while the overall seroprevalence for KSHV was 45% (772/1740). The biology of the expression of KSHV antibodies is still to be explored, and it remains unclear what drives the interchangeable expression of the lytic and latent KSHV antibodies. It should also be considered that assays used for KSHV serology still require advancement and most are in-house developed assays that have been carefully established. Evidently, using both the lytic K8.1 and latent Orf73 assays to determine KSHV seroprevalence allows for a more precise detection of the KSHV infection. This approach has been commonly adopted in most studies describing KSHV seroepidemiology (Chapter 1; Section 1.4).

4.8.2 KSHV and socio-demographic factors

This study showed no association between KSHV and age. The lack of association between KSHV and age noted in this study may be attributed to the narrow age range studied (mean (± SD) 26.0 (6.2)) or the fact that in this study KSHV infection rate in women < 20 years was already within the peak range at 42% and much higher compared to the 24% noted in women < 25 years studied in the previous study (Chapter 3).

Several studies in Africa have shown a stronger association between KSHV infection and age. However, in most of these studies the association is clear when both children and adults form part of the study and therefore the age range is broader. This association with age was clearer in the mother and child study described in Chapter 3 where KSHV infection increased from 14% to 33% in children 1.6 – 3 years and mothers > 35 years of age, respectively (Malope et al, 2008). A similar pattern was noted in a study by Butler et al, 2011, which showed an increased trend in age from 16% to 49% in young children 1.5 to 2 years and adults aged 50 years, respectively. In Chapter 3, age was also associated with

KSHV when only the mothers were considered but their age range was wider, than of women in this study (from 14 to 62 years of age vs. 13 to 42 years).

KSHV infection was inversely associated with level of education. Pregnant women with the highest levels of education had the lowest rates of KSHV infection at 42% compared to 53% in women with no formal education or less than 2 years of formal education (Table 4.3). The inverse association between KSHV and increasing education has been shown in other studies. Education is often reported to be a surrogate marker of socio-economic status, implying that those with lower socio-economic status are at a higher risk for KSHV infection, consistent with previous reports (Biryahwaho et al, 2010).

4.8.3 KSHV and geographic factors

Significant variation in the prevalence of KSHV between the regions within the Gauteng province of South Africa which remained after adjustment for several factors including age, HIV, syphilis status and education (*p4df = 0.0095*) (Table 4.3 and Figure 4.3). Except for Tshwane, the significant difference in KSHV infection amongst the region was only clearer in all the other regions when compared to Soweto. However, the risk for KSHV was similar between all the other regions. A marginal regional association has also been shown in a study in Uganda, with only one of the 8 regions compared to the Central Region showing a significant odds ratio (Biryahwaho et al, 2010).

The publication based on this chapter was the first study in sub-Saharan Africa to demonstrate such a significant geographical variation within a province (Malope-Kgokong et al, 2010). In the apartheid era, most South African townships were segregated according to ethnic and, therefore, the geographic origin of the residents. It follows that some of this variation may be explained by differences in cultural practices. However, the study was not set up to explore the variation further.

Living in close proximity to rivers and streams has previously been associated with increased risk for KSHV (Mbulaiteye et al, 2005; Tanzi et al, 2005). The study setting did not provide information on place of residence for the pregnant women, only the clinic that they attended. Therefore, we were unable to test this hypothesis. Further studies are warranted to clarify the nature of the geographical variation in KSHV prevalence observed in this study.

4.8.4 KSHV, HIV and Syphilis

HIV was strongly associated with increased risk for KSHV seropositivity as well as the risk of having both lytic and latent antibodies, as previously reported in a study of mothers and children from throughout South Africa (Chapter 3; Malope et al, 2007). However, as will be noted in the next chapter and a related publication, based on a mining community (Carletonville) with very high prevalence of both HIV and KSHV, it is clear that KSHV was not associated with HIV infection nor was it associated with other sexually transmitted infections (Malope et al, 2008). The reasons for this discrepancy are unclear but may relate to the very high HIV prevalence in the Carletonville study population. None of these studies (Chapters 4 & 5) found any association between KSHV infection and other STI's (Malope et al, 2008 & 2010). This is discussed further in Chapter 5 of this thesis.

HIV has been established as a significant co-factor in the pathogenesis of Kaposi's sarcoma. This study showed that KSHV was associated with HIV infection in pregnant women. Of more significance was the lack of association between KSHV and syphilis infection, even though syphilis infection was clearly associated with HIV infection (Tables 4.3). The presence of syphilis infection was confirmed in 17% of all the pregnant women. Also in this study, HIV positive subjects had significantly higher KSHV antibody levels than their HIV negative counterparts, an association not noted for syphilis. Higher antibody levels in HIV positive subjects may reflect poor immune control of KSHV resulting in more frequent reactivation.

Several studies have suggested that KSHV is acquired via sexual transmission among HIV negative and HIV positive individuals (Chapter 1, Section 1.8.1). However, other studies on the epidemiology of KSHV from endemic African and Mediterranean countries have also established that the virus is transmitted via non-sexual routes (Chapter 1; Section 1.8.2). The lack of association with syphilis in this study strongly supports suggestions that KSHV transmission may involve non-sexual factors. This concept is explored more fully in the next chapter, which focuses on the association between KSHV, HIV and other sexually transmitted infections in a heterosexual community.

Although HIV is a sexually transmitted infection (STI), it should also be appreciated that HIV plays a major role in diminishing the immune system of those who are affected. The biology of HIV is that it replicates in the body of those who are infected and the higher viral load destroys the immune system leading to an immunodeficient status. Thus, HIV leads to an array of diseases in those who are affected, mainly indirectly as the immune system cannot function optimally and fight opportunistic diseases and stabilize related metabolic dysfunctions. Therefore, to extrapolate that when a disease is common in HIV infected persons and when it is associated with the presence of HIV infection, it is acquired sexually is a risky conclusion. It should be noted that the viral replication status of KSHV may play a significant role in HIV infected people who are immune-compromised. Therefore, the association of KSHV with HIV could just imply that immunodeficiency modifies virus expression levels. This should be taken in consideration with invasion of the body by opportunistic infections and other HIV related cytokine changes.

4.9 Conclusion

Women attending routine ante-natal clinics are frequently used in surveys of HIV prevalence in South Africa and other African countries. Assessing KSHV prevalence in this population therefore allows for valid comparisons between this study and future studies in other parts of South Africa or other African countries. Using secondary information and conducting testing

for all available blood samples for KSHV lytic K8.1 and latent Orf73 antibodies was considered a rational decision. This was based on the fact that the original survey is one of a series of annual HIV and STI surveys that have been conducted by the South African Department of Health, to estimate the HIV and Syphilis incidence and prevalence in pregnant women. HIV prevalence in South African women attending public sector antenatal clinics is well described, however little was known about their KSHV status. Therefore the findings of this study can be used to extrapolate KSHV seroepidemiology findings to the total South African female population. Also the overall seroprevalence for KSHV in these populations can be compared to the local HIV statistics and be used to gauge the South African KSHV infection status.

This study adds to findings in South Africa and other African countries (Brayfield et al, 2003) which suggest a lack of evidence for sexual transmission of KSHV in heterosexual African populations. The study provides further evidence for non-sexual horizontal transmission of KSHV, likely via saliva as suggested by other studies. However, risk factors for KSHV infection, the exact mode of KSHV transmission, and other epidemiological cofactors that may promote KSHV infection need further elucidation. A longitudinal study of KSHV transmission in South Africa is needed as well as studies aiming to identify geographical, cultural and/or lifestyle and environmental factors that may predispose people to KSHV infection.

5 The Carletonville Community KSHV Sero-epidemiology Study

5.1 Introduction

Studies in Africa and Mediterranean regions continue to show an association between KSHV and nonsexual factors (Chang et al, 1994; Whitby et al, 1995). However the role of sexual mode of transmission of the virus remains uncertain (Cesarman et al, 1995; Soulier et al, 1995). In AIDS patients, Kaposi's sarcoma occurs rarely in those who acquired HIV via parenteral routes leading to the suggestion, even before the discovery of KSHV, that the causative agent must be sexually transmitted (Beral et al, 1990). Studies of risk factors for KSHV infection in men who have sex with men (MSM) demonstrate an association with markers of sexual activity including the number of partners, unprotected sexual practices and markers of sexually transmitted infections (STI) (Martin et al, 1998; Grulich et al, 2005).

Many studies have tried to identify specific sexual practices associated with the transmission of KSHV among MSM with little success, possibly because most MSM will report multiple sexual practices [Grulich et al, 2005; Martin et al, 2000). KSHV can be detected in the semen of infected participants (Horward et al, 1997; Diamond et al, 1997), but detection is less common than in saliva [Pauk et al, 2000] indicating that infected saliva is the most likely source of KSHV during transmission between MSM (Martin et al, 2003).

