Predictors of HIV Acquisition in High Risk Women in Durban, South Africa

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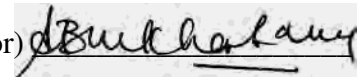
Abstract

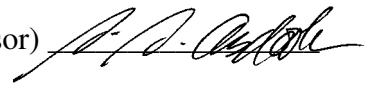
In South Africa young women bear a disproportionate burden of HIV infection however, risk factors for HIV acquisition are not fully understood in this setting. In a cohort of 245 HIV negative women, we used proportional hazard regression analysis to examine the association of demographic, clinical and behavioural characteristics with HIV acquisition. The overall HIV incidence rate (IR) was 7.20 per 100 women years (wy), 95% Confidence Interval (CI) 4.20–9.80]. Women 18 to 24 years had the highest HIV incidence [IR 13.20 per 100 wy, 95% CI 6.59–23.62] and were almost three times more likely to acquire HIV compared to women 25 years and older [adjusted Hazard Ratio (aHR) 2.61, 95% CI 1.05–6.47]. Similarly, women in relationships with multiple sex partners [IR 8.97 per 100 wy, 95% CI 5.40–14.0] had more than twice the risk of acquiring HIV when compared to women who had no partner or who had a husband or stable partner (aHR 2.47, 95% CI 0.98–6.26). HIV prevention programmes must address young women's vulnerability and promote safer sex practices for high risk women.

Preface

This dissertation represents original work, supervised by Professors Ayesha BM Kharsany and Salim S Abdool Karim and is submitted in partial fulfilment of the requirements of Master of Public Health degree. The dissertation is based on a secondary analysis of data collected in the CAPRISA 002 Study (Viral Set Point and Clinical Progression in HIV-1 subtype C infection: The role of immunological and viral factors during acute and early infection). No participant contact was required for the analysis undertaken in this dissertation.

Nivashnee Naicker (Student)  Date: 03 March 2015

Ayesha BM Kharsany (Supervisor)  Date: 03 March 2015

Salim S Abdool Karim (Co-supervisor)  Date: 03 March 2015

Declaration

I, Nivashnee Naicker, declare that:

This Master of Public Health dissertation is my own work and all primary and secondary sources have been appropriately acknowledged. The dissertation has not been submitted to any other institution as part of an academic qualification.

This dissertation is in partial fulfilment of the requirement of the Master of Public Health degree at the School of Family and Public Health Medicine, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban South Africa.

Signature:  Date: 03 March 15

Permissions and Ethics Approval

The CAPRISA 002 Study protocol was reviewed and approved by the ethics committees of the University of Natal (E013/04), University of Cape Town (025/2004) and University of the Witwatersrand (MM040202). Ethics approval for a secondary analysis of data was granted from the Biomedical Research Ethics Committee, University of KwaZulu-Natal (BE 092/11) and permission to review data stored in the CAPRISA 002 database was approved through the CAPRISA data sharing policy.

Dedication

I would like to dedicate this work to my parents Shan and Prem Naicker. Through your hard work and personal sacrifices I have been able to realise my dreams. I am forever indebted to you.

And to God almighty, for making everything possible.

Publications or Presentations

A conference abstract entitled '***Predictors of HIV Acquisition in High Risk Women in Durban, South Africa'*** was presented at the 6th South African AIDS Conference, Durban ICC, 18th – 21st June 2013 as a poster presentation.

The manuscript entitled '***Risk Factors for HIV Acquisition in High Risk Women in a Generalised Epidemic Setting'*** was accepted for publication by the journal AIDS and Behavior on 10 January 2015 (reference AIBE-D-14-00551R2) and was published online on 11 February 2015.

Acronyms and Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral treatment
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CCR5	Chemokine receptor 5
CI	Confidence Interval
CXCR4	C-X-C chemokine receptor type 4
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HR	Hazard Ratio
HSV	Herpes simplex virus
HSRC	Human Sciences Research Council
IRR	Incidence Rate Ratio
KZN	KwaZulu-Natal
OR	Odds Ratio
STI	Sexually transmitted infection
SSA	Sub-Saharan Africa
TRIM5αhu	Human TRIM5α
Wy	Women years

Acknowledgements

My sincere gratitude to my supervisor Professor Ayesha Kharsany for your guidance and mentorship over the past three years. Your patience, kindness and encouragement have made this project an enjoyable experience for me. I am forever grateful for the knowledge you have imparted to me and I hope to continue learning from you in future.

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Professor Abdool Karim is the Principal Investigator of the CAPRISA 002 study.

My heartfelt gratitude to the CAPRISA 002 team, of which I have been a part of since 2008. Your dedication and commitment to the study and to the participants is unparalleled.

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Ms Werner is the statistician on the CAPRISA 002 study.

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Dr Francois van Loggerenberg, for your assistance with this project. Dr van Loggerenberg worked as Study Coordinator on the CAPRISA 002 study previously and was responsible for establishing the original CAPRISA 002 cohort.

To my husband Eugene, thank you for allowing me the opportunity to pursue my dreams and for your love and support during this journey.

To my daughter Hridaya, you have filled my life with immeasurable joy and happiness since the day you were born. I am truly blessed to have you.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The Human Immunodeficiency Virus (HIV) epidemic remains one of the greatest public health challenges of the 21st century. The discovery of the disease in 1981, among young homosexual men presenting with atypical pneumonia in North America, led to the global spread of HIV (1). To date, an estimated 75 million infections resulting in 36 million deaths have occurred globally. HIV is the third leading cause of deaths worldwide (2, 3). Recent advances in the provision and scale up of antiretroviral treatment (ART) has transformed this previously fatal disease to that of a chronic manageable condition, substantially reducing morbidity and mortality (4).

Despite the availability of ART, the majority of new HIV infections occur in sub-Saharan Africa (SSA) with SSA bearing the greatest global burden of HIV (4). Within SSA, southern Africa, including South Africa, has been disproportionately burdened by the epidemic with sustained high HIV prevalence and incidence rates particularly in women (5, 6). Risk for HIV acquisition in high HIV burden settings has been attributed to a combination of structural, socio-demographic, behavioural and biological factors, however, better understanding these factors could be used to design interventions to shape behaviour and reduce vulnerability and risk of HIV.

The overall aim of this research was to assess risk factors for HIV acquisition in a cohort of high risk women, comprising female sex-workers and women with at least three sexual partners, in Durban, KwaZulu-Natal; with a focus on behavioural, biological and socio-demographic characteristics.

Chapter 2: Literature Review

The purpose of the literature review is to explore current evidence relating to the research topic '**Predictors of HIV acquisition in high risk women in Durban, South Africa**' in order to provide baseline information on the subject which may be used to compare and contrast findings of this study with other studies undertaken locally and in other regions.

The following literature review provides an overview of HIV epidemiology and factors contributing to the HIV epidemic and explores in-depth risk factors at the individual level which are associated with HIV infection. These include socio-demographic, behavioural and biological factors.

A computerized search of PubMed and Medline databases was done using the key words 'HIV', 'risk' and the subheading of interest eg. 'pregnancy'. Google Scholar searches and references from relevant articles were also used. Studies were limited to those published in English from 1985 to present.

1. HIV Epidemiology

1.1. The Global Epidemiology of HIV

At the end of 2012, an estimated 35.3 million people were living with HIV globally (7). The distribution of the HIV epidemic varies between continents, regions, countries and even within countries. Sub-Saharan Africa remains the worst afflicted region contributing approximately 70% of all HIV infections worldwide yet accounting for only 12% of the world's population (7, 8). In this region, women are disproportionately burdened by the disease, comprising almost 60% of adults living with HIV (8). Within SSA, southern Africa remains the worst affected region contributing over a third of global HIV infections and 40% of infections are in women (8). Other regions heavily burdened by the epidemic include Eastern Europe, Central Asia, South and South-East Asia, Latin America and North America (7).

Recent trends have shown a decline in new HIV infections globally by 33% since 2001 due to a scale up of HIV prevention and treatment efforts and the natural course of the epidemic, however variations in trends exist (7, 8). While SSA has experienced a 34% decline in new infections between 2001 and 2012, in almost half the countries in the region, areas such as the Middle East, North Africa, Eastern Europe and Central Asia have experienced an increase in the number of new infections during the same period (7, 8).

With the expansion of antiretroviral treatment (ART) programmes globally, a greater number of people are now living with HIV. An estimated 5.5 million deaths related to Acquired Immune Deficiency Syndrome (AIDS) have been averted in low and middle income countries and life expectancy has significantly increased (7). Fewer children have acquired HIV due to the provision of antiretroviral chemoprophylaxis to pregnant women for the

prevention of mother to child transmission of HIV, the greatest benefit of which has been seen in SSA (8). However, regions such as Eastern Europe, Central and East Asia, the Middle East, North Africa have experienced an increase in AIDS-related deaths between 2001 and 2010 (8). Furthermore, despite the substantial gains made in SSA over the past decade, 70% of all new infections among adults and children occurred in SSA in 2012 (7).

1.2. Transmission of HIV

Sexual transmission is the predominant route of HIV transmission, accounting for approximately 80% of all infections globally (4). Sex acts between heterosexual couples discordant for HIV infection, where one partner is HIV positive and the other is HIV negative, perpetuates the epidemic in regions such as SSA and the Caribbean, and among men who have sex with men (MSM), in Latin America and North America (8). Injection drug use, common in Asia and Eastern Europe, contributes to about 10% of infections globally and is becoming increasingly more common in SSA (4, 8). Approximately 6% of HIV transmission occurs through vertical transmission from mother to child during the antepartum period, intrapartum period or through breastfeeding in the postpartum period (4). A high-level of ART coverage for prevention of mother-to-child transmission has seen significant declines in the number of children newly infected with HIV in the last decade (7). Transmission through blood and other infected bodily fluids accounts for the remaining 4% of infections which occur in healthcare settings (4).

1.3. HIV Epidemic Typologies

HIV epidemic typologies may be described as low-level, concentrated or generalised epidemics (4). In low-level epidemics, HIV may be present for a long period but never reaches significantly elevated levels in the population (4). The HIV prevalence remains

between 1% and 5% in the general population (4). West Africa is an example of a region with a low-level epidemic (4). Concentrated epidemics are characterised by high rates of HIV within specific subgroups such as MSM, injection drug users or sex workers (4). In this type of epidemic, HIV is not well established in the general population (4). HIV prevalence is above 5% in these at risk populations and this may vary between countries (9). North America, Canada, South America and China are some of the countries characterised by this epidemic typology (9). Most countries in SSA are experiencing a generalised epidemic where HIV is firmly established in the general population with prevalence exceeding 5% in adults (4). Heterosexual transmission is the main mode of transmission. Southern Africa which is the worst affected region in SSA, is considered to be the epicentre of the HIV epidemic and accounts for 40% of all women infected with HIV globally (8). This region is characterised by a hyperendemic generalised epidemic with HIV prevalence exceeding 15% in the adult population (4). Countries affected include Swaziland, Botswana, Lesotho, Mozambique, South Africa, Zambia, Zimbabwe and Malawi (4). HIV transmission in the SSA region is predominantly heterosexual and young women remain at high risk of HIV, acquiring infection five to seven years earlier than males with three to six times higher rates of infection (10).

1.4. Monitoring the HIV epidemic

HIV prevalence is a useful measure of existing HIV infections in a population. Increasing HIV prevalence in a population signifies increased survival rates, mainly due to effective antiretroviral treatment which leads to reduced morbidity and mortality. HIV incidence is a more sensitive measure to monitor the epidemic, indicating the rates of new infections arising in a population. It is a useful tool to gauge the rate of HIV transmission and changes in HIV incidence over time may be used to evaluate the impact of prevention measures on the HIV

epidemic. HIV incidence may be measured directly by longitudinal follow up of at-risk persons through prospective cohort studies (11). However, some of the limitations of prospective cohort studies are that they are logistically difficult to implement and prone to biases due to the sample selected, changes in behaviour in subjects due to repeat HIV testing and losses to follow up over time (11). Furthermore they are costly to undertake. Another method of measuring HIV incidence is through indirect measurement using HIV prevalence and mortality data in a population, however this method is also subject to limitations regarding the availability of reliable prevalence data (11). A third method of measuring HIV incidence is through the use of laboratory-based assays which distinguish recent from established HIV infection in cross-sectional surveys however misclassification of established HIV infection as recent infect remains a drawback (11). In developing countries, a combination of the above methods are used to measure HIV incidence, depending on the availability of resources.

1.5. HIV Epidemiology in South Africa

South Africa's HIV epidemic is the largest in the world, with an estimated 6.1 million people living with HIV in 2012 (7). PMTCT roll-out using single dose nevirapine was first initiated in late 2002 followed by wide-scale ART roll-out in 2004 (12). South Africa has one of the largest ART programmes in the world which has led to a concomitant decline in AIDS related morbidity and mortality. Between 2001 and 2012, new HIV infections among adults 15 years and older was estimated to decline by 63% (7). Despite the substantial gains made with HIV prevention programmes and ART provision in the past decade, young women continue to be at high risk of HIV in this setting (6, 13, 14).

Antenatal HIV prevalence data are a useful tool to monitor the epidemic trajectory and burden of disease. The national antenatal HIV prevalence was 29.5% [95% Confidence

Interval (CI) 28.8-30.2%] in 2012 (5). The provinces of KwaZulu-Natal (KZN), Mpumalanga and Free State experience higher rates of infection, and among women aged 15 to 49 years, the HIV prevalence in these three provinces exceeded 30% in 2012 in contrast to the Western Cape which had a lower prevalence of 16.9% (95% CI 13.8-20.5%) (5, 15).

Among young pregnant women, in the 15 to 24 year age group, the overall HIV prevalence was 20.1% (95% CI 19.5-20.8%) in 2011 and 19.3% (95% CI 18.7-19.9) in 2012 (5). The HIV prevalence in the 15 to 24 year age group is recognised as a reliable proxy measure for HIV incidence, as infections in young people represent new infections (16). Despite the high HIV prevalence reported among young pregnant women, national population based surveys such as the South African National HIV Prevalence, Incidence and Behaviour Survey undertaken by the Human Sciences Research Council (HSRC) indicated a 60% reduction in HIV incidence among 15 to 24 year old women between 2002 and 2008 (17, 18). Notwithstanding these reported declines, HIV incidence among young women remains unacceptably high (6, 13).

Given the high burden of HIV in this setting, particularly among women, it is important to examine the contributing factors which may determine susceptibility to HIV. These may be broadly categorised as structural factors and, at the host level, the socio-demographic, behavioural and biological factors.

2. Structural Factors Contributing to HIV Risk

Structural factors may be considered to be the physical, social, cultural, organisational, community, economic, legal or policy aspects of the environment which facilitate HIV transmission (19). These factors influence behaviour at the individual level and the association between structural factors and HIV risk is complex and direct causality is not always easy to establish (19).

Social aspects include but are not limited to population mobility, gender discrimination and gender violence, racism and segregation, and social marginalisation of vulnerable populations (19, 20). Globally, mobile populations bear a greater risk of HIV infection (20). Population mobility may be due to labour migration, as seen in South Africa, refugee migration, internal migration, resettlement or commuting (20). Drug trade and long distance truck routes have also contributed to the spread of HIV in countries such as India, South America, Asia and Africa (20, 21). Gender discrimination leading to economic disempowerment, sexual violence and overall diminished rights for women all contribute to HIV risk. Racial discrimination and segregation are implicated in HIV risk. In North America, African Americans have the highest HIV incidence, contributing to 44% of all new infections in 2010 despite accounting for only 12% of the population (22). Vulnerable populations, which include people with disabilities, remain at increased risk of HIV due to social marginalisation. The 2012 HSRC survey conducted in South Africa found that people with disabilities had a reported HIV prevalence of 16.7% (95% CI 12.9-21.4), higher than the national prevalence of 12.2% (95% CI 11.4 – 13.1) (18).

Economic policy and income at a national level are also thought to impact HIV risk since the majority of global infections occur in SSA where most countries are low-income countries. However, a study exploring per capita income of African countries and HIV risk found no

association (23). Instead middle-income countries including South Africa are known to have higher prevalence of HIV (23). Economic policy relating to resource allocation to healthcare particularly for HIV and AIDS also impacts risk. In countries like Uganda increased resource allocation for HIV and AIDS and high level political commitment has led to a dramatic decline in new infections (24). National policy regarding housing and sanitation, education, income, social organisation and law enforcement continue to impact HIV risk. Specific laws relating to sex work, illicit drug use and the criminalisation of HIV also impact HIV risk at a structural level (20).