Evidence for the sexual transmission of KSHV in heterosexual populations is less convincing. In the United Kingdom and the United States KSHV is more common among sexually transmitted disease clinic attendees than among blood donors (Kedes et al, 1996; Cannon et al, 2009) and some groups have reported an association of KSHV infection and sexual risk factors (Greenblat et al, 2001; Tedeschi et al, 2000) However, other studies have reported a lack of evidence for heterosexual transmission [Engels et al, 2007].

In African and Mediterranean countries where KSHV is endemic, KSHV infection is common in children, and there is good evidence for the non-sexual horizontal transmission of KSHV (Mayama et al, 1998; Mbulaiteye et al, 2003; Dedicoat et al, 2004). In chapter three of this thesis and other local KSHV studies, it is clear that KSHV is common in South African children and that KSHV infection in children is related to the KSHV status of the biological mother. Other African studies confirm these findings and further indicates that KSHV acquisition in non-sexually active children may be complex. Evidence for sexual transmission of KSHV in endemic countries is conflicting. In our South African study, KSHV was marginally associated with increased numbers of sexual partners, but not with HIV (Malope et al, 2007). In addition, several reports have shown associations between KSHV, STI and HIV in Ugandan and Zambian populations (Sitas et al, 1999; Klaskala et al, 2005). Other studies conducted in the same African countries have not, shown these associations (Wilkinson et al, 1999; Wawer et al, 2001; Olsen et al, 1998).

In this study, we evaluated risk factors for KSHV infection in a South African community with a high prevalence of HIV and other STI. Prevalence rates of HIV in this community are unusually high; with a peak of 67% in those aged 17-24 years of age and around 30% among the mineworkers (Williams et al 2000, Auvert et al, 2001). We postulate that such a setting, where both KSHV infection and high-risk sexual behavior are prevalent, provides a unique opportunity to gain insight into the sexual transmission of KSHV, particularly when compared with HIV and other STI in which sexual transmission is firmly established.

5.2 Objectives

- To determine the seroprevalence rate of KSHV antibodies in residents in Khutsong Location near Carletonville in the Gauteng Province
- To determine the seroprevalence rate of KSHV in mineworkers in the Carletonville vicinity

- To identify the relationship between HIV positivity and KSHV seropositivity in these high-risk groups
- To identify the relationship between antibody response to selected sexually transmitted diseases and KSHV seropositivity
- To measure the association between HIV positivity and KSHV transmission.
- To identify risk factors of KSHV seropositivity in both residents and mineworkers.

5.3 Study Design

The participants were initially recruited in 2001 for a longitudinal study examining lifestyle changes, sexual behavior and STI associated with a community-based HIV prevention intervention (the Mothusimpilo Project) near Carletonville. Carletonville is a mining town situated on the borders of Gauteng and North West provinces, South Africa. The mines employ workers from the surrounding townships, neighbouring towns and from other parts of southern Africa. The method of selection of participants was based on randomly selected houses or index rooms in mine hostels within clusters and is described in detail elsewhere (Gilgen & Williams, 2000; Williams et al, 1999; Horizon Program, 2007)

The project involved a door to door recruitment of women and men from the Khutsong location near Carletonville within the selected houses and from mineworkers staying in the mining hostels within the Carletonville vicinity. Sex workers who offered their services to the mineworkers and men within the Carletonville vicinity were recruited form identified hotspots. Recruitment of participants was done during the day and in the case of township residents some of the working population may have been missed, which may bias socioeconomic measurements. Refusal to participate and non-inclusion in the study due to absence of people in the household for over a 5-day period was estimated at 11%. Ethical clearance and approval to conduct the research was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Medical) (Protocol Number - M970235) – (Appendix C).

5.4 Study Participants

A total of 2103 study participants were included in this study. The participants included 862 male mineworkers residing in mine hostels, 95 female sex workers, and 415 male and 731 female residents of the nearby Khutsong Township. Sera were available for all the 2103 individuals. The overall mean (±SD) age of all the participants was 33.2(10.1) years, ranging between 16 and 63 years. Permission to conduct the study was obtained from the University of the Witwatersrand Research Ethics Committee (Medical). Permission was also obtained from the National Health Laboratory Services, Sexually Transmitted Infection Group who was retaining the stored sera, Population Council who funded the project and managed the data and from the Mothusimpilo Group who managed, planned and implemented the original project.

5.5 Questionnaire

A questionnaire used for this study was initially adapted from UNAIDS and modified to suit the local setting, as described elsewhere (Auvert et al, 2001; Williams et al, 2003). The questionnaire was designed to determine risk, attitudes and sexual behaviour in relation to sexually transmitted infections and HIV positivity. A one-on-one interview was conducted by trained interviewers in the language of preference of the participants. This was taking in consideration the diversity of spoken languages in South Africa. Available information on social and demographic factors, as well as sexual and risk behavior for HIV and STI was used to determine risk factors for KSHV in the community. A signed informed consent was obtained from all study participants.

5.6 Laboratory Analysis

All leftover sera were stored at -20ºC. Laboratory analysis for HIV and STI were performed at the National Health Laboratory Services, Johannesburg, South Africa. The following STI were tested: gonococcal infection, herpes simplex virus type 2 (HSV-2), syphilis and

chlamydia. A single Capillus HIV-1/HIV-2 IgG latex aggregation test (Cambridge Biotech Corporation, Galway, Ireland), with sensitivity and specificity greater than 99.9% and 99.6%, respectively, was subsequently used to screen for HIV infection.

Syphilis serology was determined by a nontreponemal carbon antigen test to detect reagin antibodies (Immutrep RPR test; Omega Diagnositcs, Alloa, Scotland, UK). Qualitative enzyme-linked immunosorbent assay (ELISA) testing was used to detect HSV-2 type-specific IgG antibodies (MRL Diagnostics, Los Angeles, California, USA). Neisseria gonorrhoea and Chlamydia trachomatis-specific DNA sequences were detected in 520 urine samples using ligase chain reactions (Abbott Laboratories, North Chicago, Illinois, USA). KSHV serology was performed on the left over stored sera at the Viral Oncology Section, AVP, NCI-Frederick. The detection of antibodies to lytic K8.1 and latent Orf73 KSHV antigens were determined as detailed in chapter 2 of this thesis. Collected questionnaire data, laboratory HIV and STI results were anonymously linked to the KSHV data using unique participant identification numbers.

5.7 Sample Size Estimation

The sample size for this study was estimated taking in consideration the known HIV prevalence of the Carletonville communities. The overall prevalence of HIV in Carletonville was about 30%. Assuming an overall KSHV prevalence rate of around 30% and about a third of the community having five or more lifetime partners, it was estimated that this study will be able to detect a relative risk of 1.5 in HIV negative individuals and 2.0 in HIV positive individuals.

5.8 Statistical analysis

Descriptive statistical analysis and measures of association were performed using SAS 9.1 and SAS Enterprise Guide 3.0 statistical software (SAS Institute Inc., Cary, North Carolina, USA). Comparisons of means were performed using the Bonferroni adjusted student t-test

between groups or analysis of variance among multiple groups. Odds ratios (OR) and 95% confidence intervals (CI) for KSHV seropositivity among the participants were calculated by fitting logistic regression models.

Multivariate analysis included adjustment for age groups 25 years and less, 26–35 years, 36–45 years, and 46 years and older, community groups, HIV or other STI. Chi-square tests for binary measures, chi-square tests for trend, chi-square tests for homogeneity were recorded and two-sided P values were always used as a measure of significance of the associations. Selected factors thought to be possible risk factors for KSHV were included in an initial multivariate model; factors used to explain risk factors for HIV and other STI were also included. Factors with closely related features (i.e. residential groups and sex) were not put in the same model to avoid redundancy. Subsequently, all factors that remained significant at $P \leq 0.1$ were identified and included in the next model and factors significant at $P<0.05$ were considered possible risk factors and included in the final model. Pearson's correlation was used as a parametric measure of correlation between the optical densities of the two assays and kappa statistic as a measure of agreement.

5.9 Results

The mean (±SD) age was 31.3(10.3), 31.8(7.1), 28.6(11.0) and 37.1(8.2) for township females, sex workers, township males and mineworkers, respectively and was significantly different amongst the study groups ($p< 0.05$). Township residents were significantly younger than non-Township residents ($p<0.05$). Generally, township males were significantly younger than all study participants, whereas mineworkers were the oldest ($p < 0.05$).

5.9.1 Lytic K8.1 and latent Orf73 Serology

The mean antibody titre (±SD) for K8.1 was similar among all non-township and township community groups ($P>0.05$). For Orf73 the mean antibody titre (±SD) was significantly

higher in sex workers than in any other community group (P<0.05), but was similar among mineworkers, female township residents and male township residents (P>0.05) (Table 5.1).

Seropositivity to lytic K8.1 antibodies (43.0%) was significantly higher than seropositivity to Orf73 antibodies (28.5%) (P<0.0001). Seropositivity to K8.1 antibodies was similar among all the community groups, ranging from 41.6% in female township residents to 47.4% in sex workers (P = 0.22; chi-square 3df 1.2; P = 0.75). No increased risks for K8.1 antibodies were noted in any of the community groups compared with male township residents (P>0.25). The prevalence of K8.1 antibodies was similar between those with or without evidence of an STI, HSV-2 and HIV (P>0.5). No increased risks for K8.1 antibodies were observed for HIV or any STI (P>0.16).

Table 5-1: Mean (Standard deviations) of Age, lytic K8.1 antibodies latent KSHV and Orf73 antibodies and seropositivity to the antibodies by community group

Community Group (total)	Mean (±SD) Age	Mean (±SD) lytic K8.1	Mean (±SD) Orf73	KSHV K8.1 n(%) Positive	KSHV Orf73 n(%) Positive
Township residents (1146)					
a. Females (731)	31.3(10.3)	1.03(0.79)	0.44(0.49)	304(41.6)	200(27.4)
b. Males (415)	28.6(11.0)	1.00(0.78)	0.46(0.49)	174 (41.9)	115(27.7)
P_{ab} value(1df)	<0.05	> 0.63	>0.50	> 0.62	> 0.95
Non Township Residents (957)					
c. Sex -workers (95)	31.8(7.1)	1.20(0.87)	0.60(0.51)	45 (47.4)	38 (40.0)
d. Mineworkers (862)	37.1(8.2)	1.09(0.84)	0.47(0.53)	381(44.2)	247(28.7)
P_{cd} value (1df)	<0.05	> 0.255	<0.0219	> 0.69	> 0.05
P_{ac} value (1df)	>0.05	>0.05	<0.05	> 0.40	< 0.02
P_{bd} value (1df)	<0.05	>0.05	>0.05	> 0.76	> 0.43
P_{a-d} value (3df)	<0.001	> 0.056	< 0.022	> 0.75	> 0.08

Seropositivity to Orf73 was highest in sex workers (40.0%) compared with male township residents (27.7%), female township residents (27.4%), and mineworkers (28.7%); chi-square 3df 6.7; P = 0.08). The risk of seropositivity to Orf73 was 1.7-fold higher in sex workers

compared with male township residents (OR 1.7, 95% CI 1.1–2.8), and similar in mineworkers (OR 1.0, 95% CI 0.8–1.3) and female township residents (OR 1.0, 95% CI 0.7–1.3), compared with male township residents (OR 1). No differences in the prevalence of latent KSHV seropositivity were noted when participants were divided by HIV status and other STI (P>0.3). A significant correlation between the optical density of the lytic K8.1 and latent KSHV Orf73 assays was noted (r = 0.53; P<0.0001). Risk factors for being positive on both assays were similar to being positive on either assay. Being positive on both assays was not associated with STI or other measures of sexual behavior. Sex workers and those residing in hotspots were, however, more likely to be positive for both assays (OR 1.7, 95% CI 1.1–2.9 and OR 2.0, 95% CI 1.2–3.4).

5.9.2 KSHV seropositivity

A total of 506 participants (24.1%) tested seropositive to both lytic K8.1 and latent KSHV Orf73 antibodies, 398(18.9%) and 94 (4.5%) were seropositive to only lytic KSHV K8.1 or latent KSHV Orf73, respectively (k = 0.50). Overall KSHV seropositivity (testing seropositive to either lytic K8.1 antibodies or latent KSHV Orf73 antibodies) was 47.5% in all participants (Table 5.2). There was no significant difference in KSHV seropositivity amongst all the community groups (p >0.72), ranging from 46.0 in township female residents to 50.5% in sex workers (table 5.2 and Figure 5.1).

5.9.3 KSHV and Sexually transmitted Infections

Table 5.2 shows that the general prevalence of STI's (presence of any STI including HIV but excluding HSV2), was high in all sections of the community. Overall HSV-2 infection rate was 65.7% in all participants, while 48.5% of all the 2103 participants had at least one other STI. The prevalence of STI was highest in sex workers (85.3%), then female township residents, mineworkers and male residents (P<0.0001). The risk for infection was OR 11.7, 95% CI 6.9–19.8; OR 3.3, 95% CI 2.5–4.3; and OR 2.0, 95% CI 1.5–2.6, in sex workers, female township residents and mineworkers, respectively compared to the township males.

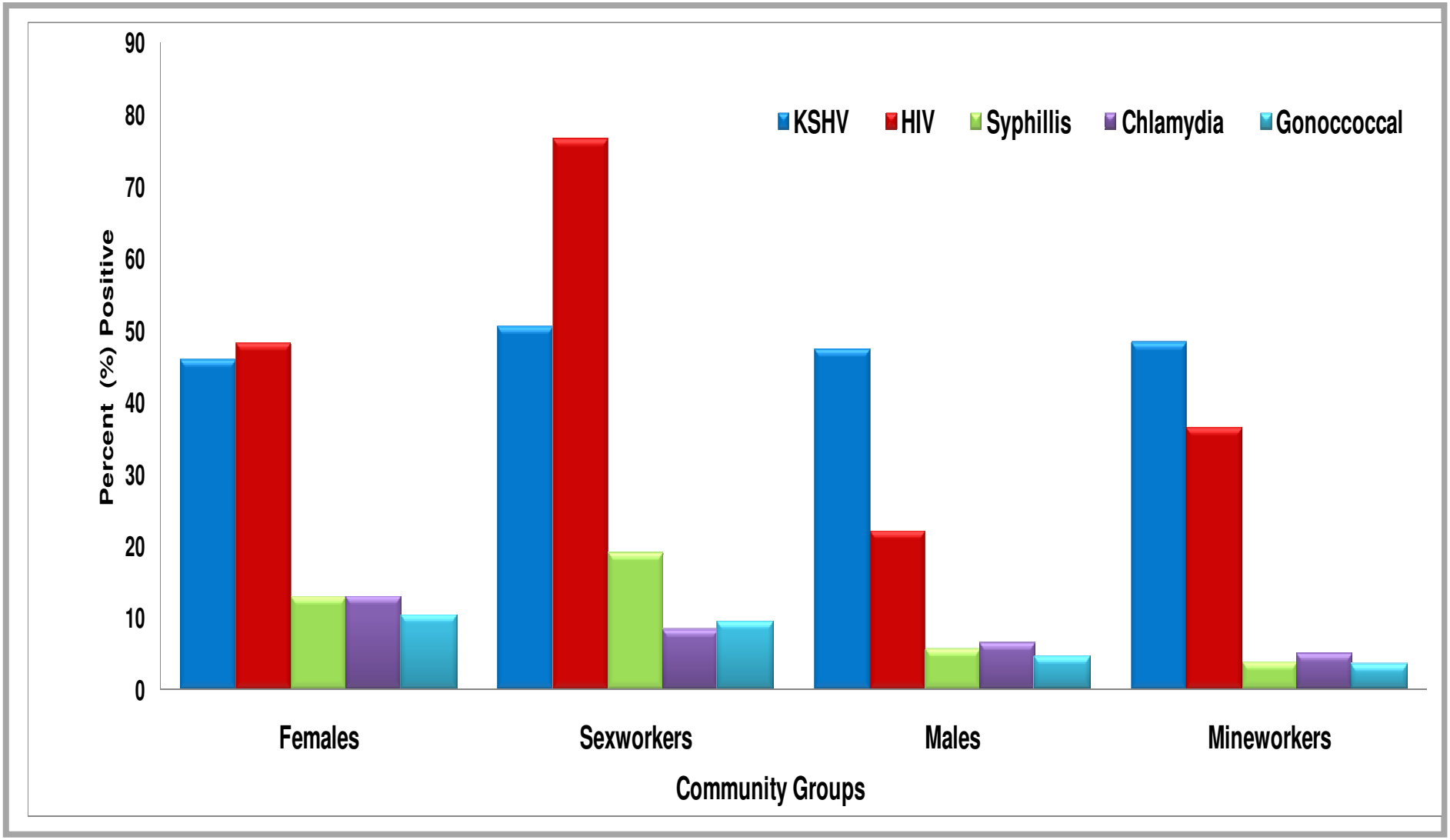


Figure 5-1: Prevalence of KSHV, HIV and other sexually transmitted infections by community group.