The differences in HIV prevalence amongst women in low to middle-income and high-income countries highlight the stark disparities across regions and countries (8, 25). Structural approaches which seek to change an individual's social, economic and political environment are recognised as an integral component of HIV policy in order to support current behavioural and biomedical prevention strategies if these are to be successful in the long-term in regions worst affected by the HIV epidemic.

3. Risk Factors at the Individual Level

3.1. Socio-demographic Risk Factors

Socio-demographic factors at the individual level are interrelated to structural factors which define an individual's environment. As opposed to structural factors, these factors are considered to be proximal to the individual thereby impacting HIV risk directly (19). Some of these factors include age, race, marital status, employment status or household income, level of education, living conditions or place of residence (18, 26, 27).

In South Africa, inequalities especially in education, access to health care, housing and employment are common due to the country's political history and the Black African race group remain the most disadvantaged population. This race group is also the worst afflicted by the epidemic as shown in the 2012 HSRC survey in which the prevalence of HIV among Black Africans was 15% (95% CI 14.0-15.9%), followed by Coloureds with a prevalence of 3.1% (95% CI 2.2-4.2) (18). The HIV prevalence among Asian and White population groups was less than 1% (18). As poverty is rife in South Africa, with the proportion unemployed at 25.4%, labour migration and urbanisation is common in this setting (28). Studies within South Africa have shown that migrant men and women as well as residents of urban informal settlements are at increased risk for HIV (15, 29-31).

At the individual level, the evidence regarding income level or employment status and HIV risk is less clear. A review of 36 studies investigating the impact of socio-economic status of women in SSA found no conclusive link between low socio-economic status and HIV risk (26). The authors concluded that HIV risk may initially rise with increased access to resources for women in low income countries while in countries with higher per capita income, increasing socio-economic status of women may reduce risk for HIV (26). Socio-

economic variables in the study included educational level, household income and employment (26). In South Africa, a large population based study which included men and women conducted in rural KwaZulu-Natal found that individuals in middle-income households were at increased risk for HIV as compared to those in the poorest households [adjusted Hazard Ratio (aHR) 1.86; p=0.002] (32). In this study the effect of educational attainment, household wealth and household expenditure on HIV incidence was evaluated (32). Among urban women in KwaZulu-Natal, unemployment or having no income was found to be a significant risk factor for HIV acquisition [Hazard Ratio (HR) 1.47, 95% CI 1.02-2.17; p=0.043] (33). These studies suggest that the impact of socio-economic status on HIV risk differs across regions and within countries and is influenced by the greater socio-economic status of the region.

The association between level of education attained and risk for HIV remains controversial. In Cameroon, among women aged 15 to 49 years participating in a national survey, HIV prevalence was found to be lowest, at 3.3% among those with no formal education (34). HIV prevalence was doubled in women reporting a primary or secondary education (34). Among women 18 years or older recruited from post natal or family planning clinics in Malawi and Zimbabwe, HIV risk increased with increasing level of education (35). In this study, higher HIV incidence correlated with a higher level of education (O-level examinations or higher versus none: HR 3.25, 95% CI 1.39–7.57; p=0.006) (35). Contrary to these findings, a prospective cohort study in Tanzania, which included men and women older than 18 years, found that achieving a secondary education was associated with lower HIV incidence and risk for HIV (secondary school versus none: HR 0.39, 95% CI 0.17-0.89; p=0.01) (36). However HIV incidence was low in that study at 1.35 per 100 person years (95% CI 1.10-1.64/100 person years). (36). Similar inconsistent findings are reported from studies in South

Africa. In a study in the Free State province found that older women reporting no formal education or a primary school education were less likely to be HIV infected (p=0.018) (37). In contrast, a national survey of sexually active young women aged 15-24 years, found that those who did not finish school were more likely to be HIV positive [adjusted Odds Ratio (aOR) 3.75, 95% CI 1.34-10.46] (27). A population based survey in rural KwaZulu-Natal, found that every additional year of school education reduced risk for HIV acquisition (aHR 0.93, p=0.022) (32). Similarly, a study of 18 to 24 year-old men and women from four provinces in South Africa found that having a grade 8 (aOR 0.04, 95% CI 0.01-0.66; p<0.05) or higher (aOR 0.04, 95% CI 0.01-0.43; p<0.01) education was protective against HIV infection (38). Overall, studies which included men, demonstrated a protective effect of higher educational level with HIV risk (32, 36, 38). However, in women, in older age groups lower educational level was found to be protective and in contrast in young women a higher educational level was protective suggesting that HIV acquisition is related to age of women (27, 34, 35, 37).

A relationship status of being married has been shown to considerably reduce the risk of HIV acquisition. In the 2012 HSRC survey, HIV incidence was lowest at 0.6% among people who were married compared to those who were single (3.4%) and people cohabiting with a sexual partner (3.7%) (18). Similar trends are seen in other African countries such as Malawi and Zimbabwe, where being unmarried was found to be a risk factor for HIV (35). In Free State province, South Africa, HIV uninfected older women were more likely to be married (p=0.01) while HIV uninfected women, irrespective of age (p=0.012 for younger women and p=0.002 for older women), reported husband headed households more frequently compared to HIV infected women (37). In rural KwaZulu-Natal, both men and women who were unmarried had twice the risk of acquiring HIV compared to those who were married

(p<0.001) (39). In urban KwaZulu-Natal, being single or lack of cohabitation with sexual partner was associated with HIV infection (HR 4.76, 95% CI 2.60-8.74; p<0.0001) among sexually active women (33). While marriage appears to be protective against HIV, this may not hold true for younger women, especially adolescents (40-42). Child and adolescent marriages are highly prevalent in developing countries (42). In Uganda, HIV incidence was increased among formerly married young women 15 to 24 years of age (40). In the same region a decline in adolescent marriage was one of the factors associated with a reduction of HIV incidence between 1999 and 2011 (41). Gender imbalances leading to non-consensual sex within marriage, partner age disparity within marriage and inability to negotiate condom use are some of the reasons why young women may be predisposed to HIV infection within marriage (42, 43).

Several studies have demonstrated that young age is associated with increased risk for HIV (33, 36, 44). Studies from SSA indicate that both young men and women are at increased risk for HIV (36, 44). In Rwanda, teenage antenatal clinic attendees were at highest risk of HIV after two years of follow up (OR 4.8, 95% CI 1.3-18.4) (45). In KwaZulu-Natal, South Africa, HIV incidence was highest among women aged less than 20 years among urban Sexually Transmitted Infection (STI) clinic attendees (6). Another study of sexually active women in urban KwaZulu-Natal found that women aged 24 years or less had more than twice the risk of acquiring HIV compared to women aged 35 years and older (HR 2.78, 95% CI 1.88-4.11; p<0.0001) (33). Similarly, the HSRC survey showed comparable trends with 24.1% of all new infections accounted for by women aged 15 to 24 years in 2012 (18). Young age as a risk factor for HIV in women may be explained by behavioural and biological factors.

3.2. Biological Risk Factors

For biological reasons, the anatomy of the female genital tract and factors which affect the genital tract epithelium may place women at increased risk for HIV (46). The stage of HIV disease, circulating viral load and ART status of an infected partner also determines HIV risk. Other biological factors which may impact HIV risk include host genetic factors, host immunity and nutritional status.

3.2.1. Anatomy, Exposure to Virus and Viral Entry

Globally, unprotected sexual intercourse between HIV discordant couples is the primary mode of HIV transmission. Studies from North America and Europe have shown that male to female transmission of HIV is more efficient than female to male transmission (47, 48). This may be attributed to anatomical differences, with the female genital tract providing a larger surface area for HIV entry (47). The presence of semen in the female genital tract is thought to provide a more favourable environment for HIV survival (47). Anal sex is associated with increased HIV risk as the rectal mucosa is prone to trauma and like the vagina; may provide a greater surface area for viral entry if exposed to infected semen. Receptive anal sex has been associated with higher levels of transmission in studies of MSM however insertive anal sex is also known to carry risk (49-51). Female sex workers engaging in anal sex were found to be at increased risk for HIV acquisition (OR 2.30, 95% CI 1.10-4.70) (52). Oral sex too poses a risk for HIV transmission, albeit lower than risk from anal or vaginal sex (53).

The stage of HIV disease in the infected partner and ART status may also impact risk for HIV. Sexual contact with a partner who recently acquired HIV or one who has AIDS is associated with increased risk for acquisition due to higher viral load exposure. A study in Uganda, found that risk of HIV transmission probability was proportional to the circulating

viral load of the infected partner (54). The recent HPTN 052 randomised controlled study supported this finding (55). Early initiation of antiretroviral treatment ($CD4^+$ cell counts between 350 cells/µl and 550 cells/µl) in HIV infected individuals conferred 96% protection against HIV in sex partners compared to those initiating ART at $CD4^+$ cell counts of 250 cells/ul or less (55). This was attributed to viral suppression in genital secretions by ART thereby reducing the viral load and risk to sexual partners (55).

3.2.2. Factors Affecting the Structure and Integrity of the Genital Tract Epithelium

As the genital tract mucosa is the first point of viral entry during heterosexual intercourse, understanding the factors which affect the integrity of the vaginal epithelial surface is paramount to understanding susceptibility to HIV in women. Physiological changes of the genital tract, which include cervical ectopy and pregnancy, and other factors such as hormonal contraception use, sexually transmitted infections and vaginal insertion practices may determine HIV susceptibility at the mucosal level.

3.2.2.1. Cervical Ectopy

Cervical ectopy occurs when the columnar epithelium of the cervical endocervix comes to lie on the ectocervix (56). It is a common occurrence in young pubescent women as this physiological change is due to increased oestrogen levels (56). The degree of ectopy varies and changes with age (57). Cervical ectopy is a common occurrence during pregnancy and with use of hormonal contraception (56, 58). It is associated with increased risk for STIs such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and herpes simplex virus (HSV) (57). In Cape Town, South Africa, women with cervical ectopy of greater than 20% were at higher risk of HIV infection (OR 2.18, 95% CI 1.01-4.69) however, since women aged 35 to 65 years old were included in the study, it is possible that the exclusion of young women at

higher risk for HIV may have biased the study findings (59). In Nairobi, Kenya, cervical ectopy was found to be a predictor of HIV among heterosexual women (OR 5.0; p=0.007) (60). In a review of observational studies, which included the South African and Kenyan study, only four studies, mainly from Kenya found that cervical ectopy was associated with HIV acquisition (61). Some of the studies which showed a positive association were undertaken in women with concomitant STIs or genital ulceration, making the temporal association of cervical ectopy and HIV acquisition less discernible. While this physiological change may offer a possible explanation for why young women are at increased risk of HIV acquisition, current empiric evidence in the African setting remains limited.

3.2.2.2. Pregnancy

Numerous studies have examined the association between pregnancy and the risk for HIV acquisition (62-65). The physiological changes in pregnancy, particularly the increase in levels of oestrogen and progesterone, are thought to increase susceptibility to HIV by inducing changes in the genital tract mucosa (63). Immunologic changes such as upregulation of HIV-1 co-receptors and changes in T-cell immunity have also been implicated (63). In Kenya HIV risk doubled during pregnancy (HR 2.34, 95% CI 1.33-4.09) although the results were not significant after controlling for sexual behaviour and clinical factors (aHR 1.71, 95% CI 0.93-3.12) (62). In the same study HIV incidence in male sexual partners was more than two-fold during pregnancy compared to partners of non-pregnant women suggesting that pregnancy may also increase transmission of HIV to male sexual partners (HR 2.31, 95% CI 1.22-4.39) (62). This finding remained significant in the adjusted analysis (aHR 2.47, 95% CI 1.26-4.85; p=0.01) (62). In Uganda, a prospective study examining HIV incidence rates during pregnancy, found that HIV incidence during pregnancy [Incidence Rate Ratio (IRR) 2.16, 95% CI 1.39-3.37] was double compared to women who were not pregnant or not

breastfeeding (63). This risk was similar (IRR 1.82, 95% CI 1.09–3.05) to women who were breastfeeding (63). In a separate prospective study conducted at family planning sites in Uganda and Zimbabwe, pregnancy was not associated with increased risk of HIV (64). However, HIV incidence and pregnancy rates were low in this study. Furthermore the authors acknowledge behavioural and biological differences in the Zimbabwean population which may explain the inconsistency with earlier findings from Uganda (63, 64). Notwithstanding, the HPTN 039 study showed similar results as pregnancy was not associated with HIV risk in HSV-2 seropositive women from Zambia, Zimbabwe and South Africa (65). Pregnancy incidence was high in this study. Overall the evidence for increased HIV risk during pregnancy is weak and needs to be explored further.

3.2.2.3. Hormonal Contraception Use

Hormonal contraception, in particular progesterone preparations, are thought to induce changes in the vaginal mucosa which may increase susceptibility to HIV (66). Steroid hormones may cause thinning of the vaginal mucosa thereby aiding viral entry; may increase target cells or may alter immune mechanisms at the mucosal level (66). A study of HIV-1 serodiscordant couples enrolled in HIV-1 incidence studies in seven African countries found an association with HIV risk (HR 1.98, 95% CI 1.06-3.68; p=0.03) among users of hormonal contraception versus non-users (67). In the same study hormonal contraception use significantly increased transmission risk to male partners (HR 1.97, 95% CI 1.12-3.45; p=0.02) (67). Contrary to this, a secondary analysis from the Carraguard study conducted in South Africa did not find an association between hormonal contraception use and HIV risk (68). A recent systematic review which included eight observational studies found no conclusive evidence for hormonal contraception use and increased HIV risk (69).

3.2.2.4. Sexually Transmitted Infections

Sexually transmitted infections are known to increase susceptibility to HIV and facilitate transmission of HIV by increased viral shedding (70). Ulcerative and non-ulcerative STIs interact synergistically with HIV. Genital ulceration may lead to increased target cells in genital tract thereby aiding HIV entry or HIV may enter directly via ruptured blood vessels in ulcerative lesions (71). Among women in Uganda and Zimbabwe, HSV type 2, the commonest ulcerative STI, increased risk for HIV by two to four-fold in women with seroprevalent HSV type 2 infection and by four to eight-fold in women with seroincident HSV type 2 infection (72). Non-ulcerative STIs are also known to increase susceptibility to HIV by causing inflammation of the genital tract thereby increasing HIV target cells (73). Genital tract infections caused by *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, prevalent in South Africa, may increase HIV acquisition from anywhere between two and five-fold (71, 74). Among women in Durban, South Africa, *Neisseria gonorrhoeae* was found to increase HIV risk by more than four-fold (HR 4.62, 95% CI 1.34-15.93; p=0.015) (73). A high level of genital inflammation was evident in women with both symptomatic and asymptomatic STIs (73). Non-STI genital infections such as bacterial vaginosis (RR 1.6, 95% CI 1.2-2.1) have been associated with increased risk for HIV acquisition (75). Despite the well-known association between STIs and HIV, only one randomised controlled trial from Mwanza, Tanzania, has shown a significant reduction in HIV acquisition following the use of STI enhanced management as an intervention at a community level (76). However, conflicting results emerged from similar trials conducted in Rakai and Masaka in Uganda (77, 78). Differences in the stage of the HIV epidemic, sexual behaviour, curable STI rates, treatment for symptomatic versus asymptomatic STIs and enhanced STI services versus intermittent mass treatment across these regions are some of

the reasons cited for the contrasting findings of these trials (79, 80). Nevertheless, enhancing treatment of STIs is an important public health measure.

3.2.3. Host Genetics

There are several genes which have been identified that are associated with HIV risk. The most commonly known genetic mutation is the 32-base pair deletion in the gene which codes for the human chemokine receptor 5 (CCR5), confined to Caucasians of Northern European descent (81). This cell receptor is used by the virus to gain entry into $CD4^+$ cells. Genetic mutations lead to a person having no CCR5 receptors or greatly reduced number of receptors however neither confers total protection against HIV as a person may still be susceptible to C-X-C chemokine receptor type 4 (CXCR4) strains (81).

Human leukocyte antigen (HLA) classes I and II are also implicated in HIV susceptibility and resistance. Commonly, HLA-B57 phenotype is associated with restriction of viral replication and non-progression of disease (82). Contrary to this, other HLA types such as HLA-B35 have demonstrated quicker progression to AIDS in a study among homosexual men (83). Other studies of host genetics and HIV have found that greater expression of human TRIM5α (TRIM5αhu) coded for by the TRIM5αhu gene, is associated with reduced susceptibility to HIV among high risk women (84). Increased production of Interleukin-10, an anti-inflammatory cytokine coded for by the IL-10 gene, has been shown to reduce HIV susceptibility in the same cohort (85). Another study using data from this cohort of women found that women with higher white blood cell counts, specifically the neutrophil subset, had a reduced risk of HIV infection while higher platelet counts increased risk for HIV infection (86). This finding was found to be linked to Duffy-null-associated low neutrophil count trait, common in African populations (86).

3.2.4. Immune Status

In HIV-1 infection, $CD4^+$ cells which are the helper T lymphocytes are the major targets for HIV infection and are able to replicate high levels of virus (87). Once infection is established, T-cells expressing $CD4^+$ cell decline leading to lowered immunity. $CD4^+$ cell counts are used clinically to determine when ART should be initiated. T-cell immunity appears to differ among races with HIV negative Caucasians having higher $CD4^+$ cell counts compared to Asians and some African populations (88). Among the African population one study found that $CD4^+$ cell loss following HIV acquisition is slow and more noticeable at higher $CD4^+$ cell counts (88). After ART initiation the $CD4^+$ cell recovery does not appear to differ between races (88). Another study found that among injection drug users low pre-seroconversion $CD4^+$ cell counts were associated with a faster rate in $CD4^+$ cell decline post infection (89). No studies were identified which evaluated pre-seroconversion $CD4^+$ cell counts and risk for HIV acquisition.

3.2.5. Nutritional status

Several studies have explored nutritional status and HIV risk. In Pune, India, patients with STIs and low B-carotene levels were 21 times more likely to become infected with HIV (OR 21.1, 95% CI 1.95-228.9) (90). A study in North America found differences in mean globulin, serum sodium, albumin and haemoglobin levels in HIV positive and HIV negative patients older than 55 years (91). A serum albumin to globulin ratio <1 predicted HIV seropositivity in patients with a background history of alcohol use or STI (91). In Rwanda, nutritional status did not differ between women who seroconverted and those who remained HIV negative (92). Pre-seroconversion levels of vitamin A, carotenoids, vitamin E, selenium, albumin, ferritin and cholesterol did not differ between the two groups (92). Among Kenyan

men with concomitant STIs, vitamin A deficiency, although common, was not associated with increased risk of HIV acquisition (93).

Thus it is important to understand biological factors and whether these contribute individually or interact to either protect against or enhance HIV acquisition.

3.3. Behavioural Factors Which Contribute to HIV Risk

Behavioural risk factors at the individual level which may determine risk for HIV acquisition include sexual risk behaviour such as early sexual debut, age disparate relationships, transactional sex, number of sex partners and partner concurrency, condom and alcohol or substance use (15, 18). Vaginal insertion practices performed either for hygiene purposes or for sexual motivation may also place women at increased risk for HIV (94, 95).

3.3.1. Sexual Risk Behaviour

3.3.1.1. Early Sexual Debut

Given the high background prevalence of HIV in Southern Africa, early sexual debut in this setting immediately places individuals at risk of HIV. In Johannesburg, South Africa, sexual debut before 16 years of age was found to be an independent risk factor for HIV among women enrolled in an HIV prevention trial (OR 2.60, 95% CI 1.30-5.17, p=0.01) (96). A national survey found that among young men and women early sexual debut was more likely if the first sexual partner was older and among women who reported forced sex (97). In KwaZulu-Natal province, sexual debut at age 15 years or younger was associated with the highest HIV incidence (IR 12.0 per 100 person years, 95% CI 8.0-18.0) however women in this study were also more likely to engage in other risky sexual behaviours (98).

3.3.1.2. Age Disparate Relationships

In Southern Africa, women aged below 20 years are almost six times more likely to be HIV infected than their male counterparts (10). While sexual activity may not necessarily be higher among young women, it is the sexual partnering of young women with older men that is thought to contribute to increased risk for HIV in this setting (10). Older men are more likely to be HIV infected than young men, thereby placing young women at increased risk for

HIV. A review of 56 studies in SSA found that among women having a sexual partner who is 10 or more years older, the relative risk of HIV was double in women aged 15 to 19 years compared to 20 to 24-year-olds (99). Similarly, the national youth survey in South Africa found that among 15 to 19-year-olds, risk for HIV was increased if the partner was five or more years older (aOR 3.20, 95% CI 1.20–8.30) and among 20 to 24-year-olds risk was increased if the partner was between one and four years older (aOR 2.30, 95% CI 1.40-3.60) when compared to women having partners the same age or younger (100). In contrast, findings from a population based study of 15 to 29-year-old women in rural Hlabisa, KwaZulu-Natal found no association between partner age disparity and HIV risk, even when the analysis was restricted to 15-19 year old women (101). Limitations cited for this study include attrition of the cohort over time, non-response and misreporting of partners age. Furthermore, economic disparities may be less common in this rural setting as compared to urban areas of South Africa, thereby minimising risk of engaging in relationships with high risk older men (101).

3.3.1.3. Transactional Sex and Commercial Sex Work

Closely linked to age disparate relationships is transactional sex which is the exchange of sex for money or goods which is not implicitly considered to be commercial sex work. Young women are thought to engage in relationships with older men for financial gain among other reasons (15, 102). In a systematic review of 68 epidemiological studies conducted in Africa, a history of transactional sex was more frequent among HIV infected women compared to HIV uninfected controls (OR 2.29, 95% CI 1.45-3.62) (103). In Western Cape province, South Africa, 26.7% of women who engaged in casual sexual relationships disclosed a history of transactional sex (104). Among antenatal clinic attendees in Soweto, South Africa, 21% of women reported ever engaging in transactional sex and this was more common in women

who had a history of partner violence, substance use, poor living conditions and urban residence (105). In the same study, transactional sex was found to increase risk for HIV acquisition (OR 1.54, 95% CI 1.07-2.21) (105).

Commercial sex work, which is defined as the exchange of money for sexual services, is estimated to range from <1% to around 4% of the female adult population in major cities and other urban areas in SSA (106). Unemployment or low socio-economic status, poverty, food insecurity, having a number of dependents or a low educational level and drug and alcohol dependence are some of the reasons women enter sex work (106). It is associated with a high burden of HIV with prevalence among sex workers and their clients estimated to be 10-20 times higher than the general population in SSA (106). There is a paucity of HIV prevalence data in South Africa with the estimate from 1998 being between 50 to 70% among female commercial sex workers (106). Sex workers are also burdened by high rates STIs including gonorrhoea which has been associated with increased HIV risk among older female sex workers in Johannesburg (107). Among female sex workers in Durban, engaging in anal sex was found to increase HIV risk by two-fold (52). In a separate study undertaken in Durban, evaluating coping mechanisms with the threat of HIV, HIV awareness was high, however this did not translate into safer sex practices (108).Vaginal insertion practices are a common occurrence among sex workers and may further predispose women to HIV (95). Alcohol and drug use before sex acts is known to reduce the likelihood of condom use in this group (95). While the number of sex clients vary across regions, emphasis on safer sex practices rather than reducing demand for commercial sex is the cornerstone of most sex worker care programmes (106). For these reasons female sex works remain at high risk group within South Africa and efforts to limit HIV incidence in this group is important to curb HIV in the context of the larger generalised epidemic.

3.3.1.4. Number of Sex Partners Including Multiple Concurrent Partnerships

Several studies have demonstrated that HIV risk increases with an increasing number of sexual partners (31, 34, 103). While multiple concurrent partnerships is thought to be a key driver of the epidemic in Southern Africa, the number of sequential lifetime partners also appears to increase risk for HIV (109). A systematic review of 68 studies conducted in Africa found that the odds of HIV infection in women reporting three or more lifetime sex partners was almost four times higher than in those reporting between zero and two partners (OR 3.64, 95% CI 2.87-4.62) (103). In Cameroon, HIV prevalence was higher among women reporting two or more sex partners in the last 12 months or four or more lifetime sex partners (34). In Johannesburg, South Africa, the risk of HIV infection was five-fold higher in women reporting two or more lifetime partners (OR 4.88, 95% CI 3.01-7.89; p=0.001) while women in KwaZulu-Natal reporting multiple sexual partners were at increased risk for HIV [adjusted odds ratio (aOR) 1.78, 95% CI 1.11-2.85; p=0.02] (31, 96). In a national survey among 15 to 24-year-old youth, having multiple concurrent partners (OR 3.40, 95% CI 1.80-6.50; p<0.001) placed women at increased risk of HIV infection although concurrent partnerships and serial monogamy were equally common in women (110). Transactional sex and an inability to negotiate condom use was more often reported in these women (110).

3.3.1.5. Condom Use

Male or female condoms are a safe and effective method of preventing HIV infection. A review of 14 observational studies of HIV discordant couples found an 80% reduction in HIV incidence in people who always used condoms versus those that never used condoms (HIV incidence 1.14 per 100 person years, 95% CI 0.56-2.04 and 5.75 per 100 person years, 95% CI 3.16-9.66 respectively) (111). In South Africa, overall condom use at last sex act among males and females was found to be 36.2% (95% CI 34.5-37.9%) in the 2012 HSRC survey

(18). Condom use was reported more frequently among males, youth and Black Africans and was higher than the national average for most high risk groups including young Black African females (18). In another study of high-risk women, those who achieved a higher level of education or who used condoms as a contraceptive method were more likely to use condoms (112). Inconsistent condom use especially with long-term or stable partners and the inability of women to negotiate condom use remain some of the drawbacks of this prevention method.

3.3.1.6. Alcohol and Substance Use

The use of alcohol and other substances has been linked to HIV risk in several studies (113-115). A systematic review of 21 studies conducted in SSA found that users of alcohol were more likely to be HIV positive than non-users, and the use of alcohol in relation to sex acts increased risk for HIV (114). A review of studies in Southern Africa confirmed these findings (113). The quantity of alcohol consumed rather than the frequency of alcohol intake was linked to risky sexual behaviour such as infrequent condom use, increased number of sex partners and partner concurrency (113). Similarly, the use of other substances eg. cannabis, cocaine, heroin and methamphetamines within and outside of the sexual context has been linked to increased HIV risk (115). A study which included non-injecting drug users in three major cities in South Africa found that substance use in relation to sexual activity led to sexual disinhibition and risky sexual behaviour such as anal sex and sex with multiple partners at the same time (116).

3.3.2. Vaginal Practices

The use of substances in the vagina may potentially change the genital micro-environment thereby compromising host defences in the genital tract or may lead to epithelial disruption (94). Douching or 'dry sex' has been implicated as a risk factor for HIV acquisition (95). Vaginal practices differ between regions and are categorised, but are not limited to the following: external washing, external application of substances, intravaginal cleansing, intravaginal insertion and oral ingestion (94). Vaginal practices are common practice among both female sex workers and women not engaging in sex work in Africa (117, 118). Among 867 women interviewed in women in KwaZulu-Natal, 90.2% of women engaged in vaginal practices which were undertaken for hygiene purposes or for sexual motivation, with intravaginal cleansing being the commonest vaginal practice (119, 120). The type of substances used to cleanse the vagina appears to influence HIV risk, with more abrasive substances likely to alter the vaginal flora to a greater extent. A ten year study of Kenyan sex workers found that women who practiced vaginal cleansing were at increased risk for HIV compared to those that did not (121). Risk for HIV was four-fold higher with intravaginal washing with soap (HR 3.84, 95% CI 1.51-9.77; p=0.005) and three-fold higher in those who washed with water only (HR 2.64, 95% CI 1.00-6.97; p=0.05) compared to women who did not practice vaginal washing, after adjusting for demographic variables, sexual behaviour and STIs (121). Among Zimbabwean and Ugandan women, vaginal cleansing was not associated with HIV acquisition (122). In South Africa, no association was found between vaginal practices and incident HIV infection (123). Vaginal practices were associated with injectable hormonal contraception use and bacterial vaginosis in two separate studies indicating that other risk factors for HIV need to be excluded when exploring the association between vaginal practices and HIV risk (119, 124).

While behavioural risk factors contribute substantially to the HIV burden, these factors must be interpreted in the context of the broader structural factors in this setting which may influence risk behaviour. Nevertheless, understanding the role of these factors to modify unsafe sex practices could substantially impact on the high disease burden.

3.4. Knowledge of HIV

An adequate knowledge of HIV prevention and transmission is key for willingness to engage HIV prevention modalities. Studies undertaken in SSA show variation in trends of HIV knowledge (125-128). A review of health surveillance data from 16 countries in SSA, found that knowledge of blood-borne transmission of HIV was inversely correlated to HIV prevalence at a national level (128). In rural Nigeria knowledge about HIV transmission was generally high among 210 adults interviewed with the majority of participants having a good or fair knowledge of HIV (125). A survey of health care workers including physicians, midwives, nurses, medical students and nursing auxiliaries in Madagascar found that knowledge of transmission of HIV was poor and misconceptions were common (126). Among young urban women in Kenya, comprehensive HIV knowledge had increased from 9% in 1993 to 54% in 2008 and 2009 (127). The 2012 HSRC survey in South Africa found that 26.8% of the population had adequate knowledge about HIV sexual transmission and prevention. Knowledge levels differed by age, race, locality and province (18). Furthermore, between the 2008 and 2012 reporting periods knowledge of HIV decreased among all high risk groups with the exception of Black African females in whom knowledge of HIV increased marginally (18). Earlier studies of female sex workers in Limpopo province, South Africa, found that knowledge of HIV prevention was inadequate and some misconceptions regarding HIV transmission prevailed while in KwaZulu-Natal province knowledge of HIV transmission and prevention was high among high risk women (112, 129).

4. Conclusion

This literature review emphasises the sustained high burden of HIV infection in women in South Africa and that while risk for HIV is multifactorial, it is incompletely elucidated in this setting. High risk behaviours such as having multiple sex partners, engaging in age–disparate relationships, using alcohol in relation to sex acts and being a sex worker may contribute significantly to HIV risk however biological factors such as STIs are also recognised as increasing HIV susceptibility in women (73, 96, 100, 106, 113). Consistent condom use is known to reduce risk for HIV and marriage also appears to attenuate risk, albeit in older age groups (18, 111). Ongoing research to further explore factors which increase susceptibility to HIV infection in women, particularly at the level of the female genital tract, may inform the development of biomedical interventions for women in future. Understanding risk for HIV in this setting is imperative to guide current prevention policy with regard to communicating the right messages and identifying and targeting groups at greatest risk of HIV. The role of structural factors and HIV risk must not be underestimated in this setting. In the long-term, structural approaches must be incorporated into current HIV policy if behavioural and biomedical interventions are to be successful.

Chapter 3: Journal Manuscript

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Title Page

Risk factors for HIV acquisition in high risk women in a generalised epidemic setting

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Main Manuscript

Risk factors for HIV acquisition in high risk women in a generalised epidemic setting

Abstract

In South Africa young women bear a disproportionate burden of HIV infection however, risk factors for HIV acquisition are not fully understood in this setting. In a cohort of 245 HIV negative women, we used proportional hazard regression analysis to examine the association of demographic, clinical and behavioural characteristics with HIV acquisition. The overall HIV incidence rate (IR) was 7.20 per 100 women years (wy), 95% Confidence Interval (CI) 4.20–9.80]. Women 18 to 24 years had the highest HIV incidence [IR 13.20 per 100 wy, 95% CI 6.59–23.62] and were almost three times more likely to acquire HIV compared to women 25 years and older [adjusted Hazard Ratio (aHR) 2.61, 95% CI 1.05–6.47]. Similarly, women in relationships with multiple sex partners [IR 8.97 per 100 wy, 95% CI 5.40–14.0] had more than twice the risk of acquiring HIV when compared to women who had no partner or who had a husband or stable partner (aHR 2.47, 95% CI 0.98–6.26). HIV prevention programmes must address young women's vulnerability and promote safer sex practices for high risk women.