Table 5-2: Prevalence of positive serology for KSHV and sexually transmitted conditions in the Carletonville Community

Community Groups (Total)	**[#] KSHV K8.1 & Orf73 n (%) Positive**	**[#] KSHV K8.1 or Orf73 n(%) Positive**	**HIV n(%) Positive**	**Syphilis n(%) Positive**	**Chlamydia n(%) Positive**	**Gonococcal Infection Positive**	**n(%)**	**STI* n(%) Positive**	**HSV2 n(%) Positive**
a. Females (731)	168(23.0)	336 (46.0)	353 (48.3)	95 (13.0)	94 (12.9)	76(10.4)		436(59.6)	562(76.9)
b. Males (415)	92(22.2)	197 (47.5)	92 (22.2)	24 (5.8)	27(6.5)	19(4.6)		132(31.8)	197(47.5)
P_{ab} value(1df)	*> 0.95*	*> 0.62*	*< 0.0001*	*< 0.0001*	*< 0.0005*	*<0.0003*		*< 0.0001*	*< 0.0001*
c. Sex -workers (95)	35 (36.8)	48 (50.5)	73 (76.8)	18 (19.0)	8(8.4)	9(9.5)		81(85.3)	91 (95.8)
d. Mineworkers (862)	211 (24.5)	417 (48.4)	315 (36.5)	34 (3.9)	43(5.0)	30(3.5)		370(42.9)	532 (61.7)
P_{cd} value (1df)	> 0.05	> 0.69	< 0.0001	< 0.0001	> 0.18	< 0.014		< 0.0001	< 0.0001
P_{ac} value (1df)	< 0.02	> 0.40	< 0.0001	> 0.14	> 0.27	> 0.34		< 0.0001	< 0.0001
P_{bd} value (1df)	> 0.43	> 0.76	< 0.0001	> 0.12	> 0.19	> 0.77		< 0.0001	< 0.0001
P_{a-d} value (3df)	> 0.11	> 0.72	< 0.0001	< 0.0001	< 0.0001	< 0.014		< 0.0001	< 0.0001
All (2103)		998(47.5)	833(39.6)	171(8.1)	172(8.2)	134 (6.4)		1019(48.5)	1382 (65.7)

[#]Positive to either lytic K8.1 or latent Orf73 KSHV antbodies. [‡] Positive to both K8.1 and Orf73 antibodies. Positive to any STI excluding HSV2. * Positive to either syphilis, gonorrhoea, Chlamydia or HIV, excluding HSV2.

Overall, HIV prevalence was 39.6% and was highest in sex workers (76.8%), followed by female township residents (48.3%), mineworkers (36.5%), and male township residents (22.2%; P<0.0001). Significant differences were seen mostly between sex workers and other groups.

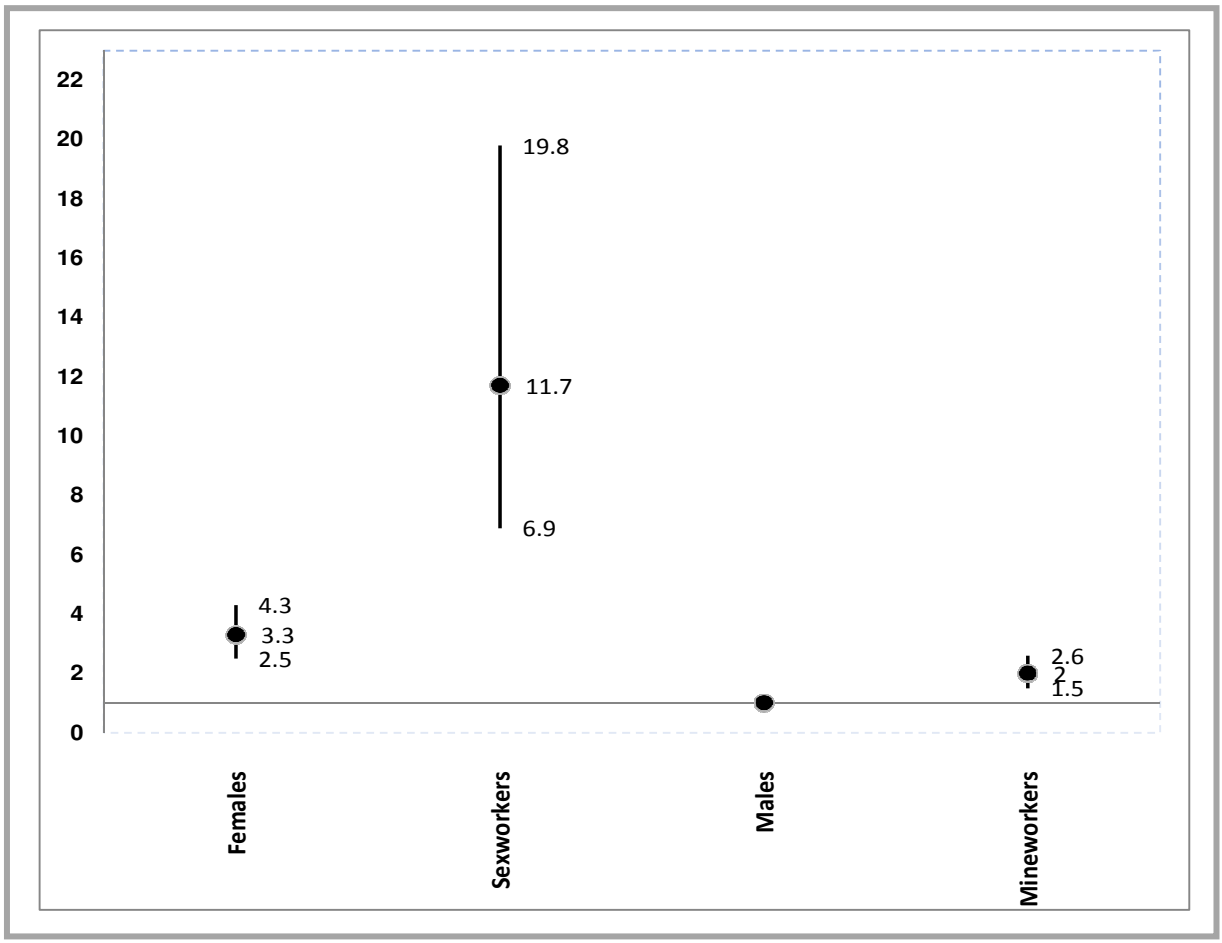


Figure 5-2: Odds ratios and 95% Confidence intervals for sexually transmitted infection in the Carletonville communities.

5.9.4 Risk factors for KSHV

5.9.4.1 Socio-demographic Risk factors

Table 5.3 shows demographic and social factors and their association with KSHV, HIV and syphilis infection. KSHV, seropositivity to either lytic K8.1 antibodies or latent KSHV Orf73 antibodies, was not associated with community groups, but was marginally associated with age and spoken home language, only if the spoken language is Zulu compared to Tswana. The profile of risk for the participants follows the expected pattern of an STI. The age group

between 26 and 45 years was at greatest risk for HIV infection compared to the younger age groups of 16 – 25 years (Table 5.3). All older age groups were also at an increased risk for KSHV infection compared to the youngest participants. Compared with male residents, sex workers and female residents were at greatest risk of HIV and syphilis (11.7 and 3.9-fold).

The risk of HIV infection, adjusted for age and other STI was sevenfold, 2.4-fold and twofold higher in sex workers, female township residents and mineworkers (OR 7.0, 95% CI 4.0–12.0; OR 2.4, 95%CI 1.8–3.2; OR 2.0, 95%CI 1.5– 2.7; p<0.001. HIV infection was strongly associated with spoken home language. The association was not noted for syphilis infection (Table 5.3).

5.9.4.2 Sexual Behavioural Risk factors

Sexual intercourse and the number of sexual partners were both strong risk factors for HIV and syphilis. Drinking alcohol was associated with a high risk of both syphilis and HIV infection. In men, circumcised participants appeared to be protected from HIV infection compared with those who were not circumcised (OR 0.8, 95% CI 0.6–1.0). Those residing in hotspots areas, sex workers were at highest risk for HIV and syphilis infection compared to those residing in council houses. Exposure to alcohol was also a risk for HIV and syphilis. Syphilis infection was not associated with marriage. KSHV infection was associated with exposure to marriage and frequent alcohol drinking (Table 5.4).

Sex workers were 29.3 times more likely to be infected with STI, followed by a 4.1-fold risk in female township residents and then a 1.2-fold non-significant risk in mineworkers (OR 29.3, 95% CI 7.1–121.1; OR 4.1, 95% CI 3.1–5.6; andOR1.2, 95% CI 0.9–1.6; P<0.01; results not shown in tables).

Table 5.5 above shows strong association between HIV and syphilis with sexual activity, number of lifetime sexual partners. Circumcision (in males) was protective for HIV infection but was not associated with syphilis infection. This association with measures of

Table 5-3: Demographic risk factors for KSHV and sexually transmitted infections

		HIV		Syphilis		KSHV K8.1 or Orf73		KSHV K8.1 and Orf73		
	Total	**% +ve**	**OR(95% CI)**	**% +ve**	**OR(95% CI)**	**% +ve**	**OR(95% CI)**	**total(n)**	**% +ve**	**OR(95% CI)**
Community Groups										
Township Males	415	22.2	1.0	5.8	1.0	47.5	1.0	310	29.8	1.0
Township Females	731	48.3	3.3(2.5 -4.3)*	13.0	2.4(1.5 -3.8)*	46.0	0.9 (0.7 -1.2)	563	29.7	1.0 (0.7 -1.3)
Mineworkers	862	36.5	2.0(1.5 -2.6)*	3.9	0.7(0.4 -1.1)	48.4	1.0 (0.8 – 1.3)	656	32.2	1.1 (0.8 -1.5)
Sex -workers	95	76.8	11.7(6.9 -19.8)*	19.0	3.9(2.0 - 7.5)*	50.5	1.1 (0.7 – 1.8)	82	42.7	1.7 (1.1 – 2.9)*
Age groups										
16 – 25	541	28.5	1.0	4.4	1.0	47.0	1.0	408	29.7	1.0
26 – 35	675	55.0	3.1 (2.4 - 3.9)*	10.8	2.6(1.6 – 4.1)*	46.4	1.0(0.8 - 1.2)	536	32.5	1.1(0.8 - 1.5)
36 - 45	650	36.3	1.4 (1.1 - 1.8)*	8.2	1.9(1.2 – 3.1)*	46.5	1.0(0.8 - 1.2)	492	29.7	1.2(0.9 - 1.7)
46 – 63	237	30.4	1.1 (0.8 - 1.5)	9.3	2.2(1.2 – 4.0)*	54.4	1.4(1.0 - 1.8)*	175	38.3	1.2(0.9 - 1.6)
Spoken Home Language										
Sotho	606	43.4	1.5(1.2– 1.9)*	9.2	1.4(0.9 – 2.2)	46.7	1.0(0.8 – 1.3)	469	31.1	1.0(0.8 – 1.4)
Tsonga	101	43.6	1.5(1.0 – 2.4)*	3.0	0.4(0.1 – 2.1)	45.5	1.0(0.6 – 1.5)	76	27.6	0.9(0.5 – 1.4)
Tswana	463	33.7	1.0	6.9	1.0	46.4	1.0	359	30.9	1.0
Xhosa	655	37.0	1.2(0.9 – 1.5)	9.6	1.4(0.9 – 2.2)	46.7	1.0(0.6 – 1.5)	499	30.1	1.0(0.7 – 1.2)
Zulu	179	47.5	1.7(1.3 – 2.5)*	5.0	0.7(0.3 – 1.5)	55.9	1.5(1.0 – 2.1)*	130	39.2	1.4(1.0 – 2.2)*
Other	99	43.4	1.5(1.0 – 2.3)*	8.1	1.2(0.5 – 2.6)	48.5	1.1(0.7 – 1.7)	78	34.6	1.2(0.7 – 2.0)

Table 5-4: KSHV and sexually transmitted infections in relation to social factors

		HIV		Syphilis		KSHV K8.1 or Orf73		KSHV K8.1 & Orf73		
	Total	**% +ve**	**OR(95% CI)**	**% +ve**	**OR(95% CI)**	**% +ve**	**OR(95% CI)**	**Total(n)**	**% +ve**	**OR(95% CI)**
Ever been married or living as married										
No	648	37.4	1.0	7.3	1.0	44.8	1.0	492	27.4	1.0
Yes	1349	43.5	1.3(1.1 – 1.6)*	9.0	1.3(0.9 – 1.8)	48.7	1.2(1.0 – 1.4)*	1119	33.2	1.3(1.1 – 1.7)*
Age of first marriage										
Unmarried	754	32.6	0.4(0.3 – 0.7)*	6.5	0.6(0.4 -1.3)	45.2	0.7(0.5 – 1.0)*	578	28.5	0.6(0.4 – 0.9)*
<18 years	139	50.4	1.0	9.4	1.0	54.7	1.0	104	39.4	1.0
18 – 25 years	635	47.1	0.9(0.6 - 1.3)	9.1	1.0(0.5 – 1.8)	49.1	0.8(0.6 - 1.2)	475	32.0	0.7(0.5 – 1.1)
26 – 35 years	511	38.0	0.6(0.4 - 0.9)*	8.6	0.9(0.5 – 1.7)	47.6	0.8(0.5 - 1.1)	401	33.2	0.8(0.5 – 1.2)
> 36 years	64	37.5	0.6 (0.3 – 1.1)	10.9	1.2(0.5 – 3.1)	40.6	0.6(0.3 - 1.0)*	53	29.3	0.6(0.3 – 1.2)
Area of residence										
Council	285	35.1	1.0	9.1	1.0	43.2	1.0	223	27.3	1.0
Mine Hostel	862	36.5	1.1(0.8 - 1.4)	3.9	0.4(0.2 – 0.7)*	48.4	1.2(0.9 – 1.6)	656	32.2	1.3(0.9 – 1.7)
Hotspots	95	76.8	6.1(3.6 – 10.5)*	19.0	2.3(1.2 – 4.5)*	50.5	1.3(0.8 – 2.1)	82	42.7	2.0(1.2 – 3.4)*
Private housing	221	25.3	0.6(0.4 – 0.9)*	8.1	0.9(0.5 – 1.7)	50.2	1.3(0.9 – 1.8)	164	32.9	1.3(0.8 – 2.0)
Site and services	217	34.1	1.0(0.7 - 1.3)	9.7	1.1(0.6 – 2.0)	43.8	1.0(0.7 – 1.4)	163	25.2	0.9(0.6 – 1.4)
Squatter settlements	423	50.8	1.9(1.4 – 2.6)*	12.8	1.5(0.9 – 2.4)	48.2	1.2(0.9 – 1.7)	323	32.2	1.3(0.9 – 1.8)
Drank alcohol in the last 4 weeks										
Never	1185	35.4	1.0	6.5	1.0	47.3	1.0	905	31.0	1.0
Less than once a week	380	43.7	1.4(1.1 - 1.8)*	10.5	1.7(1.1 – 2.5)*	45.8	0.9(0.7 - 1.2)	287	28.2	0.9(0.7 - 1.2)
Less than once a day but at least once a week (weekly)	429	45.5	1.5 (1.2 – 1.9)*	7.9	1.2(0.8 – 1.9)	47.1	1.0(0.8 -1.2)	336	32.4	1.1(0.8 -1.4)
At least once a day (daily)	109	48.6	1.7(1.2 – 2.6)*	18.3	3.2(1.9 – 5.5)*	56.0	1.4(1.0 - 2.1)*	83	42.2	1.6(1.0 - 2.6)*

Table 5-5: KSHV and sexually transmitted infections in relation to sexual behavioural factors

	Total	HIV		Syphilis		KSHV K8.1 or Orf73		KSHV K8.1 and Orf73		
		% +ve	OR(95% CI)	% +ve	OR(95% CI)	% +ve	OR(95% CI)	total(n)	% +ve	OR(95% CI)
Have you ever had sex										
No	105	1.9	1.0	1.9	1.0	46.7	1.0	85	34.1	1.0
Yes	1998	41.6	36.6(9.0 – 148.7)*	8.5	1.6(1.2 – 19.5)*	47.5	1.0(0.7 - 1.5)	1526	31.3	0.8(0.6 - 1.4)
Number of lifetime sexual partners										
0	111	5.4	1.0	2.7	1.0	50.0	1.0	89	37.1	1.0
1 - 2	427	32.8	8.5(3.6 – 19.9)*	8.9	3.5(1.0 – 11.6)*	47.5	0.9(0.6 – 1.4)	315	28.9	0.7(0.4 – 1.3)
3 - 15	1318	43.4	13.4(5.8 – 30.7)*	8.2	3.2(1.0 – 10.3)*	46.7	0.9(0.6 - 1.3)	1012	30.5	0.7(0.4 - 1.1)
> 15	247	46.7	15.2(6.5 – 36.0)*	8.9	3.5(1.0 – 12.0)*	50.6	1.0(0.7 - 1.6)	195	37.4	1.0(0.6 – 1.7)
Circumcision (Males only)										
No	695	34.7	1.0	4.6	1.0	45.2	1.0	545	30.0	1.0
Yes	578	28.6	0.8(0.6 – 1.0)*	4.5	1.0(0.6 – 1.7)	51.4	1.3(1.0 – 1.6)*	420	33.1	1.0(0.9 – 1.2)

Table 5-6: Seroprevalences and Risks (odds ratios) of HIV and KSHV in all subjects in relation to sexually transmitted infections.