Keywords: Risk factors, HIV acquisition, women

Introduction

Into the fourth decade of the Human Immunodeficiency Virus (HIV) epidemic, South Africa has an estimated 6.1 million people living with HIV and contributes 17% of the global HIV burden, yet accounts for only 0.7% of the world's population (1, 2). With the majority of infections heterosexually acquired, young women bear a disproportionate burden of HIV. Sentinel surveillance among pregnant women shows that in 1990, HIV prevalence was 0.7% and by 1998 increased exponentially to 22.8%, peaking at 30.2% in 2010 (3). Data from national population based surveys show similar trends with high HIV prevalence in young women compared to young men (4, 5). HIV prevalence peaked at 36.0% among women aged 30 to 34 years and at 28.8% among men in the 35 to 39 year–old age group (5). HIV prevalence in women aged 15 to 19 years was 5.6%, eight times higher than males in the same age group, and increased to 17.4% among women aged 20 to 24 years and 28.4% in the 25 to 29 year age group (5). Within South Africa there is significant geographical variation in the distribution of HIV and the province of KwaZulu–Natal remains the worst affected (3, 6). Whilst HIV prevalence is an important tool to establish the burden of existing infections and to ascertain survival rates over time, HIV incidence rate is a more sensitive measure to monitor the epidemic as it indicates the rates of new infections as well as ongoing HIV transmission. Amongst rural and urban women in KwaZulu-Natal, the HIV incidence rates have been 6.5 and 6.4 per 100 women years (wy) respectively, and in young women younger than 18 years, 4.7 per 100 wy (7, 8). These data underscore the need to understand the risk factors contributing to these high HIV prevalence and incidence rates.

The key risk factors for HIV in this setting include a combination of structural, behavioural and biological factors. Poverty, labour migration, urbanisation, gender inequalities and gender–based violence contribute to an increased susceptibility to HIV (6, 9).

At the individual level, being single, unemployed or not achieving a high school education increase vulnerability and predispose young women to HIV (5, 10, 11). High risk sexual behaviour such as engaging in multiple concurrent partnerships, transactional sex and age–disparate relationships, has contributed significantly to enhancing HIV risk and is exacerbated by the inability of women to negotiate condom use even in long–term partnerships (9, 12-15). Physiological changes of the genital tract as well as factors which affect the integrity of the genital tract epithelium such as sexually transmitted infections (STIs) and intra–vaginal insertion practices, either for cleansing or enhancing sex, may increase susceptibility to HIV by facilitating HIV viral entry (9, 16-19). Recent data suggests that genital tract inflammation in women with symptomatic and asymptomatic STIs may upregulate HIV susceptible target cells at the mucosal level thereby aiding transmission (20). The association between hormonal contraception use, in particular progestogen–only injectable preparations, and HIV risk remains contentious as current biological and epidemiological evidence is limited (21, 22).

As the HIV epidemic evolves, it is important to understand the factors that contribute to vulnerability and risk so that the design of HIV prevention programmes are tailored taking these into account. In this prospective cohort study we explored factors associated with HIV acquisition in a generalised epidemic setting.

Methods

Study Setting and Study Population

This study is a secondary analysis of data collected in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 002 study. The CAPRISA 002 study was initiated to advance the understanding of HIV–1 subtype C acquisition, pathogenesis and

disease progression. Between August 2004 and May 2005 volunteers from the city of Durban, KwaZulu–Natal, South Africa were recruited for study participation. Eligibility criteria and screening and enrolment procedures have been described (23). Briefly women 18 years and older, self–identifying as sex workers or having had at least three partners in the three months prior to recruitment, and testing HIV negative were eligible for study participation. Although all women residing in KwaZulu-Natal province are known to be at high risk of HIV infection, the aim of the CAPRISA 002 study was to recruit women at greatest risk of HIV infection (23). Women were recruited from known sex worker sites in Durban (23). One of the challenges encountered in the development of this cohort is that the term sex worker or client was not always culturally appropriate when recruiting women (23). Using broader terms such as having 'multiple partners who provide material needs in exchange for sex' was used therefore used as a recruitment strategy (23).

Study procedures

Volunteers were provided with verbal and written information about the study objectives and procedures after which written consent for screening procedures and for long–term storage of specimens were obtained. Women agreeing to participate in the study received pre and post–test counselling for HIV testing and risk reduction counselling and had blood samples collected for HIV antibody testing. Women testing HIV positive were referred to support services for ongoing care and psychosocial support. All women testing HIV antibody negative or indeterminate were enrolled until the study endpoint of HIV infection or for a period of 24 months.

At baseline, trained nurses administered questionnaires to all participants to obtain information regarding socio–demographic history, risk behaviour, knowledge about HIV and more direct questions related to sex work. A complete physical examination was undertaken

at baseline and at each monthly follow up visit. Urine pregnancy testing was done if clinically indicated or upon request by a participant (23). Pelvic examination for genital sample collection was undertaken at baseline and at six monthly intervals. Blood specimen collection was done at baseline (safety monitoring tests, serological testing for STIs and long–term storage), at each monthly visit (HIV testing) and at each six–monthly visit (safety monitoring tests and serological testing for STIs).

Laboratory Evaluations

HIV testing was done using two rapid antibody tests using the Determine HIV–1 test (Abbott Diagnostics, Johannesburg, South Africa) and the Capillus HIV–1/HIV–2 test (Trinity Biotech, USA) followed by HIV–1 RNA polymerase chain reaction (PCR) testing using the Cobas AmpliScreen Multiprep HIV–1 test version 1.5 and the Cobas AmpliPrep/Cobas Amplicor HIV–1 Monitor test version 1.5 (Roche Diagnostics, Branchburg, New Jersey, USA) if at least one rapid antibody test was negative (24). Confirmatory HIV ELISA testing was done on PCR positive samples (Enzynost anti HIV1/2 plus Dade Behring, Deerfield, Illinois, USA) (24). HIV infection as endpoint was based on a positive HIV–1 antibody test with a previously documented negative HIV–1 antibody test; or the presence of HIV–1 RNA in the absence of HIV antibodies (23).

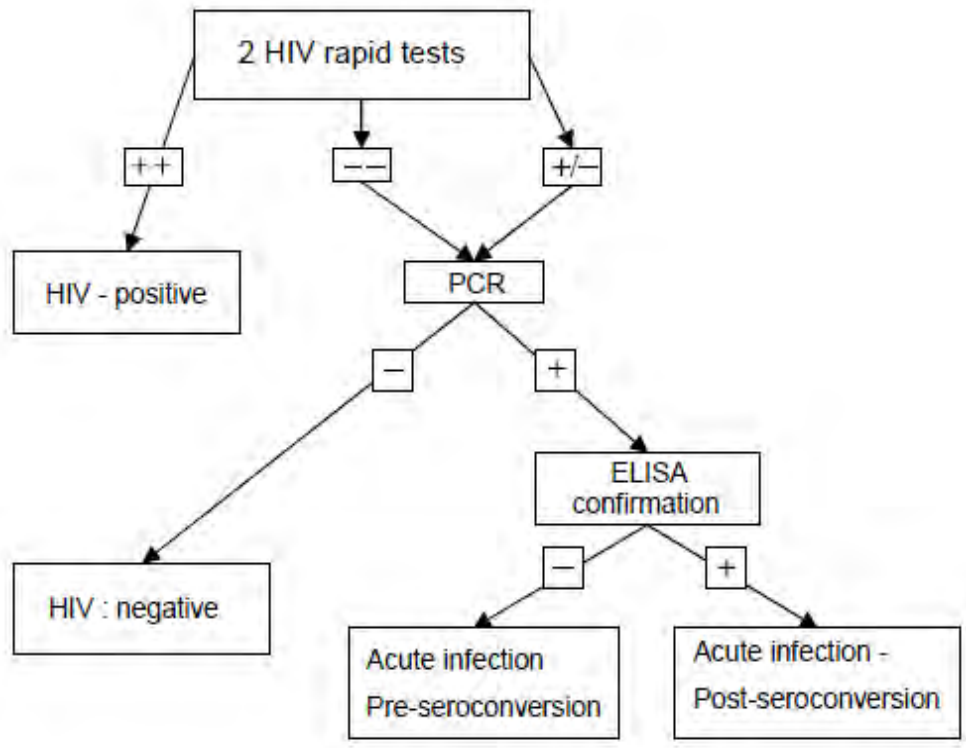

Figure 1. HIV testing algorithm. ELISA, Enzyme-linked immunosorbent assay; PCR, polymerase chain reaction (24).

Safety monitoring tests comprised haematological and biochemical evaluations. Genital specimens were tested for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Mycoplasma genitalium* and herpes simplex virus (HSV) type 2 using PCR and bacterial vaginosis (BV) was diagnosed on Gram–stained smears using Nugent's criteria. Syphilis [Becton Dickinson Macro–Vue RPR (rapid plasma reagin) card test and Omega ImmuTrep TPHA test], HSV infection (HerpeSelect–1 and HerpeSelect–2) and hepatitis B (HBV) infection (CENTAUR XP) were diagnosed serologically.

Data Management

Demographic, behavioural, clinical and laboratory data were captured onto standardised case report forms (CRFs) which were coded with a participant identification number in order to maintain participant confidentiality. CRFs were faxed using the DataFax

system (Clinical DataFax Sytems Inc., Ontario, Canada), with all data verified by data encoders for quality checks and stored in a secure study specific database.

Measures

HIV–1 infection was the main outcome measure. Baseline information on socio–demographic factors (age, number of dependents, educational level), behavioural factors (age at sexual debut, relationship status, frequency and type of sex acts, male and or female condom use [defined as condom use at last sex act], contraception type, douching, alcohol and substance use during sex), sex for compensation (time period involved in sex work, age started sex work, days per week engaged in sex work, number of sites worked at, number of sex clients, short sessions or overnight stays per week with clients and condom use), clinical factors, including pregnancy and laboratory measures (full blood counts, liver function tests, electrolytes, vitamin B12, folate, iron, glucose, calcium, phosphate, $CD4^+$, $CD3^+$ and $CD8^+$ cell counts and STIs), and knowledge questions on HIV prevention and transmission were obtained.

Statistical Analysis

This was an observational cohort at high risk of acquiring HIV, designed to answer questions regarding HIV pathogenesis during acute HIV infection. Thus for the purposes of this analysis, the cohort was analysed as an observational cohort, including all HIV negative women in the original design, and therefore no sample size calculation was performed. Baseline data were summarised using descriptive statistics, with continuous variables reported as means and standard deviations or medians and interquartile ranges, while categorical variables are reported as percentages and actual numbers. Unadjusted and adjusted proportional hazards regression analysis was performed to assess the impact of socio–demographic factors, risk behaviour, sex for compensation and clinical factors on HIV acquisition. Estimated time of HIV infection was defined as the midpoint between the last

negative HIV ELISA and the first positive HIV ELISA or 14 days prior to a positive HIV–1 RNA test if the HIV ELISA is negative on the same day (23). Time to HIV infection was calculated from the date of enrolment until the estimated time of HIV infection. HIV incidence rate was calculated as [Incidence Rate (IR) = number of HIV cases / total person years at risk X 100] and confidence intervals (CI) for IR assumed a Poisson distribution. Participants who remained HIV negative were censored at their last visit. Factors with a p–value of less than 0.2 in the unadjusted analysis were included in the adjusted model. As anal sex is known to be a risk factor for HIV acquisition, even among high risk women, this variable was included in a multivariable model (25, 26). Although current evidence for contraception and HIV risk is limited, this variable was also included given the biological plausibility of this variable with HIV risk (9, 27, 28). The first multivariable model included all women, sex workers and women reporting more than three sexual partners (Table 2). In this model some variables relating to sex work had a p–value of less than 0.2 in the unadjusted analysis, however these were not included in the adjusted model, because a large proportion of the cohort did not identify themselves as sex workers. The second multivariable model (Table 2) was restricted to women reporting sex for compensation. All variables related to sex work with a p-value of less than 0.2 in the unadjusted model were included in the final adjusted model.

P–values less than 0.05 were considered statistically significant. Analysis was performed using SAS software version 9.3 (SAS Institute Inc., Cary).

Ethics Approval

Ethics approval for the CAPRISA 002 study was obtained from the Universities of Natal (E013/04), Cape Town (025/2004) and Witwatersrand (MM040202). The ethical approval for the secondary analysis of data was granted from the Biomedical Research Ethics

Committee, University of KwaZulu–Natal (BE 092/11) and permission to review data stored in the CAPRISA 002 database was approved through the CAPRISA data sharing policy.

Results

A total of 775 women were screened of whom 509 (65.7%) were ineligible for the following reasons: tested HIV positive (n=462; 59.6%), reported less than three sexual partners in the previous three months (n=22; 2.8%), were pregnant (n=16; 2.1%), planned to relocate (n=4; 0.5%), younger than 18 years (n=3; 0.4%) or afraid of testing procedures (n=2; 0.3%) (23). Twenty–one (2.7%) women were eligible for study participation but did not return for enrolment and 245 (31.6%) were enrolled into the study.

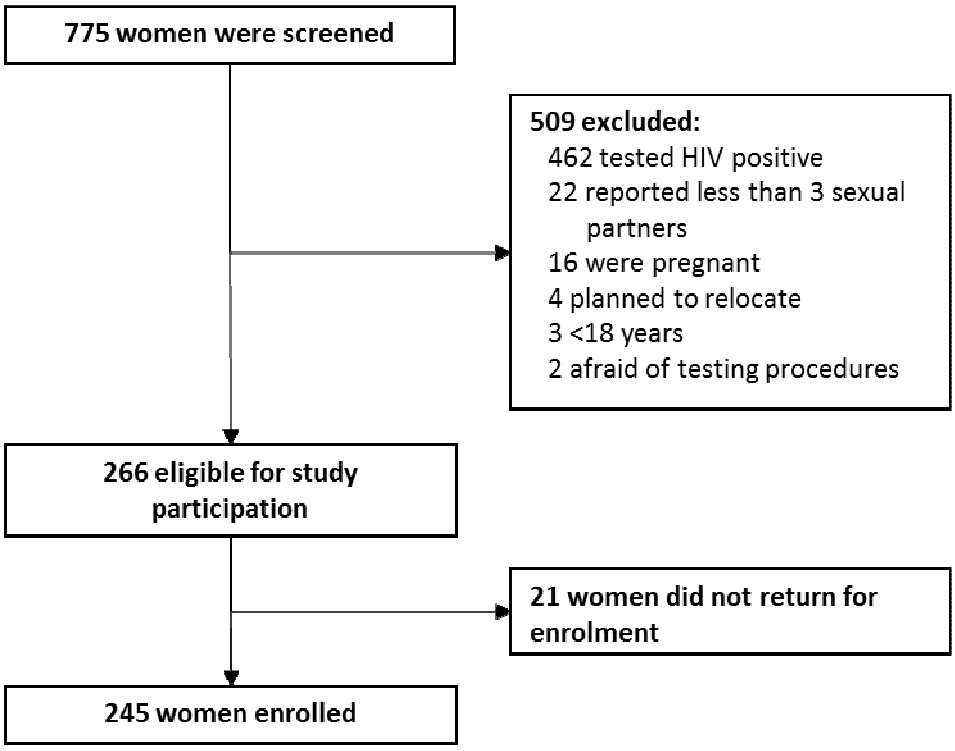

Figure 2. CAPRISA 002 Screening and Enrolment

After 390 wy of follow up, 28 women acquired HIV, yielding an incidence rate (IR) of 7.20 per 100 wy [95% CI 4.20–9.80]. Women aged 18 to 24 years had the highest HIV incidence (IR 13.20 per 100 wy, 95% CI 6.59–23.62). The HIV incidence among women 25 years and older was 4.58 per 100 wy (95% CI 2.50–7.68). Three women were excluded from any risk factor analyses as they were in the window period of HIV infection at study entry.

The baseline characteristics overall and of those acquiring HIV are shown in Table I. The mean age of 242 women was 34.3 years [standard deviation (SD) 10.47, range 18–58], 185 (76.8%) women had one or more dependents and 156 women (64.5%) had an educational level which was above grade 8. The mean age at sexual debut was 17 (SD 2.38, range 12–26) years. Although a large proportion (191; 78.9%) self–identified themselves as sex workers, having spent a median of three years [interquartile range (IQR) 1.3–8.3] in sex work, only 137 (56.6%) women reported being in a relationship with multiple partners at the time of the study.

Women who acquired HIV were approximately five years younger than those who remained HIV negative [t statistic (t)=2.20, p=0.029], reported being in relationships with multiple partners more frequently [Fisher's exact test hypergeometric probability (Fprob)=0.02, p=0.054] and had higher baseline serum vitamin B12 levels (t=-2.00, p=0.047).