		HIV (Total subjects, n = 2103)		KSHV (K8.1 or Orf73)‡ (Total Subjects, n = 2103)		KSHV (K8.1 and Orf73) *# (All subjects, n = 1611)		
	Total(n)	(%) Positive	OR(95% CI)#	(%) Positive	OR (95% CI)#	Total (n)	(%) Positive	OR (95% CI)#
Chlamydia								
No	1931	38.9	1	47.4	1			1
Yes	172	47.7	1.3(0.9 -1.8)	47.7	1.0(0.8 – 1.4)	131		1.0(0.7 – 1.4)
P Value (1d.f)			0.5		0.81			0.81
Gonococcal Infection								
No	1969	38.4	1	47.6	1	1508		1
Yes	134	57.5	1.9(1.3 -4.2)*	45.5	0.9(0.7 – 1.3)	103		0.8(0.6 – 1.3)
P Value (1d.f)			0.01		0.58			0.58
Syphilis								
No	1932	37.7	1	47.2	1	1478		1
Yes	171	60.8	2.1(1.5 -3.0)*	50.9	1.2(0.9 – 1.6)	133		1.3(0.9 – 1.9)
P Value (1d.f)			0.0002		0.37			0.37
HSV -2								
No	721	20.0	1	46.1	1	553	29.7	1
Yes	1382	49.9	3.5 (2.8 -4.4)*	48.2	1.1(0.9 – 1.3)	1058	32.3	1.1(0.9 – 1.4)
P Value (1d.f)			< 0.0001		0.54			0.54
All subjects by HIV status								
No	1270	0	-	46.8	1	970	30.3	1
Yes	833	100	-	48.5	1.1(0.9 – 1.3)	641	30.1	1.1(0.9 – 1.4)
P Value (1d.f)					0.57			0.57
Any STI(excluding HSV -2)								
No	1084	0	-	47.1	1	831	31.1	1
Yes	1019	81.6	-	47.8	1.0(0.9 – 1.2)	780	31.8	1.0(0.8 – 1.3)
P Value (1d.f)					0.76			0.74

‡ KSHV/KSHV seroprevalence = lytic KSHV K8.1 seropositive or Latent KSHV Orf73 seropositive. *# Seropositive to both KSHV K8.1 and Orf73 antibodies, excluded subjects who were seropositive to only one of the tested KSHV antibodies, associations are done in those who were seropositive to both K8.1 and Orf73 antibodies compared to those who were seronegative to both K8.1 and Orf73 antibodies(n = 1611). # odds ratios adjusted for all STIs, community groups and age group. * Statistically significant association.

sexual behavior is not noted for KSHV infection. However in contract males who are circumcised are at an increased risk for KSHV infection.

5.9.4.3 Sexually Transmitted Infections and Risk for KSHV infection

Table 5.6 shows the risk for KSHV and HIV in relation to sexually transmitted infections. Positive serology for another STI was a strong risk factor for HIV infection (Table 5.6). The risk of HIV infection is higher in participants with evidence of gonococcal, syphilitic or HSV-2 infection. The risk associated with Chlamydial infection was not significant. The risk for HIV infection was highest in those with HSV2 infection, followed by those with syphilis infection and then those with gonococcal infection, compared to those without the same infection. While HIV was clearly associated with infection by an STI, this association was not noted for KSHV.

5.10 Discussion

The association between KSHV and a sexually transmitted infection is currently one of the subjects that are explored to understand the mode of transmission of this virus. If KSHV is a sexually transmitted infection, it is anticipated that its epidemiological patterns will match that of other sexually transmitted infections especially HIV. These possibilities were explored in this study.

As expected from previous work (Williams et al, 2003), the high prevalence of HIV infection in Carletonville was significantly associated with other STI, measures of sexual behavior and being a sex worker. In marked contrast, none of the factors associated with these known sexually transmitted agents was applicable to KSHV infection. The prevalence of KSHV infection was nearly as common as HIV infection (47.5 versus 40%) in this community. In agreement with other reports, KSHV infection was associated with an increase with age (Sitas et al, 2000), whereas HIV infection was highest in 26–35 year olds, a pattern well defined in South Africa (Shisana et al, 2004). Although still high,

the prevalence of other STI in this study was up to six fold lower than the prevalence of HIV infection (Table 5.1). This may be a reflection of on-going intervention programmes in the Carletonville area aimed at reducing STI through syndromic treatment and other preventive measures (Williams et al, 2003)

Defining modes of transmission of KSHV remains a challenge, especially in African endemic countries, where contrasting information for sexual transmission and non-sexual modes of transmission is common. In this study we were able to compare the well-known pattern of sexually related risk factors for HIV, with that seen for KSHV in a community at risk of both. The presence of other STI in this study was clearly associated with an increased risk of HIV infection.

In contrast, KSHV seropositivity in this population was not associated with the presence of chlamydia, gonococcal, syphilitic or HSV-2 infection (Table 5.3). This is in agreement with a study in Uganda that showed no association between STI and KSHV (Wawer et al, 2001). Another study conducted in Zimbabwe have also shown no association between KSHV and measures of sexual behavior including number of recent sexual partners, marital status, education, condom use, previously diagnosed sexually transmitted infections, payment for sex, chronic hepatitis B infection, or incident HIV infection in Zimbabwean males. Also in the same study KSHV seropositivity in wives was not associated with KSHV seropositivity in their husbands (Campbell et al, 2009). Zimbabwe is a neighboring country to South Africa with recent influx of Zimbabwean immigrants into South Africa seeking better employment opportunities. Thus similarities of the lack of associations between KSHV and sexually transmitted infections and measures of sexually behavior, strongly suggests that within the southern African region non sexual modes of KSHV transmission play the most significant role than sexual routes.

In our study, KSHV infection did not differ significantly by HIV status. This was in contrast to a recent study in South African children, in which HIV co-infection was associated with KSHV seropositivity (Wilkinson et al, 1999). KSHV infection was not associated with any measures of sexual activity, including the number of lifetime sexual partners. Most significantly, being a sex worker carried no greater risk of KSHV infection than other township residents, a finding supported by work in Djibouti (Marcelin et al, 2002).This lack of association between KSHV, STI and measures of sexual behavior in this population indicates that sexual transmission is not an important transmission route in the Carletonville population. This may be attributed to the existing high background of KSHV exposure in the population before sexual activity, thus masking the role of sexual transmission. KSHV is, however, prevalent to a similar degree in MSM in the United States and United Kingdom, where a clear role for sexual risk factors has been reported (Martin et al, 1998)

Evidently, in this population and other African and Mediterranean populations, non-sexual modes of transmission play an important role in KSHV infection (Sitas et al, 2000; Plancouline et al, 2000; Whitby et al, 2000). In this study, a number of interesting associations have emerged. Whereas the reduced risk of HIV infection conferred by circumcision shown in this study (OR 0.8, 95% CI 0.6–1.0) is well recognized [43, 44], circumcision appeared to carry a significant risk of KSHV infection (OR 1.3, 95% CI 1.0–1.6). This was also noted in a previous report from Kenya (Beaten et al, 2002). In our study, circumcision was related to the home language, with most of the circumcised subjects speaking isiXhosa (70%) or Sesotho (50%) and only approximately 20% of those speaking isiZulu, Setswana and other languages. In South Africa, these languages are indicative of different social practices and geographical origins. Unexpectedly, the risk of KSHV infection was significant if the home language was isiZulu compared with Setswana, and no association was noted with other languages (Table 5.2). It follows that the association with circumcision found in this study may have more to do with

geographical and cultural factors than with the absence of a foreskin. Other associations are difficult to explain. Drinking alcohol was a risk factor for both HIV and KSHV, and although this may be linked to sexual behavior it could also be related to the common practice of sharing drinking vessels and KSHV transmission via saliva, which is thought to be an important route of KSHV infection (Pauk et al, 2000; Cattani et al, 1999; Mbulaiteye et al, 2004).

The highest prevalence of latent KSHV Orf73 antibody was seen in sex workers who also had a very high prevalence of HIV infection, an association not seen for lytic KSHV K8.1. Sex workers also had the highest latent Orf73 KSHV antibody titers compared with any other community group and were also more likely to express both lytic and latent antibodies together. The biological significance of this finding is unclear. The risk factors relating to the transmission of KSHV in African populations require considerable further study. Having an infected family member, especially an infected mother, is clearly an important risk factor (Mbulaiteye et al, 2006). A role for environmental risk factors, such as the source of household water and insect vectors, has been proposed (Mbulaiteye et al, 2005; Coluzzi et al, 2003; Whitby et al, 2007).

5.11 Conclusion

This study serves a significant role in epidemiological studies aiming to define the seroepidemiology and mode of transmission of KSHV in African populations. The study clearly shows that KSHV is not related to sexually transmitted infections and other measures of sexual behavior in the Carletonville vicinity. In addition, this study shows that although KSHV and HIV infections are very prevalent in this setting, they follow different epidemiological patterns. The lack of association between KSHV infection and the other community groups and sex workers, which is also corroborated by the lack of association with areas of residence including hotspots -- where most of the sex workers

temporarily resides when on duty, strongly shows that sexual behavior plays a non-significant role in this regard.