Table II shows the socio–demographic, behavioural and biological variables associated with risk of HIV acquisition overall and by sex work. Younger women, aged 18 to 24 years were almost three times more likely to acquire HIV compared to women 25 years and older [Hazard Ratio (HR) 2.85, 95% CI 1.29–6.28; Chi-square test statistic (χ^2)=6.73; p=0.010] and similarly women reporting many partners were also at almost three times greater risk of HIV acquisition (HR 2.61, 95% CI 1.04–6.52; χ^2=4.18; p=0.041). A higher educational level, above grade 8, was weakly associated with increased risk for HIV (HR

2.45, 95% CI 0.92–6.52; χ^2=3.21; p=0.073) as were STIs (HR 2.47, 95% CI 0.93 – 6.58; χ^2=3.26; p=0.071), but no significant associations were found for HIV risk and engaging in anal sex (HR 1.49, 95% CI 0.68–3.29; χ^2=0.99; p=0.321) or delayed sexual debut (HR 0.88, 95% CI 0.73–1.06; χ^2=1.88; p=0.170).

Among women reporting sex for compensation, HIV risk remained three–fold higher among 18 to 24 year–old women (HR 3.27, 95% CI 1.29–8.30; χ^2=6.24; p=0.013) and ten–fold higher among those reporting many partners (HR 10.32, 95% CI 1.37–77.55; χ^2=5.15; p=0.023). Weak associations were found between increased HIV risk and every additional overnight stay per week with clients (HR 1.33, 95% CI 0.94–1.88; χ^2=2.61; p=0.106) and reduced risk for every additional year spent in sex work (HR 0.93, 95% CI 0.85–1.02; χ^2=2.34; p=0.126).

In the final multivariable model overall, young age remained significant for HIV risk (HR 2.61, 95% CI 1.05–6.47; χ^2=4.27; p=0.039) and by sex work, having multiple partners was significantly associated with HIV risk (HR 8.55, 95% CI 1.11 – 6.02; χ^2=4.23; p=0.040).

Table I: Baseline socio–demographic, behavioural and biological characteristics of women, overall and by HIV status[a], Durban, South Africa, 2004/2007

Variable	Total (n = 242)	HIV Positive (n = 25)	HIV Negative (n = 217)	Test statistic = value[b]	p–value
Demographic data					
Age in years (Mean, ±SD)	34.3 (10.47)	30.0 (10.37)	34.8 (10.39)	t=2.20	**0.029**
Age group (in years) % (n)					
18– 24	26.54% (64)	44.0% (11)	24.4% (53)	Fprob=0.0230	0.053
≥ 25	73.6% (178)	56.0% (14)	75.6% (164)		
Number of dependents % (n)					
0	23.2% (56)	16.0% (4)	24.1% (52)	Fprob=0.1429	0.460
≥ 1	76.8% (185)	84% (21)	75.9% (164)		
Educational level % (n)					
≤Grade 8	35.5% (86)	20.0% (5)	37.3% (81)	Fprob=0.0416	0.121
> Grade 8	64.5% (156)	80.0% (20)	62.7% (136)		
Risk behaviour					
Age in years at sexual debut (Mean, ±SD)	17.0 (2.38)	16.5 (1.76)	17.1 (2.44)	t=1.18	0.239
Relationship status % (n)					
No partner or Stable/Married partner	43.2% (104)	24.0% (6)	45.4% (98)	Fprob=0.0212	0.054
Many partners	56.6% (137)	76.0% (19)	54.4% (118)		
Mean sex acts per month (±SD)	10.3 (6.78)	10.3 (5.03)	10.3 (6.97)	t=-0.01	0.994
Type of sex act % (n)					
Ever had vaginal sex	100% (242)	100% (25)	100% (217)	–	–
Ever had anal sex	34.6% (83)	44.0% (11)	33.5% (72)	Fprob=0.0997	0.374
Ever had oral sex	25.3% (61)	24.0% (6)	25.5% (55)	Fprob=0.1915	1.000
Condom use at last sex act % (n)					
Yes	58.7% (142)	64.0% (16)	58.1% (126)	Fprob=0.1469	0.670
No	41.3% (100)	36.0% (9)	41.9% (91)		
Any contraception % (n)	79.7% (192)	68.0% (17)	81.0% (175)	Fprob=0.0635	0.185
Contraception type % (n)					
Condom only	41.5% (100)	36.0% (9)	42.1% (91)	Fprob=0.0296	0.808
Hormonal	30.7% (74)	32.0% (8)	30.5 (66)		
None/Other[c]	27.8% (67)	32.0% (8)	27.2% (59)		
Douching after sex % (n)	9.5% (23)	4.0% (1)	10.2% (22)	Fprob=0.2096	0.483
Sex after alcohol or substance use % (n)					
Yes	26.9% (65)	20.0% (5)	27.7% (60)	Fprob=0.1441	0.484
No	73.1% (177)	80.0% (20)	72.4% (157)		
Sex for compensation					
Self–reported sex workers % (n)	78.9% (191)	72.0% (18)	79.7% (173)	Fprob=0.1287	0.436
Years in sex work (Median, IQR)	3.0 (1.3 – 8.3)	2.5 (1.0 – 7.0)	3.0 (1.3 – 9.0)	Z=-1.36	0.179
Age at start of sex work (Mean, ±SD)	28.2 (9.02)	27.2 (10.16)	28.3 (8.92)	t=0.49	0.625
Days per week perform sex work (Mean, ±SD)	2.9 (1.40)	2.9 (1.55)	2.9 (1.39)	t=-0.21	0.835
Sites worked per year (Mean, ±SD)	2.6 (1.30)	2.8 (0.86)	2.6 (1.33)	t=-1.08	0.291

Table I: Baseline socio–demographic, behavioural and biological characteristics of women, overall and by HIV status[a], Durban, South Africa, 2004/2007

Variable	Total (n = 242)	HIV Positive (n = 25)	HIV Negative (n = 217)	Test statistic = value[b]	p–value
Clients per day (Mean, ±SD)	2.5 (1.19)	2.6 (1.24)	2.5 (1.19)	t=-0.36	0.722
Clients in the past week (Mean, ±SD)	3.1 (3.83)	2.4 (2.53)	3.1 (3.94)	t=1.01	0.323
Short sessions per week (Mean, ±SD)	4.2 (4.05)	3.6 (4.68)	4.3 (3.99)	t=0.63	0.527
Overnight stays per week (Mean, ±SD)	1.1 (1.20)	1.4 (1.34)	1.0 (1.19)	t=-1.46	0.147
Condom use in the last month % (n)					
Always	38.2% (73)	27.8% (5)	39.3% (68)	Fprob=0.0332	0.650
Sometimes	42.9% (82)	50.0% (9)	42.2% (73)		
Never	18.9% (36)	22.2% (4)	18.5% (32)		
HIV knowledge					
How safe is anal sex compared to peno–vaginal sex? % (n)					
Same or more risk	62.8% (152)	56.0% (14)	63.6% (138)	Fprob=0.0021	0.278
Less risk or don't know	37.2% (90)	44.0% (11)	36.4% (79)		
How safe is oral sex compared to peno–vaginal sex? % (n)					
Same or more risk	71.8% (173)	68.0% (17)	72.2% (156)	Fprob=0.1629	0.644
Less risk or don't know	28.2% (68)	32% (8)	27.8 (60)		
If you have an STI are you more likely to get HIV? % (n)					
Yes	71.1% (172)	80.0% (20)	70.1% (152)	Fprob=0.0850	0.665
No	1.7% (4)	0.0% (0)	1.8% (4)		
Only sometimes/unsure	27.3% (66)	20.0% (5)	28.1% (61)		
Do you think HIV can be treated? % (n)					
Yes	95.0% (230)	96.0% (24)	94.9% (206)	Fprob=0.1587	0.516
No	2.1% (5)	4.0% (1)	1.8% (4)		
Unsure	2.9% (7)	0.0% (0)	3.2% (7)		
Clinical evaluation					
Ever pregnant prior to HIV % (n)					
Yes	10.3% (25)	12.0% (3)	10.1% (22)	Fprob=0.2441	0.730
No	89.7% (217)	88.0% (22)	89.9% (195)		
Laboratory parameters					
Haemoglobin g/dL (Mean, ±SD)	12.7 (1.29)	12.8 (1.43)	12.7 (1.27)	t=-0.51	0.613
Eosinophils 1 x 10^9/L (Mean, ±SD)	0.2 (0.24)	0.3 (0.25)	0.2 (0.24)	t=-0.81	0.420
Albumin g/L(Mean, ±SD)	44.7 (3.57)	44.8 (3.32)	44.7 (3.60)	t=-0.02	0.981
Vitamin B12 pg/mL (Mean, ± SD)	291.0 (93.14)	327.6 (112.19)	287.0 (90.25)	t=-2.00	**0.047**
Folate ng/mL (Mean, ±SD)	22.4 (8.45)	20.8 (8.54)	22.6 (8.45)	t=0.94	0.346
Serum iron µmol/L (Mean, ±SD)	12.3 (6.41)	12.1 (5.36)	12.3 (6.53)	t=0.16	0.877
Random glucose mmol/L (Mean, ±SD)	5.2 (2.02)	4.7 (0.85)	5.3 (2.11)	t=2.72	**0.008**
Sodium mmol/L (Mean, ±SD)	137.1 (2.22)	138.2 (2.08)	137.0 (2.21)	t=-2.66	**0.008**
Potassium mmol/L (Mean, ± SD)	4.0 (0.32)	4.1 (0.41)	4.0 (0.32)	t=-0.35	0.730
Chloride mmol/L (Mean, ±SD)	103.0 (2.55)	103.9 (1.98)	102.9 (2.59)	t=-1.93	0.054
Calcium mmol/L (Mean, ±SD)	2.4 (0.12)	2.4 (0.09)	2.4 (0.12)	t=-0.67	0.501

Table I: Baseline socio–demographic, behavioural and biological characteristics of women, overall and by HIV status[a], Durban, South Africa, 2004/2007

Variable	**Total (n = 242)**	**HIV Positive (n = 25)**	**HIV Negative (n = 217)**	**Test statistic = value[b]**	**p–value**
Phosphate mmol/L (Mean, ±SD)	1.1 (0.18)	1.1 (0.17)	1.0 (0.18)	t=-1.43	0.155
HIV negative $CD4^+$ count cells/µl (Median, IQR)	888 (742 – 1132)	969 (855 – 1068)	878 (738 – 1132)	Z=0.76	0.446
HIV negative $CD3^+$ count cells/µl (Median, IQR)	1493 (1202 – 1839)	1522 (1324– 1857)	1486 (1197– 1822)	Z=0.58	0.564
HIV negative $CD8^+$ count cells/µl (Median, IQR)	517 (402– 688)	500 (420– 760)	520 (402– 680)	Z=-0.04	0.966
Bacterial vaginosis % (n)	52.7% (127/241)	68.0% (17)	50.9% (110/216)	Fprob=0.0465	0.139
Sexually transmitted infection[d] % (n)	31.3% (75/240)	44.0% (11)	29.8% (64/217)	Fprob=0.0625	0.172

[a] HIV status as at end of follow-up period

[b]Test statistic are: t=t-tests or Z=Wilcoxon Rank Sums (Normal Approximation), for continuous variables; Fprob=Fisher's exact test hypergeometric probability, for categorical data

[c] Female sterilisation or rhythm/calendar method

[d] Any STI present if participant tested positive for syphilis antibodies or *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Chlamydia trachomatis* or herpes simplex virus (HSV) type II (PCR)

Table II: Socio–demographic, behavioural and biological risk factors for HIV acquisition in women, overall and by sex work, Durban, South Africa, 2004/2007

Variable	Overall						By Sex Work					
	#HIV Cases/ PY at risk	HIV Incidence (95% CI)	Univariate Hazard Ratio (95% CI)	p-value (χ^2)	Multivariable Hazard Ratio (95% CI)	p-value (χ^2)	#HIV Cases/ PY at risk	HIV Incidence (95% CI)	Univariate Hazard Ratio (95% CI)	p-value (χ^2)	Multivariable Hazard Ratio (95% CI)	p-value (χ^2)
Demographic characteristics												
Age group (in years)												
≥25	14/305.90	4.58 (2.50 – 7.68)	1.00	–	1.00	–	10/248.35	4.03 (1.93 – 7.40)	1.00	–	1.00	–
18–24	11/83.32	13.20 (6.59 – 23.62)	2.85 (1.29 – 6.28)	**0.010** ***(χ^2=6.73)***	2.61 (1.05 – 6.47)	**0.039** ***(χ^2=4.27)***	8/59.36	13.48 (5.82 – 26.56)	3.27 (1.29 – 8.30)	**0.013** ***(χ^2=6.24)***	2.44 (0.79 – 7.56)	0.123 *(χ^2=2.38)*
Number of dependents												
None	4/79.27	5.05 (1.37 – 12.92)	1.00	–			3/62.58	4.79 (0.99 – 14.01)	1.00	–		
1 or more	21/309.13	6.79 (4.21 – 10.38)	1.36 (0.47 – 3.95)	0.576 *(χ^2=0.31)*			15/244.31	6.14 (3.44 – 10.13)	1.29 (0.37 – 4.46)	0.686 *(χ^2=0.16)*		
Educational level												
Grade ≤8	5/148.20	3.37 (1.10 – 7.87)	1.00	–	1.00	–	4/115.79	3.45 (0.94 – 8.85)	1.00	–	1.00	–
Grade >8	20/241.02	8.30 (5.07 – 12.82)	2.45 (0.92 – 6.52)	0.073 *(χ^2=3.21)*	1.92 (0.65 – 5.68)	0.241 *(χ^2=1.37)*	14/191.92	7.29 (3.99 – 12.24)	2.10 (0.69 – 6.39)	0.190 *(χ^2=1.72)*	1.45 (0.40 – 5.20)	0.570 *(χ^2=0.32)*
Behavioural characteristics												
Age at sexual debut (per 1 year increase)			0.88 (0.73 – 1.06)	0.170 *(χ^2=1.88)*	0.86 (0.71 – 1.05)	0.149 *(χ^2=2.08)*			0.96 (0.78 – 1.19)	0.707 *(χ^2=0.14)*		
Relationship status												
No partner or stable/ married partner	6/175.52	3.40 (0.09 – 18.92)	1.00	–	1.00	–	1/117.30	0.85 (0.02 – 4.75)	1.00	–	1.00	–
Many Partners	19/211.75	8.97 (5.40 – 14.01)	2.61 (1.04 – 6.52)	**0.041** ***(χ^2=4.18)***	2.47 (0.98 – 6.26)	0.056 *(χ^2=3.65)*	17/190.41	8.93 (5.20 – 14.29)	10.32 (1.37 –77.55)	**0.023** ***(χ^2=5.15)***	8.55 (1.11 – 6.02)	**0.040** ***(χ^2=4.23)***
Average no. of sex acts per month (per 1 act increase)			1.00 (0.94 – 1.06)	0.954 *(χ^2<0.01)*					0.99 (0.93 – 1.06)	0.801 *(χ^2=0.06)*		
Ever had anal sex												
No	14/253.73	5.52 (3.02 – 9.26)	1.00	–	1.00	–	9/192.44	4.68 (2.14 – 8.88)	1.00	–	1.00	–
Yes	11/133.33	8.25 (4.12 – 14.76)	1.49 (0.68 – 3.29)	0.321 *(χ^2=0.99)*	1.65 (0.73 – 3.74)	0.230 *(χ^2=1.44)*	9/115.11	7.82 (3.57 – 14.84)	1.66 (0.66 – 4.19)	0.281 *(χ^2=1.16)*	1.68 (0.63 – 4.47)	0.301 *(χ^2=1.07)*
Ever had oral sex												
No	19/293.53	6.47 (3.90 – 10.11)	1.00	–			13/225.89	5.76 (3.06 – 9.84)	1.00	–		
Yes	6/94.75	6.33 (2.32 – 13.78)	0.97 (0.39 – 2.44)	0.955 *(χ^2<0.01)*			5/80.88	6.18 (2.01 – 14.43)	1.07 (0.38 – 2.99)	0.902 *(χ^2=0.02)*		
Condom use at last sex act												
Yes	16/225.00	7.11 (4.06 – 11.55)	1.00	–			11/173.32	7.11 (6.35 – 11.36)	1.00	–		
No	9/164.22	5.48 (2.51 – 10.40)	0.77 (0.34 – 1.75)	0.534 *(χ^2=0.39)*			7/134.39	5.21 (2.09 – 10.73)	0.82 (0.32 – 2.12)	0.684 *(χ^2=0.17)*		
Contraception type												
Condom only	9/161.76	5.56 (2.54 – 10.56)	1.00	–	1.00	–	7/127.41	5.49 (2.21 – 11.32)	1.00	–	1.00	–
Hormonal	8/118.78	6.75 (2.91 – 13.31)	1.21 (0.47 – 3.14)	0.692 *(χ^2=0.16)*	1.03 (0.38 – 2.76)	0.958 *(χ^2<0.01)*	5/91.16	5.48 (1.78 – 12.80)	1.00 (0.32 – 3.15)	0.999 *(χ^2<0.01)*	0.86 (0.27 – 2.75)	0.799

Table II: Socio–demographic, behavioural and biological risk factors for HIV acquisition in women, overall and by sex work, Durban, South Africa, 2004/2007

Variable	Overall						By Sex Work					
	#HIV Cases/ PY at risk	HIV Incidence (95% CI)	Univariate Hazard Ratio (95% CI)	p-value (χ^2)	Multivariable Hazard Ratio (95% CI)	p-value (χ^2)	#HIV Cases/ PY at risk	HIV Incidence (95% CI)	Univariate Hazard Ratio (95% CI)	p-value (χ^2)	Multivariable Hazard Ratio (95% CI)	p-value (χ^2)
												(χ^2=0.07)
None or other[a]	8/108.16	7.40 (3.19 – 14.57)	1.33 (0.51 – 3.45)	0.558 *(χ^2=0.34)*	1.51 (0.57 – 4.03)	0.411 *(χ^2=0.68)*	6/88.32	6.79 (2.49 – 14.79)	1.24 (0.42 – 3.69)	0.699 *(χ^2=0.15)*	1.52 (0.49 – 4.77)	0.469 *(χ^2=0.52)*
Douching after sex												
No	24/356.29	6.73 (4.32 – 10.02)	1.00	–			18/280.89	6.41 (3.80 – 10.13)	1.00	–		
Yes	1/32.11	3.11 (0.08 – 17.35)	0.46 (0.06 – 3.40)	0.448 *(χ^2=0.58)*			0/25.99	0.00	–	–		
Sex after alcohol or substance use												
No	20/285.24	7.01 (4.28 – 10.83)	1.00	–			13/215.56	6.03 (3.21 – 10.31)	1.00	–		
Yes	5/103.98	4.81 (1.56 – 11.22)	0.68 (0.26 – 1.82)	0.447 *(χ^2=0.58)*			5/92.15	5.43 (1.76 – 12.66)	0.90 (0.32 – 2.51)	0.834 *(χ^2=0.04)*		
Sex for compensation												
Sex worker												
No	7/81.51	8.59 (3.45 – 17.69)	1.00	–								
Yes	18/307.71	5.85 (3.47 – 9.25)	0.68 (0.28 – 1.63)	0.390 *(χ^2=0.74)*								
Time in sex work (per 1 year increase)			0.93 (0.85 – 1.02)	0.126 *(χ^2=2.34)*					0.93 (0.85 – 1.02)	0.126 *(χ^2=2.34)*	0.94 (0.85 – 1.05)	0.289 *(χ^2=1.12)*
Age at start of sex work (per 1 year increase)			0.98 (0.93 – 1.04)	0.564 *(χ^2=0.33)*					0.98 (0.93 – 1.04)	0.564 *(χ^2=0.33)*		
Days per week perform sex work (Per 1 day increase)			1.08 (0.79 – 1.48)	0.648 *(χ^2=0.21)*					1.08 (0.79 – 1.48)	0.648 *(χ^2=0.21)*		
Number of sites per year (per 1 site increase)			1.12 (0.80 – 1.57)	0.503 *(χ^2=0.45)*					1.12 (0.80 – 1.57)	0.503 *(χ^2=0.45)*		
Clients per day (per 1 client increase)			1.10 (0.76 – 1.59)	0.628 *(χ^2=0.23)*					1.10 (0.76 – 1.59)	0.628 *(χ^2=0.23)*		
Clients past week (per 1 client increase)			0.93 (0.75 – 1.16)	0.536 *(χ^2=0.38)*					0.93 (0.75 – 1.16)	0.536 *(χ^2=0.38)*		
Short sessions per week (per 1 session increase)			0.97 (0.83 – 1.12)	0.651 *(χ^2=0.20)*					0.97 (0.83 – 1.12)	0.651 *(χ^2=0.20)*		
Overnight stays per week (per 1 stay increase)			1.33 (0.94 – 1.88)	0.106 *(χ^2=2.61)*					1.33 (0.94 – 1.88)	0.106 *(χ^2=2.61)*	1.22 (0.84 – 1.77)	0.291 *(χ^2=1.11)*
Condom use with client in the last month												
Always	5/119.27	4.19 (1.36 – 9.78)	1.00	–			5/119.27	4.19 (1.36 – 9.78)	1.00	–		
Sometimes	9/132.17	6.81 (3.11 – 12.93)	1.61 (0.54 – 4.82)	0.391 *(χ^2=0.74)*			9/132.17	6.81 (3.11 – 12.93)	1.61 (0.54 – 4.82)	0.391 *(χ^2=0.74)*		
Never	4/56.27	7.11 (1.94 – 18.20)	1.68 (0.45 – 6.26)	0.440 *(χ^2=0.60)*			4/56.27	7.11 (1.94 – 18.20)	1.68 (0.45 – 6.26)	0.440 *(χ^2=0.60)*		
Clinical evaluation												
Ever pregnant prior to HIV												
No	22/342.24	6.43 (4.03 – 9.73)	1.00	–			22/342.24	5.89 (3.37 – 9.57)	1.00	–		

Table II: Socio–demographic, behavioural and biological risk factors for HIV acquisition in women, overall and by sex work, Durban, South Africa, 2004/2007

	Overall						By Sex Work				
Variable	**#HIV Cases/ PY at risk**	**HIV Incidence (95% CI)**	**Univariate Hazard Ratio (95% CI)**	**p-value (χ^2)**	**Multivariable Hazard Ratio (95% CI)**	**p-value (χ^2)**	**#HIV Cases/ PY at risk**	**HIV Incidence (95% CI)**	**Univariate Hazard Ratio (95% CI)**	**p-value (χ^2)**	**Multivariable Hazard Ratio (95% CI)**	**p-value (χ^2)**
Yes	3/46.99	6.38 (1.32 – 18.66)	0.99 (0.30 – 3.32)	0.990 *(χ^2<0.01)*			2/36.15	5.53 (0.67 – 19.98)	0.93 (0.22 – 4.06)	0.927 *(χ^2<0.01)*		
Laboratory evaluations												
Sexually transmitted infection[b] or bacterial vaginosis												
No	5/148.75	3.36 (1.09 – 7.84)	1.00	–	1.00		4/116.34	3.44 (0.94 – 8.80)	1.00	–	1.00	
Yes	20/238.49	8.39 (5.12 – 12.95)	2.47 (0.93 – 6.58)	0.071 *(χ^2=3.26)*	2.49 (0.91 – 6.82)	0.077 *(χ^2=3.13)*	14/189.38	7.39 (4.04 – 12.40)	2.13 (0.70 – 6.46)	0.184 *(χ^2=1.77)*	1.89 (0.59 – 6.08)	0.287 *(χ^2=1.14)*

[a] Female sterilisation or rhythm/calendar method

[b] Any STI present if participant tested positive for syphilis antibodies or *Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium, Chlamydia trachomatis* or herpes simplex virus (HSV) type II (PCR)

Discussion

In this study young age and having multiple sex partners were associated with risk of HIV acquisition. Despite the introduction of HIV prevention and treatment programmes, the HIV incidence rate of 7.2 per 100 wy remains unacceptably high. South Africa and many southern African countries continue to experience similar high HIV incidence rates (7, 10, 29, 31, 32). Longitudinal studies among women in South Africa have shown HIV incidence rates of 6.6, 8.5 and 6.5 per 100 wy particularly amongst young women in the province of KwaZulu–Natal (7, 10, 29). Among pregnant women from the same region, HIV incidence was 10.7 per 100 wy (30). In Malawi and Zimbabwe, among women recruited from postnatal or family planning clinics, the HIV incidence rates were 4.20, 4.86 and 4.78 per 100 wy in Lilongwe, Blantyre and Harare respectively (31). Overall, the highest incidence rate of 5.78 per 100 wy was among women younger than 25 years, whilst in Rwanda, among antenatal clinic attendees HIV incidence was 10.5 per 100 wy among women younger than 20 years (31, 32). These studies demonstrate young women's vulnerability and greater risk of acquiring HIV compared to older women. Our study shows that young age carries a three–fold greater risk of HIV acquisition.

The importance of measuring HIV incidence is key to understanding the dynamics of HIV disease to shape and modify effective responses. The persistence of high HIV incidence rates and the vulnerability of young women is incompletely elucidated in this setting. Recent studies have shown that residing in urban informal settlements, being unmarried or unemployed was associated with higher HIV incidence highlighting the underlying structural and social factors driving the epidemic (5, 10). Although common in this setting, high risk behaviours such as engaging in age-disparate relationships, was not found to predict HIV risk in a population-based study and may be less likely to contribute to high HIV incidence in young women than previously thought (33). However, in this study we did not explore the

age of sexual partner as risk for HIV acquisition as risk through multiple partners with possible different ages is likely to be less apparent. Sexually transmitted infections are known to contribute to HIV risk in women in this region and ongoing research into the immunology of the female genital tract may provide further insight into the biological susceptibility to HIV infection particularly in young women (10, 20).

Although the vulnerability of young women is well recognised in this setting, risk for HIV is often underestimated since the perceived risk of HIV remains low (5). South Africa's epidemic is generalised with HIV prevalence at an unprecedented high level, in excess of 15% in the adult population, and new infection rates around 2% per year (5, 34). However, the majority of HIV–infected individuals are unaware of their HIV status, and this remains a barrier for both treatment access and prevention and helps sustain the epidemic. Poor knowledge of HIV transmission and limited access to and availability of health care services further promotes risk (5).

Our study demonstrates that women in relationships with multiple partners were more likely to acquire HIV. While multiple concurrent partnerships is recognised as a key driver of the epidemic in the Southern African region, the total number of lifetime partners has also been found to be a significant predictor of HIV in this region (12, 35). A meta–analysis of 68 studies from sub–Saharan Africa assessing risk factors for HIV acquisition reported that the number of lifetime partners increased the risk of HIV by almost four–fold [Odds Ratio (OR) 3.64, 95% CI 2.87–4.62] (35). In an urban mining community in Carletonville risk for HIV was almost five–fold higher in women reporting two or more lifetime partners (OR 4.88, 95% CI 3.01–7.89) (36). This risk was almost double for women reporting more than one partner three months prior to enrolment into HIV prevention intervention studies (adjusted OR 1.78, 95% CI 1.11–2.85) and similar for young women 15 to 24 years (OR 2.0, 95% CI 1.1–3.7)

participating in the national household survey of South African youth, reporting more than one lifetime partner (4, 37).

In this setting unemployment levels remain high and resources are constrained (9, 38). Marriage is also uncommon in this setting, leading to instability of relationships (9). Transactional sex, which may range from serial monogamous relationships to occasional exchange of sex for money or goods, provides a means of survival for women and their dependents, resulting in women engaging in multiple partnerships, either sequential or concurrent, in order to meet their basic needs (6, 9). Hence, emphasis and efforts on monogamy and multiple partner reduction cannot stand alone. The integration of structural approaches into current HIV policy, which address the socio-economic needs of women and which create an environment of equal opportunities and rights for women, is crucial if behavioural and biomedical prevention efforts are to succeed in the long-term.

For female sex workers, increasing access to sexual and reproductive health services and sensitisation of service providers to sex worker vulnerability and health care need should be promoted. Increasing availability of condoms, reducing substance and alcohol use and access to multicomponent HIV prevention packages including ART as pre-exposure prophylaxis as a women initiated method of HIV prevention is key in this population if HIV incidence is to be reduced. Rigorously including HIV counselling and testing (HCT) across all levels is important and key to knowledge of HIV status. HCT is especially important to help people learn their own and their partners' status so as to protect individuals whose partners are HIV–positive. Furthermore, repeat HCT must be promoted in hyperendemic regions.

The use of hormonal contraception was not found to be associated with HIV acquisition. Results from similar studies exploring the use of hormonal contraception increasing the risk of HIV acquisition remain inconsistent (21, 22, 27, 39). Major limitations of these studies have been the failure to test the hypotheses through robust study designs. Earlier studies showed some signals of an association between hormonal contraception and HIV acquisition (27). Similarly intra–vaginal insertion practices and pregnancy were not associated with HIV risk, though the outcome may be limited by the small sample size.

Whilst elevated serum vitamin B12 levels was associated with HIV acquisition, its causal role requires further exploration. Recent evidence from critically ill elderly medical patients suggests that elevated serum vitamin B12 levels are associated with increased mortality (40, 41). Although increased levels have been associated with systemic inflammatory markers, its role in mediating HIV risk remains unclear (40). While genital tract inflammation associated with HIV risk in this cohort has been reported, the role of vitamin B12 as a marker of systemic inflammation and HIV risk requires further evaluation (20).

The major strength of the study was the prospective cohort study design which allowed assessment of risk factors prior to HIV acquisition; however, there are several limitations as well. Firstly, participants had monthly study visits with risk reduction counselling, male and female condom provision, HIV testing with interviewer administered questionnaires; it is therefore possible that participants could have provided socially desirable responses given the sensitive nature of the sexual risk behaviour questions which could have biased the associations. Furthermore, given the wide age range of this cohort (18 to 58 years), it was difficult to adequately assess some risk behaviours which may be age dependent. Ideally, we would have liked to follow up behaviour in those women who remained HIV negative to assess HIV risk over time. Secondly, the small sample size of the study is an

important limitation, impacting on the precision of the study findings, therefore the particularly large confidences intervals around some of the point estimates. This may have also limited our ability to demonstrate stronger associations with some variables such as higher educational level and HIV acquisition and reduced HIV risk with every additional year spent in sex work. Thirdly, the recruitment of high risk women limits the generalizability of the study findings as these may not be representative of women from elsewhere in the province. Finally, as the study excluded women younger than 18 years, it was not possible to measure HIV incidence and risk factors in this young age group in whom HIV incidence is high (8). Whilst there are ethical challenges in conducting research in adolescents, it is important that young people, especially girls are included into research studies in order to better understand the high rates of HIV acquisition (8, 42).

In conclusion, the findings of this study confirm that young women continue to bear the brunt of the HIV epidemic in this region. It is important that as transmission dynamics change over time, especially in hyperendemic settings, investments towards large–scale, fundamental changes in behaviour, social practices and community norms, address young women's vulnerability to HIV.

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References

1. UNAIDS. UNAIDS Report on the Global HIV Epidemic 2013. Available from: http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed April 22, 2014.

2. Abdool Karim SS, Churchyard GJ, Abdool Karim Q, Lawn SD. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. Lancet. 2009;374(9693):921-33.

3. National Department of Health. The 2012 National Antenatal Sentinel HIV and Herpes Simplex type-2 prevalence Survey, South Africa. Available from: http://www.health-e.org.za/wp-content/uploads/2014/05/ASHIVHerp_Report2014_22May2014.pdf. Accessed July 14, 2014.

4. Pettifor AE, Rees HV, Kleinschmidt I, Steffenson AE, MacPhail C, Hlongwa-Madikizela L, et al. Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. AIDS. 2005;19(14):1525-34.

5. Shisana O, Rehle, T, Simbayi LC, Zuma, K, Jooste, S, Zungu N, Labadarios, D, Onoya, D. et al. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town: HSRC Press; 2014.

6. Kelly K, Mkhwanazi N, Nkhwashu N, Rapiti R, Mashale R. (2012) HIV prevention situation analysis in KwaZulu-Natal, Mpumalanga and Gauteng provinces, South Africa. USAID Sexual HIV Prevention Programme in South Africa (SHIPP). Available from: http://futuresgroup.com/files/publications/Synthesis_of_Research_on_Prevention_of_Sexual_Transmission_of_HIV_in_SA.pdf. Accessed March 20, 2013.