HIV was clearly associated with other STI's and measures of sexual behavior. However, KSHV was not associated with presence of any these infections. These findings correspond to findings in the previous chapter of this thesis that show no association between KSHV and STI. Nevertheless this study HIV infection was not associated with KSHV infection, in contrast to the previous studies. The role of HIV infection in facilitating the transmission of KSHV needs careful study because changes in the prevalence of KSHV as a result of the HIV epidemic will have important public health implications. Further longitudinal epidemiological studies specifically designed to identify risk factors for KSHV are required in African populations.

6 Discussion

In Africa, the AIDS epidemic has greatly increased the incidence of KS. Within the same era the causative agent, KSHV, was discovered and correlated with the distribution of KS. While evidence of KSHV infection was noted to be widespread in many populations, KS remains relatively rare. Even in severely immunosuppressed individuals and in regions where classic and African endemic KS is prevalent, the incidence of KS is lower than might be expected from the seroprevalence of KSHV. The sudden appearance of KS in MSM led researchers in the Western world to conclude that KSHV is transmitted sexually. However, the high prevalence of KSHV infection in the general population of African and Mediterranean regions made non-sexual modes of transmission more plausible. The challenge is to identify the precise mode of transmission of KSHV and the risk factors associated with acquiring the virus in these populations.

6.1 KSHV in children

In chapter 3 of this study KSHV seroprevalence was 16% in 1287 children up to 16 years of age, with seroprevalences range between 14% and 18% reported in children 1.6 to 10 years of age. This correlated with findings by Dedicoat et al, 2004, who reported prevalences on 18% in South African children. However, there are recognizable geographic differences in KSHV seroprevalences in children across Africa (De Sanjose et al, 2009; Dollard et.al, 2010). Higher prevalences of KSHV have been reported in other African countries such as Cameroon, Egypt, Tanzania and Uganda, (Mayama et a, 1998; Gessain et al, 1999; Andreoni et al, 1999; Pfeiffer et al, 2011) compared to South African children.

In addition, lower but substantial seroprevalence of KSHV infection has been reported in children in the United States and higher prevalence of 20% in Italian children adopted from Eastern European Countries (Anderson et al, 2008; Viviano et al, 2009). Clearly, KSHV infection is common in African children of all ages and does exist in children in

lower prevalence countries. This strongly suggests a non-sexual mode of KSHV transmission. The main challenge is identifying the exact mode and risk factors for KSHV transmission to children. It is evident that horizontal transmission plays a significant role. Shedding of KSHV in saliva may best describe how children acquire or transmit the virus and the significance of intrafamilial transmissions has been highlighted in few studies (Mbulaiteye et al, 2004; Guech-Ongeny et al, 2010; Mancuso et al, 2011).

In chapter 3 of this thesis, KSHV seropositivity in children was strongly associated with KSHV seropositivity in the biological mother (Malope et al, 2007), a finding that is in accordance with other South African studies (Sitas et al, 1999, Dedicoat et al, 2004). The observation in this study, and many other African and Mediterranean studies, that KSHV increases with age strongly suggests horizontal rather than a vertical mother to child transmission of KSHV.

Minhas et al, 2008 reports high KSHV sero-conversion rates per 100 child years by 48 months of age in Zambian children. Molecular evidence for mother to child KSHV transmission has also been reported, in one study, samples obtained from mother child pairs shared the same KSHV subtype and other pairs had the same KSHV strains with exact nucleotide homology (Mbulaiteye et al, 2006).

Other factors associated with KSHV transmission to children include low socioeconomic status, using communal water resources, clustering and other person to person interactions (Mbulaiteye et al, 2005). The work in this thesis was limited to describing the associations between KSHV serology in mothers and children and could not throw light on the other interactions. However, the study provides strong evidence of high KSHV prevalence in children and an increased risk of infection if the mother is KSHV seropositive.

6.2 KSHV in heterosexual adult populations

KSHV seroprevalence was high in all the adult populations included in the studies in this thesis. The lowest seroprevalence was 30%, noted in mothers of children who were attending the dispute paternity clinics, and increased significantly by age group ($p = 0.03$). The seroprevalence was higher (45%) in women attending antenatal clinics and matched that of the Carletonville community with an overall seroprevalence of 48%. KSHV seroprevalence was similar amongst all the Carletonville community groups including township females (46%) and males (48%), miners (48%) and sexworkers (50%), ($p > 0.72$). Again the prevalence of KSHV infection in the Carletonville community increased with age. This association was not clear in the women attending antenatal clinics and was attributed to the limited age distribution (mean age 26, ±6.2 years).

KSHV seroprevalence in the antenatal women varied significantly by the municipal region in which the clinics were situated. Women in the West Rand, Ekhurhuleni and Emfuleni regions had the highest KSHV seroprevalences ranging between 46% and 49%, while women in Soweto and Tshwane regions had lower seroprevalences at 35% and 37%, respectively ($p_{4df} = 0.0015$). The risk for KSHV seropositivity was highest in women in the high prevalence regions (West Rand, Ekhurhuleni and Emfuleni) compared to Soweto. This was the first study in sub-Saharan Africa to demonstrate such geographical variation within a province. While the study was not planned to explain these variations, it is acknowledged that in the apartheid era most South African townships were segregated according to ethnic groups and therefore, geographic origin of the residents. It follows that some of this variation may be explained by differences in cultural practices.

The high KSHV seroprevalence in South African populations is consistent with findings of other local studies (Wojcicki et al, 2004, Dedicoat et al, 2005). However, even higher seroprevalence has been reported in other African countries. All the three studies of this

thesis were based on large sample sizes and were able to show consistent high KSHV seroprevalence in South African heterosexual populations.

6.3 KSHV and HIV

The overall prevalence of HIV in the Carletonville community was 40% and, unlike KSHV, HIV prevalence was significantly different amongst the community groups. As expected, sex workers had the highest HIV prevalence at 76%. Township females had prevalences of 48% followed by miners at 37% and township males at 22%. HIV prevalence in mothers attending paternity clinics and the antenatal women were similar at 23%. The prevalence was 9% in children of mothers attending paternity dispute clinics up to 16 years of age. Carletonville is one of the townships with highest HIV burdens within the Gauteng province, with prevalences of up to 70% previously reported in young females between 25 to 30 years of age (Williams et al, 2003).

HIV infection was associated with KSHV seropositivity in mothers and their children and in women attending antenatal clinics, with significantly higher KSHV seroprevalence noted in HIV positive than HIV negative subjects. In chapter 3, the HIV status of mothers was marginally associated with an increased risk of KSHV seropositivity in their children. However, when children were divided by the mother's HIV status, in HIV negative mothers the risk of the children being KSHV positive was greater if the mother was also KSHV positive. This association was not noted in HIV positive mothers. This may be that children of HIV positive mothers are more likely to also be HIV positive and may therefore be more susceptible to acquiring the KSHV infection from other persons.

The role played by HIV in the transmission of KSHV needs to be studied further. No association was found between HIV and KSHV in the Carletonville community (OR 95% CI: 1.0 (0.9–1.2); p1df = 0.57) (Malope et al, 2008, Chapter 5). Carletonville had the highest HIV prevalence with at least 40% of the population infected. Contrasting

information on the association between KSHV and HIV has also been reported in other African studies. There are national and other African studies which have shown a clear association between KSHV and HIV (Sitas et al, 1998 & 1999, Gambus et al, 2001; Dedicoat et al, 2004), but also contradictory studies that showed no clear association between the two infections (Plancouline et al, 2000; De Santis et al, 2002).The role of HIV in causing immunosuppression and subsequently leading to the development of KS is well described. However it is crucial to understand other biological and epidemiological implications that HIV status may have on KSHV infection. For example there has been indication that some of the HIV proteins such as the Tat protein may promote KSHV transmission (Aoki & Tosato et al, 2004).

6.4 Lytic and latent KSHV antibodies and HIV

The significance of the expression of lytic K8.1 or latent Orf73 KSHV antibodies is not clear. While expression of the K8.1 antigen by the virus is associated with active replication, high lytic K8.1 antibody titers are not necessarily indicative of active KSHV disease. There is an overlap in the expression of the antibodies with some infected subjects expressing lytic or latent antibodies only and others expressing both.

In the mother to child study, HIV-infected subjects had significantly higher lytic and latent KSHV antibody levels than HIV-negative subjects. Also KSHV lytic and latent antibody titers were higher in HIV-positive mothers compared with HIV-negative mothers. Whether HIV infection promotes increased shedding of KSHV among mothers, and therefore transmission to children, or whether HIV-infected children are more susceptible to infection by KSHV, remains uncertain. A significant association between lytic K8.1 and latent Orf73 KSHV antibodies was also noted in women attending antenatal clinics. However, in the Carletonville study lytic K8.1 and latent Orf73 antibodies were not associated with HIV infection. On the other hand, expression of latent but not lytic K8.1 antibodies was associated with being a sex worker.