7. Abdool Karim Q, Kharsany ABM, Frohlich JA, Werner L, Mashego M, Mlotshwa M, et al. Stabilizing HIV prevalence masks high HIV incidence rates amongst rural and urban women in KwaZulu-Natal, South Africa. Int J Epidemiol. 2011;40(4):922-30.

8. Abdool Karim Q, Kharsany AB, Frohlich JA, Werner L, Mlotshwa M, Madlala BT, et al. HIV incidence in young girls in KwaZulu-Natal, South Africa--public health imperative for their inclusion in HIV biomedical intervention trials. AIDS Behav. 2012;16(7):1870-6.

9. Abdool Karim Q, Sibeko S, Baxter C. Preventing HIV infection in women: a global health imperative. Clin Infect Dis. 2010;50 Suppl 3:S122-9.

10. Wand H, Ramjee G. Combined impact of sexual risk behaviors for HIV seroconversion among women in Durban, South Africa: implications for prevention policy and planning. AIDS Behav. 2011;15(2):479-86.

11. Pettifor AE, Levandowski BA, MacPhail C, Padian NS, Cohen MS, Rees HV. Keep them in school: the importance of education as a protective factor against HIV infection among young South African women. Int J Epidemiol. 2008;37(6):1266-73.

12. Halperin DT. Why is HIV prevalence so severe in Southern Africa? The Southern African Journal of HIV Medicine. 2007;8(1):19-25.

13. Leclerc-Madlala S. Age-disparate and intergenerational sex in southern Africa: the dynamics of hypervulnerability. AIDS. 2008;22 Suppl 4:S17-25.

14. Chimbindi NZ, McGrath N, Herbst K, San Tint K, Newell ML. Socio-Demographic Determinants of Condom Use Among Sexually Active Young Adults in Rural KwaZulu-Natal, South Africa. Open AIDS J. 2010;4:88-95.

15. van Loggerenberg F, Dieter AA, Sobieszczyk ME, Werner L, Grobler A, Mlisana K. HIV prevention in high-risk women in South Africa: condom use and the need for change. PloS one. 2012;7(2):e30669.

16. Salvana EMT. HIV and STIs: Interactions in resource-limited settings. Available from: http://www.medscape.com/viewarticle/754637. Accessed March 12, 2014.

17. McClelland RS, Lavreys L, Hassan WM, Mandaliya K, Ndinya-Achola JO, Baeten JM. Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study. AIDS. 2006;20(2):269-73.

18. Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, et al. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. Lancet. 2005;366(9492):1182-8.

19. Myer L, Wright TC, Jr., Denny L, Kuhn L. Nested case-control study of cervical mucosal lesions, ectopy, and incident HIV infection among women in Cape Town, South Africa. Sex Transm Dis. 2006;33(11):683-7.

20. Mlisana K, Naicker N, Werner L, Roberts L, van Loggerenberg F, Baxter C, et al. Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. J Infect Dis. 2012;206(1):6-14.

21. Polis CB, Curtis KM. Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence. Lancet Infect Dis. 2013;13(9):797-808.

22. WHO. Hormonal contraception and HIV Technical statement. Available from: http://whqlibdoc.who.int/hq/2012/WHO_RHR_12.08_eng.pdf. Accessed July 18, 2014.

23. van Loggerenberg F, Mlisana K, Williamson C, Auld SC, Morris L, Gray CM, et al. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. PLoS One. 2008;3(4):e1954.

24. Abdool Karim SS, Mlisana K, Kharsany A, Williamson C, Baxter C, Karim Q. Utilizing nucleic acid amplification to identify acute HIV infection. AIDS. 2007;21(5):653-5.

25. Kingsley LA, Detels R, Kaslow R, Polk BF, Rinaldo CR, Jr., Chmiel J, et al. Risk factors for seroconversion to human immunodeficiency virus among male homosexuals. Results from the Multicenter AIDS Cohort Study. Lancet. 1987;1(8529):345-9.

26. Abdool Karim SS, Ramjee G. Anal sex and HIV transmission in women. Am J Public Health. 1998;88(8):1265 - 6.

27. Heffron R, Donnell D, Rees H, Celum C, Mugo N, Were E, et al. Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. Lancet Infect Dis. 2012;12(1):19-26.

28. Hel Z, Stringer E, Mestecky J. Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. Endocr Rev. 2010;31(1):79-97.

29. Ramjee G, Kapiga S, Weiss S, Peterson L, Leburg C, Kelly C, et al. The value of site preparedness studies for future implementation of phase 2/IIb/III HIV prevention trials: experience from the HPTN 055 study. J Acquir Immune Defic Syndr. 2008;47(1):93-100.

30. Moodley D, Esterhuizen TM, Pather T, Chetty V, Ngaleka L. High HIV incidence during pregnancy: compelling reason for repeat HIV testing. AIDS. 2009;23(10):1255-9.

31. Kumwenda N, Hoffman I, Chirenje M, Kelly C, Coletti A, Ristow A, et al. HIV incidence among women of reproductive age in Malawi and Zimbabwe. Sex Transm Dis. 2006;33(11):646-51.

32. Bulterys M, Chao A, Habimana P, Dushimimana A, Nawrocki P, Saah A. Incident HIV-1 infection in a cohort of young women in Butare, Rwanda. AIDS. 1994;8(11):1585-91.

33. Harling G, Newell ML, Tanser F, Kawachi I, Subramanian SV, Barnighausen T. Do Age-Disparate Relationships Drive HIV Incidence in Young Women? Evidence from

a Population Cohort in Rural KwaZulu-Natal, South Africa. J Acquir Immune Defic Syndr. 2014;66(4):443-51.

34. Statistics South Africa. Mid-year population estimates 2014. Available from: http://beta2.statssa.gov.za/publications/P0302/P03022014.pdf. Accessed August 04, 2014.

35. Chen L, Jha P, Stirling B, Sgaier SK, Daid T, Kaul R, et al. Sexual risk factors for HIV infection in early and advanced HIV epidemics in sub-Saharan Africa: systematic overview of 68 epidemiological studies. PLoS One. 2007;2(10):e1001.

36. Zuma K, Gouws E, Williams B, Lurie M. Risk factors for HIV infection among women in Carletonville, South Africa: migration, demography and sexually transmitted diseases. Int J STD AIDS. 2003;14(12):814-7.

37. Mavedzenge SN, Weiss HA, Montgomery ET, Blanchard K, de Bruyn G, Ramjee G, et al. Determinants of differential HIV incidence among women in three southern African locations. J Acquir Immune Defic Syndr. 2011;58(1):89-99.

38. Statistics South Africa. Available from: http://beta2.statssa.gov.za/. Accessed September 07, 2014.

39. Morrison CS, Skoler-Karpoff S, Kwok C, Chen PL, van de Wijgert J, Gehret-Plagianos M, et al. Hormonal contraception and the risk of HIV acquisition among women in South Africa. AIDS. 2012;26(4):497-504.

40. Sviri S, Khalaila R, Daher S, Bayya A, Linton DM, Stav I, et al. Increased Vitamin B12 levels are associated with mortality in critically ill medical patients. Clin Nutr. 2012;31(1):53-9.

41. Salles N, Herrmann F, Sakbani K, Rapin CH, Sieber C. High vitamin B12 level: a strong predictor of mortality in elderly inpatients. J Am Geriatr Soc. 2005;53(5):917-8.

42. Nelson RM, Lewis LL, Struble K, Wood SF. Ethical and regulatory considerations for the inclusion of adolescents in HIV biomedical prevention research. J Acquir Immune Defic Syndr. 2010;54 Suppl 1:S18-24.

Chapter 4: Discussion

In this study we have shown that young age and having many partners placed women at increased risk for HIV infection. However, we were unable to demonstrate any association between other biological or behavioural factors such as hormonal contraception use, vaginal insertion practices and substance use with HIV. Among sex workers, no significant risk factors were identified related to sex work. These findings are however, subject to several limitations with regard to the internal validity and external validity of this retrospective analysis.

Overall this study demonstrated an HIV incidence of 7.2 per 100 wy, similar to incidence rates of 6.6, 8.5 and 6.5 per 100 wy found in other studies in KwaZulu-Natal and elsewhere in Africa (6, 33, 35, 45, 130). The HIV incidence of 13.2 per 100 wy among women aged 18 to 24 years was almost three-fold higher, as was risk for HIV acquisition, compared to women aged 25 years and older. These findings are consistent with earlier studies undertaken locally and in other African countries in which younger women were found to be most vulnerable to HIV infection (6, 33, 35, 45). The HSRC survey in South Africa found that young women aged between 15 and 24 years contributed almost a quarter of all new infections in 2012 (18). Antenatal prevalence surveys show similar results with HIV prevalence in the 15 to 24 year age group being 19.3% in the same reporting period (5). The high incidence of HIV particularly among adolescent girls is of particular concern, as current research, prevention and biomedical strategies are not inclusive of younger age groups, more importantly those less than 18 years of age, but remain at high risk for HIV acquisition (13). Highlighting the burden of disease in this age group is imperative to shift focus of prevention efforts to younger women and to incorporate these into improving adolescent health

including HIV prevention programmes through school-based health services (131). A limitation to this analysis is that women younger than 18 years were not included in this study because of the ethical and regulatory challenges for the inclusion of adolescents in research studies, despite the increased risk of HIV in this group (132). It is now increasingly recognised that adolescents are able to and should be included in HIV prevention and treatment research activities if they are to benefit from such research (132, 133).

This study also shows that women in relationships with multiple partners were at a higher risk for HIV acquisition. Although the percentage of women having multiple partners is reported to be around 8% for 15 to 24 year-old women and around 4% for women ≥25, having multiple concurrent partnerships or a high number of lifetime sexual partners increases women's vulnerability to HIV in this setting (18, 31, 103, 109). Earlier studies and the HSRC survey found that women in non-stable partnerships had a considerably higher HIV incidence when compared to married women (18, 35, 37, 39). While reducing the number of sexual partners and promotion of stable partnerships, and possibly even marriage, should be included in HIV prevention messaging for women in the general population, this may not be a practical option for women such as female sex workers. Instead, increasing access to sexual and reproductive health services and sensitisation of service providers to sex worker vulnerability and health care need should be promoted. Knowledge of HIV status through frequent HIV testing, increasing availability of condoms, reducing substance and alcohol use and access to multicomponent HIV prevention packages including ART as pre-exposure prophylaxis as a women initiated method of HIV prevention is key in this population if HIV incidence is to be reduced.

As this particular cohort enrolled high risk women, defined by being a female sex workers or having at least three sex partners in the three months prior to study entry; the association of

many partners and increased risk for HIV must be interpreted with caution as the inclusion criteria may have biased the association. Nevertheless, this association was strengthened when the analysis was restricted to sex workers only. This association did not remain significant in the overall multivariable analysis suggesting that this association may be explained partly by other demographic, behavioural or biological factors.

A major strength of the study lies in the prospective cohort design which allowed us to assess risk factors prior to HIV acquisition in high risk women, wherein access to such groups is limited in this setting. Furthermore we were able to demonstrate that even among high risk women, young age is a risk factor for HIV with high HIV incidence rates. It is also important to note that almost 60% of women were ineligible for study participation due to being HIV seropositive at screening and only a third of those screened were eligible to enrol. A subgroup analysis of those excluded further showed the high burden of prevalent infections in young women although risk behaviour of women who screened out may have differed substantially from women enrolled into the study. Nevertheless the prevalence in women screened out and the high HIV incidence rates demonstrate the impact of HIV, more importantly in young women.

An important consideration in this secondary analysis is the age of the dataset. This study enrolled women between August 2004 and May 2005, at a time when HIV prevention and treatment services were limited and at a time when HIV stigma and discrimination were likely to be more common. Women participating in this study may have actively sought HIV prevention services and we may have therefore selected a cohort of women with health seeking behaviour or who engage in risky behaviour more frequently. Furthermore, since the introduction of ART only began around 2004, the benefits of expanded ART coverage on HIV incidence and reduced transmission risk may not be apparent in this cohort. However,

more recent studies undertaken in KwaZulu-Natal indicate no significant declines in HIV incidence in women following scale-up of ART services (6, 13).

As risk behaviour questionnaires were interviewer-administered and women were provided with risk reduction counselling, condoms and HIV testing at each study visit, the reported responses may have resulted in social desirability bias and may have influenced the responses to questions on risk behaviour. Women may have under-reported sexual activity and high risk sexual behaviours, particularly those women not self-identifying as female sex workers. Furthermore, observer or interviewer bias was also introduced as interviewers were aware of the participants' sex worker status and this may have influenced the capture of responses as well. These biases could have been minimised had the questionnaires been self-administered and anonymous. As risk behaviour questionnaires were administered at baseline only, we were unable to assess changes in risk behaviour over time. Importantly it would have been useful to assess the temporal association of risk behaviours at follow up visits for women who remained HIV negative.

The small sample size of the study is an important limitation, impacting on the precision of the study findings, therefore the particularly large confidences intervals around the point estimates. While there were weak associations identified such as increased risk for HIV with a higher level of education, these findings were not found to be statistically significant. Furthermore, the small sample size may not have allowed us to adequately assess other variables such as delayed sexual debut and HIV risk.

There are several possible confounding variables which may have impacted the analysis of data. Sexually transmitted infections have been evaluated in a separate analysis and was found to increase risk for HIV overall by three-fold (73). This variable, because of its biological plausibility as a risk factor for HIV, was therefore included in the multivariable

analysis. Host genetic and immunological factors that were not included in the multivariable analysis, some of which include TRIM5αhu, Interleukin-10 and Duffy-null-associated low neutrophil count trait, were previously found to reduce risk for HIV in this cohort (84-86). Viral factors were also not accounted for in this analysis. Furthermore, from laboratory evaluations, some variables were found to be significantly different between women who acquired HIV and those who did not e.g. vitamin B12 and serum sodium were raised among women who acquired HIV while random glucose levels were lower in the same group. These variables were not included in the multivariable analysis as the clinical significance and biological plausibility of these findings are uncertain. However, better designed studies are needed to further explore these variables and HIV risk.

As large proportion of women in this cohort were self-reported female sex workers and were recruited from a very specific geographical location in KwaZulu-Natal province, the external validity or generalisability of this cohort is limited to similar high risk women from the same region. Furthermore the study enrolled women with HIV-1 subtype C infection which makes the findings less generalisable to regions with other HIV subtypes. Results from this study may not be extrapolated to other areas in KwaZulu-Natal province or elsewhere in South Africa as this is a very well defined cohort in an urban setting. Nevertheless, this high risk cohort provides useful information which may be compared with other high risk cohorts in South Africa and in other regions in Africa.

Notwithstanding these limitations, the study remains relevant as the province of KwaZulu-Natal remains a region of highly burdened by HIV and knowledge of risk behaviour among women is critical to informing HIV policy.

Chapter 5: Conclusion and Implications for Future Research and Policy

This research highlights the following key findings and recommendations for policy and future research undertaken:

- Young women in the province of KwaZulu-Natal bear a high burden of HIV infection, even amongst high risk groups.
- This study confirms that having multiple partners contributes to enhancing HIV risk in this setting.
- As HIV prevention technologies for women remain limited in this setting, enhancing currently available multicomponent HIV prevention packages including HIV testing with counselling, increasing male and female condom use and reducing substance and alcohol use are important tools for preventing HIV acquisition. Access to ART as pre-exposure prophylaxis when licenced and available for public sector use should be promoted as a women initiated method to prevent HIV acquisition.
- ART programmes through early initiation of treatment at higher $CD4^+$ cell counts and expanded ART coverage through existing services provide substantial benefit by reducing HIV related morbidity at the individual level and more importantly impact on HIV incidence at a population level over the long-term.
- As newer HIV prevention tools are developed, such as long acting injectable ART for pre-exposure prophylaxis, which are still in the early phases of research, these may become more practical prevention options for women in the future.