6.5 KSHV and sexually transmitted diseases

Chapters 4 and 5 investigated the association between KSHV and STI's. The Carletonville study looked at the community groups that included male and female township residents, miners from the surrounding mines and sex workers that mainly offers their services to the miners and the township male community. The inclusion of sex workers in this study provided distinct references as they are clearly a high risk group who are susceptible to increased risk for contracting STIs including HIV. The difference between sex workers and other community members was expected for HIV, STIs and measures of sexual behaviour but not well defined for KSHV.

KSHV was not associated with the presence of chlamydia, gonococcal infection, syphilis and HSV2. In addition KSHV was not associated with any measures of sexual behaviour including "ever had sex" and "number of lifetime sexual partners". Of more significance is that the risk for KSHV infection was similar in all community groups and being a sex worker was not associated with increased KSHV seropositivity. This lack of association between KSHV and sexually transmitted infections was also noted in chapter 4, in which KSHV infection was not associated with syphilis infection.

The Carletonville study was one of the first studies to clearly show no association between KSHV, sexually transmitted infections and measures of sexual behavior in the Southern African regions. Similar findings were also noted in other studies (Marcelin et al, 2002; Wawer et al, 2001). This lack of association between KSHV, STIs and measures of sexual behavior in these populations indicates that sexual transmission is not an important transmission route. This may be attributed to the existing high background of KSHV exposure in the African population before sexual activity, thus masking the role of sexual transmission.

6.6 Is KSHV sexually transmitted?

This is one of the most significant focuses that need to be studied further and determined. If KSHV is a sexually transmitted infection it would be expected to follow the similar patterns as other STIs. Moreover KSHV would be expected to be associated with other STI's. Chapters 4 and 5 of this thesis show no association between KSHV and sexually transmitted infections and measures of sexual behaviour. These findings have been shown in other studies. Contradictory to our findings there are studies in Africa that show the association between KSHV and other sexually transmitted infections and higher risk for KSHV infections in sex workers.

Biological samples have been tested in several studies to try to detect KSHV DNA on saliva, semen, other genital samples and breast milk. These have been done in different populations groups with confirmed clinical KS and KSHV infection including MSM, bisexual and heterosexual populations. From these studies, KSHV DNA has been detected in saliva of most patients (80 – 100%) with KS. However, very few studies have detected KSHV in semen or genitourinary samples and from breast milk.

If KSHV is sexually transmitted then it is expected that it should be detected in semen and genitourinary samples. More and more studies failed to detect substantial DNA from these sexual specimens. While studies have also shown sex workers and MSM to be promiscuous, it should be appreciated that during sexual acts there is increased exchange of saliva between partners through oro-genital sexual acts that may include salivary exposure. In a study conducted in MSM in San Franscisco, of 283 MSM, 87% indicated they have used saliva as a lubricant in insertive or receptive penile–anal intercourse or fingering/fisting at some point during their lifetime. In the same study 26% of MSM who avoids unprotected penile–anal intercourse, had reported anal exposure to saliva via use as a lubricant (Butler L et al, 2009). Of significant consideration is that many MSM do use saliva as a lubricant (Butler L et al, 2009) and therefore it is more likely that oro-genital sex

may be playing the most significant role in KSHV transmission amongst MSM. For that reason can it be that KSHV infection in these population groups is spread through the oral sexual routes?

In Africa KSHV infection is present in young children who are not sexually active and this has also been reported in low prevalence countries. Also literature on the association between KSHV and sexual acts in adult populations raises conflicting results. The differences in the KSHV patterns between endemic and non-endemic countries and the fact that in countries where KSHV is elevated in MSM, KSHV is described as a sexually transmitted infection, does not seem to fit with the observations in African and Mediterranean countries were KSHV is common in children, teenagers, adults and the elderly.

7 Conclusion

This thesis involved three crossectional studies that aimed to define the seroepidemiology of KSHV in the South African population. As part of this thesis three international journal articles have been published that have highlighted some of the important factors about KSHV seroepidemiology and associated risk factors. The thesis has without any doubt indicated that non sexual modes play significant roles in the transmission of KSHV in heterosexual populations and that a significant number of young children are also infected by the virus. This thesis therefore has clearly put an end to the idea that KSHV is only sexually transmitted. Following the publication based on chapter 5 of this thesis other studies have been published internationally substantiating these findings.

The studies have also given a very clear picture of the prevalence of KSHV exposure in southern Africa amongst children and adults. It continued to show the role of mother to child transmission and highlighted the significance of understanding the role of HIV in KSHV epidemiology. It has also highlighted the question as to why, with so many Southern African people coinfected with KSHV and HIV there are so few with KS in spite of drastically impaired immunity.

While the seroepidemiology of KSHV in the Southern African populations is now known, more focus should be on understanding the mode of acquisition of the virus. It is therefore thought that a longitudinal study looking at children from birth followed up to adulthood is necessary to clear up the question of when KSHV is acquired.

The major limitations of the thesis are that all secondary data was used for socio-demographic factors, defining risk factors and measures of sexual behaviour. However because both the mother to child study and the Carletonville studies were initially planned to measure sexual behaviour and STI infection rate in selected communities, they were best suited to be used for the KSHV study to link the findings to the already well known HIV and STI trends in the local settings. For all these studies all available samples were

tested for KSHV specifically for this thesis which was the main objective of the study. Also for the paternity study all samples were tested for HIV.

Now that we know the extent of the spread of KSHV in the Southern African populations, further understanding of the interaction of the virus is required. It is recommended that longitudinal studies are conducted that will aim to look at questions that this thesis could not answer. Questions of interest include determining the importance of lytic K8.1 and latent Orf73 antibodies and the patterns of antibody detection in children with acute infection, followed over time. A longitudinal study could also identify additional risk factors for KSHV infection and further our understanding of KSHV transmission and epidemiology in Southern Africa.

8 Appendix

8.1 Appendices A: Copy of Ethics Clearance Certificate 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Sitas

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M990808

PROJECT Effect Of HIV On Mother To Child Transmission Of Human Herpesvirus 8

INVESTIGATORS Dr F Sitas

DEPARTMENT National Cancer Registry, SAIMR, Central

DATE CONSIDERED 990827

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 990829 **CHAIRMAN**.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Dr F Sitas
Dept of National Cancer Registry, SAIMR, Central

Works2\lain0015\HumEth97.wdb\M 990808

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE .....SIGNATURE .....

PROTOCOL NO.: M 990808

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

8.2 Appendices B: Copy of Ethics Clearance Certificate 2

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Sitas

CLEARANCE CERTIFICATE PROTOCOL NUMBER M 961024

PROJECT	Prevalence of Kaposi's sarcoma herpes virus in South African populations of varying HIV risk
INVESTIGATORS	Dr F Sitas
DEPARTMENT	Anatomical Pathology, SAIMR
DATE CONSIDERED	961025
DECISION OF THE COMMITTEE	Approved unconditionally
DATE	961031

CHAIRMAN. ..... pp (Professor P E Cleaton-Jones)

c c Supervisor: Dr F Sitas
Dept of Anatomical Pathology, SAIMR

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 6 November 1996 SIGNATURE .....

8.3 Appendices C: Copy of Ethics Clearance Certificate 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Williams/Ballard

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M970235

PROJECT Evaluation of the Carletonville HIV/STD intervention

INVESTIGATORS Professors Williams/Ballard

DEPARTMENT ERU and Med Microbiology, ERU and SAIMR

DATE CONSIDERED 970228

DECISION OF THE COMMITTEE *

Approved with extension of additional blood testing and to be done with funding from ANRS (use of swabs included)

DATE 970310 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Professors Williams/Ballard
Dept of ERU, Med Microbiology, ERU and SAIMR

Works2\lain0015\HumEth97.wdb\M 970235

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

8.4 Appendices D: Copy of Ethics Clearance Certificate 4

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Malope

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-10-18

PROJECT Recruitment of Volunteers for Calibration of Serological Assays

INVESTIGATORS Ms BI Malope

DEPARTMENT National Cancer Registry, NHLS

DATE CONSIDERED 02-10-25

DECISION OF THE COMMITTEE Approved unconditionally

Unles otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 30 July 2007.

DATE 02-11-13 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof F Sitas
Dept of National Cancer Registry, NHLS
Works2\Iain0015\HumEth97.wdb\M 02-10-18
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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

DATE 21/11/2002 SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NO IN ALL QUERIES .: M 02-10-18

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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[i] ELISA = Enzyme-Linked Immunosoebent Assay
[ii] Orf = Open-reading Frame
[iii] PCR = Polymerase Chain Reaction
[iv] PVDF = Polyvinylidene Fluoride
[v] FITC = Fluorescein Isothiocyanate
[vi] EBV = Ebstein Bar Virus
[vii] SIR = Standardized Incidence Ratios