- For women engaging in sex work emphasis and efforts on safer sex practices, easy access to sexual and reproductive health services and in the long-term, social and economic upliftment of women, remain key priorities.
- For future studies assessing risk factors for HIV acquisition in women, we recommend that younger adolescent women be included as they are becoming increasingly more vulnerable to HIV infection and knowledge of HIV risk in this group is critical to informing targeted HIV prevention programmes. Furthermore assessing risk over time is imperative as risk behaviour evolves over time.
- The role of vitamin B12 as a marker of systemic inflammation requires further exploration in this cohort. Ongoing research to better understand immunological and biological responses in the female genital tract will be key to provide greater insight into young women's susceptibility to HIV

Chapter 6: References

1. Avert. History of AIDS up to 1986. Available from: http://www.avert.org/aids-history-86.htm. Accessed March 19, 2013.

2. WHO. Global Health Observatory: HIV/AIDS. Available from: http://www.who.int/gho/hiv/en/. Accessed August 29, 2014.

3. Fauci AS, Morens DM. The perpetual challenge of infectious diseases. N Engl J Med. 2012;366(5):454-61.

4. Abdool Karim Q, Kharsany ABM. Epidemiology of HIV/AIDS. In: Hall JC, Hall BJ & Cockerell CJ, eds. HIV/AIDS in the Post-HAART Era: Manifestations,Treatment, and Epidemiology. People's Medical Publishing House -USA; 2011.

5. National Department of Health. The 2012 National Antenatal Sentinel HIV and Herpes Simplex type-2 prevalence Survey, South Africa. Available from: http://www.health-e.org.za/wp-content/uploads/2014/05/ASHIVHerp_Report2014_22May2014.pdf. Accessed July 14, 2014.

6. Abdool Karim Q, Kharsany ABM, Frohlich JA, Werner L, Mashego M, Mlotshwa M, et al. Stabilizing HIV prevalence masks high HIV incidence rates amongst rural and urban women in KwaZulu-Natal, South Africa. Int J Epidemiol. 2011;40(4):922-30.

7. UNAIDS. UNAIDS Report on the Global HIV Epidemic 2013. Available from: http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/ep

idemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed April 22, 2014.

8. WHO, UNAIDS and UNICEF. Global HIV/AIDS Response. Epidemic update and health sector progress towards Universal Access. Progress Report 2011. Available from: http://www.who.int/hiv/pub/progress_report2011/hiv_full_report_2011.pdf. Accessed August 12, 2013.

9. Kharsany ABM, Abdool Karim Q. HIV Epidemiology and Prevention. In: Barros E, ed. Tech-HIV-infection - Impact, Awareness and Social Implications of living with HIV/AIDS. InTech; 2011.

10. Abdool Karim Q, Humphries H. Reducing HIV Infection in Young Women in Southern Africa: The Key to Altering Epidemic Trajectories in a Generalised Hyperendemic Setting. Available from: http://www.aidstar-one.com/sites/default/files/additional_resources/AIDSTAR-One_Pev_Spotlight_Issue_3.pdf. Accessed March 12, 2013.

11. Mastro TD, Kim AA, Hallett T, Rehle T, Welte A, Laeyendecker O, et al. Estimating HIV Incidence in Populations Using Tests for Recent Infection: Issues, Challenges and the Way Forward. Journal of HIV AIDS surveillance & epidemiology. 2010;2(1):1-14.

12. Simelela NP, Venter WD. A brief history of South Africa's response to AIDS. S Afr Med J. 2014;104(3 Suppl 1):249-51.

13. Abdool Karim Q, Kharsany ABM, Frohlich JA, Werner L, Mlotshwa M, Madlala BT, et al. HIV incidence in young girls in KwaZulu-Natal, South Africa--public health

imperative for their inclusion in HIV biomedical intervention trials. AIDS Behav. 2012;16(7):1870-6.

14. Nel A, Mabude Z, Smit J, Kotze P, Arbuckle D, Wu J, et al. HIV incidence remains high in KwaZulu-Natal, South Africa: evidence from three districts. PLoS One. 2012;7(4):e35278.

15. Kelly K, Mkhwanazi N, Nkhwashu N, Rapiti R, Mashale R. (2012) HIV prevention situation analysis in KwaZulu-Natal, Mpumalanga and Gauteng provinces, South Africa. USAID Sexual HIV Prevention Programme in South Africa (SHIPP). Available from: http://futuresgroup.com/files/publications/Synthesis_of_Research_on_Prevention_of_Sexual_Transmission_of_HIV_in_SA.pdf. Accessed March 20, 2013.

16. Ghys PD, Kufa E, George MV. Measuring trends in prevalence and incidence of HIV infection in countries with generalised epidemics. Sex Transm Infect. 2006;82 Suppl 1:i52-6.

17. Rehle TM, Hallett TB, Shisana O, Pillay-van Wyk V, Zuma K, Carrara H, et al. A decline in new HIV infections in South Africa: estimating HIV incidence from three national HIV surveys in 2002, 2005 and 2008. PLoS One. 2010;5(6):e11094.

18. Shisana O, Rehle, T, Simbayi LC, Zuma, K, Jooste, S, Zungu N, Labadarios, D, Onoya, D. et al. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town: HSRC Press; 2014.

19. Gupta GR, Parkhurst JO, Ogden JA, Aggleton P, Mahal A. Structural approaches to HIV prevention. Lancet. 2008;372(9640):764-75.

20. Poundstone KE, Strathdee SA, Celentano DD. The social epidemiology of human immunodeficiency virus/acquired immunodeficiency syndrome. Epidemiol Rev. 2004;26:22-35.

21. Abdool Karim Q, Abdool Karim SS. The evolving HIV epidemic in South Africa. Int J Epidemiol. 2002;31(1):37-40.

22. Centers for Disease Control and Prevention. HIV among African Americans. Available from: http://www.cdc.gov/hiv/risk/racialethnic/aa/facts/. Accessed August 02, 2014.

23. Nattrass N. Poverty, sex and HIV. AIDS Behav. 2009;13(5):833-40.

24. WHO. HIV/AIDS. Available from: http://www.who.int/immunization/topics/hiv/en/index1.html. Accessed March 02, 2014.

25. Centers for Disease Control and Prevention. HIV among women. Available from: http://www.cdc.gov/HIV/risk/gender/women/facts/index.html. Accessed August 02, 2014.

26. Wojcicki JM. Socioeconomic status as a risk factor for HIV infection in women in East, Central and Southern Africa: a systematic review. J Biosoc Sci. 2005;37(1):1-36.

27. Pettifor AE, Levandowski BA, MacPhail C, Padian NS, Cohen MS, Rees HV. Keep them in school: the importance of education as a protective factor against HIV infection among young South African women. Int J Epidemiol. 2008;37(6):1266-73.

28. Statistics South Africa. Available from: http://beta2.statssa.gov.za/. Accessed September 07, 2014.

29. Lurie MN, Williams BG, Zuma K, Mkaya-Mwamburi D, Garnett G, Sturm AW, et al. The impact of migration on HIV-1 transmission in South Africa: a study of migrant and nonmigrant men and their partners. Sex Transm Dis. 2003;30(2):149-56.

30. Camlin CS, Hosegood V, Newell ML, McGrath N, Barnighausen T, Snow RC. Gender, migration and HIV in rural KwaZulu-Natal, South Africa. PLoS One. 2010;5(7):e11539.

31. Zuma K, Gouws E, Williams B, Lurie M. Risk factors for HIV infection among women in Carletonville, South Africa: migration, demography and sexually transmitted diseases. Int J STD AIDS. 2003;14(12):814-7.

32. Barnighausen T, Hosegood V, Timaeus IM, Newell ML. The socioeconomic determinants of HIV incidence: evidence from a longitudinal, population-based study in rural South Africa. AIDS. 2007;21 Suppl 7:S29-38.

33. Wand H, Ramjee G. Combined impact of sexual risk behaviors for HIV seroconversion among women in Durban, South Africa: implications for prevention policy and planning. AIDS Behav. 2011;15(2):479-86.

34. Kongnyuy EJ, Soskolne V, Adler B. Hormonal contraception, sexual behaviour and HIV prevalence among women in Cameroon. BMC Womens Health. 2008;8:19.

35. Kumwenda N, Hoffman I, Chirenje M, Kelly C, Coletti A, Ristow A, et al. HIV incidence among women of reproductive age in Malawi and Zimbabwe. Sex Transm Dis. 2006;33(11):646-51.

36. Geis S, Maboko L, Saathoff E, Hoffmann O, Geldmacher C, Mmbando D, et al. Risk factors for HIV-1 infection in a longitudinal, prospective cohort of adults from the Mbeya Region, Tanzania. J Acquir Immune Defic Syndr. 2011;56(5):453-9.

37. Hattingh Z. Socio-demographic risk factors for HIV infection in women living in Mangaung, Free state. S Afr J Clin Nutr. 2009;22(4):203-7.

38. Naidoo P, Chirinda W, McHunu G, Swartz S, Anderson J. Social and structural factors associated with vulnerability to HIV infection among young adults in South Africa. Psychology, health & medicine. 2014,Jul 15:1-11.

39. Barnighausen T, Tanser F, Gqwede Z, Mbizana C, Herbst K, Newell ML. High HIV incidence in a community with high HIV prevalence in rural South Africa: findings from a prospective population-based study. AIDS. 2008;22(1):139-44.

40. Santelli JS, Edelstein ZR, Mathur S, Wei Y, Zhang W, Orr MG, et al. Behavioral, biological, and demographic risk and protective factors for new HIV infections among youth in Rakai, Uganda. J Acquir Immune Defic Syndr. 2013;63(3):393-400.

41. Santelli JS, Edelstein ZR, Wei Y, Mathur S, Song X, Schuyler A, et al. Trends in HIV acquisition, risk factors and prevention policies among youth in Uganda, 1999-2011. AIDS. 2015;29(2):211-9.

42. Clark S, Bruce J, Dude A. Protecting young women from HIV/AIDS: the case against child and adolescent marriage. International family planning perspectives. 2006;32(2):79-88.

43. Nyamhanga TM, Frumence G. Gender context of sexual violence and HIV sexual risk behaviors among married women in Iringa Region, Tanzania. Global health action. 2014;7:25346.

44. Ruzagira E, Wandiembe S, Abaasa A, Bwanika AN, Bahemuka U, Amornkul P, et al. HIV incidence and risk factors for acquisition in HIV discordant couples in Masaka, Uganda: an HIV vaccine preparedness study. PLoS One. 2011;6(8):e24037.

45. Bulterys M, Chao A, Habimana P, Dushimimana A, Nawrocki P, Saah A. Incident HIV-1 infection in a cohort of young women in Butare, Rwanda. AIDS. 1994;8(11):1585-91.

46. Abdool Karim Q, Sibeko S, Baxter C. Preventing HIV infection in women: a global health imperative. Clin Infect Dis. 2010;50 Suppl 3:S122-9.

47. Nicolosi A, Correa Leite ML, Musicco M, Arici C, Gavazzeni G, Lazzarin A. The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. Epidemiology. 1994;5(6):570-5.

48. Padian NS, Shiboski SC, Jewell NP. Female-to-male transmission of human immunodeficiency virus. Jama. 1991;266(12):1664-7.

49. Kingsley LA, Detels R, Kaslow R, Polk BF, Rinaldo CR, Jr., Chmiel J, et al. Risk factors for seroconversion to human immunodeficiency virus among male homosexuals. Results from the Multicenter AIDS Cohort Study. Lancet. 1987;1(8529):345-9.

50. Winkelstein W, Jr., Lyman DM, Padian N, Grant R, Samuel M, Wiley JA, et al. Sexual practices and risk of infection by the human immunodeficiency virus. The San Francisco Men's Health Study. Jama. 1987;257(3):321-5.

51. Detels R, English P, Visscher BR, Jacobson L, Kingsley LA, Chmiel JS, et al. Seroconversion, sexual activity, and condom use among 2915 HIV seronegative men followed for up to 2 years. J Acquir Immune Defic Syndr. 1989;2(1):77-83.

52. Abdool Karim SS, Ramjee G. Anal sex and HIV transmission in women. Am J Public Health. 1998;88(8):1265 - 6.

53. Lifson AR, O'Malley PM, Hessol NA, Buchbinder SP, Cannon L, Rutherford GW. HIV seroconversion in two homosexual men after receptive oral intercourse with ejaculation: implications for counseling concerning safe sexual practices. Am J Public Health. 1990;80(12):1509-11.

54. Gray RH, Wawer MJ, Brookmeyer R, Sewankambo NK, Serwadda D, Wabwire-Mangen F, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet. 2001;357(9263):1149-53.

55. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365(6):493-505.

56. Nel JT. Ectopy and related conditions of the cervix. In: Nel JT, ed. Core Obstetrics and Gynaecology. Heinemann; 1995.

57. Critchlow CW, Wolner-Hanssen P, Eschenbach DA, Kiviat NB, Koutsky LA, Stevens CE, et al. Determinants of cervical ectopia and of cervicitis: age, oral contraception, specific cervical infection, smoking, and douching. Am J Obstet Gynecol. 1995;173(2):534-43.

58. Bright PL, Norris Turner A, Morrison CS, Wong EL, Kwok C, Yacobson I, et al. Hormonal contraception and area of cervical ectopy: a longitudinal assessment. Contraception. 2011;84(5):512-9.

59. Myer L, Wright TC, Jr., Denny L, Kuhn L. Nested case-control study of cervical mucosal lesions, ectopy, and incident HIV infection among women in Cape Town, South Africa. Sex Transm Dis. 2006;33(11):683-7.

60. Moss GB, Clemetson D, D'Costa L, Plummer FA, Ndinya-Achola JO, Reilly M, et al. Association of cervical ectopy with heterosexual transmission of human immunodeficiency virus: results of a study of couples in Nairobi, Kenya. J Infect Dis. 1991;164(3):588-91.

61. Venkatesh KK, Cu-Uvin S. Assessing the relationship between cervical ectopy and HIV susceptibility: implications for HIV prevention in women. Am J Reprod Immunol. 2013;69 Suppl 1:68-73.

62. Mugo NR, Heffron R, Donnell D, Wald A, Were EO, Rees H, et al. Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1-serodiscordant couples. AIDS. 2011;25(15):1887-95.

63. Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, et al. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. Lancet. 2005;366(9492):1182-8.

64. Morrison CS, Wang J, Van Der Pol B, Padian N, Salata RA, Richardson BA. Pregnancy and the risk of HIV-1 acquisition among women in Uganda and Zimbabwe. AIDS. 2007;21(8):1027-34.

65. Reid SE, Dai JY, Wang J, Sichalwe BN, Akpomiemie G, Cowan FM, et al. Pregnancy, contraceptive use, and HIV acquisition in HPTN 039: relevance for HIV prevention trials among African women. J Acquir Immune Defic Syndr. 2010;53(5):606-13.

66. Hel Z, Stringer E, Mestecky J. Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. Endocr Rev. 2010;31(1):79-97.

67. Heffron R, Donnell D, Rees H, Celum C, Mugo N, Were E, et al. Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. Lancet Infect Dis. 2012;12(1):19-26.

68. Morrison CS, Skoler-Karpoff S, Kwok C, Chen PL, van de Wijgert J, Gehret-Plagianos M, et al. Hormonal contraception and the risk of HIV acquisition among women in South Africa. AIDS. 2012;26(4):497-504.

69. Polis CB, Curtis KM. Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence. Lancet Infect Dis. 2013;13(9):797-808.

70. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect. 1999;75(1):3-17.

71. Salvana EMT. HIV and STIs: Interactions in resource-limited settings. Available from: http://www.medscape.com/viewarticle/754637. Accessed March 12, 2014.

72. Brown JM, Wald A, Hubbard A, Rungruengthanakit K, Chipato T, Rugpao S, et al. Incident and prevalent herpes simplex virus type 2 infection increases risk of HIV acquisition among women in Uganda and Zimbabwe. AIDS. 2007;21(12):1515-23.

73. Mlisana K, Naicker N, Werner L, Roberts L, van Loggerenberg F, Baxter C, et al. Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. J Infect Dis. 2012;206(1):6-14.

74. Kapiga S, Kelly C, Weiss S, Daley T, Peterson L, Leburg C, et al. Risk factors for incidence of sexually transmitted infections among women in South Africa, Tanzania, and Zambia: results from HPTN 055 study. Sex Transm Dis. 2009;36(4):199-206.

75. Atashili J, Poole C, Ndumbe P, Adimora A, Smith J. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS. 2008;22(12):1493-501.

76. Grosskurth H, Mosha F, Todd J, Mwijarubi E, Klokke A, Senkoro K, et al. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet. 1995;346(8974):530-6.

77. Kamali A, Quigley M, Nakiyingi J, Kinsman J, Kengeya-Kayondo J, Gopal R, et al. Syndromic management of sexually-transmitted infections and behaviour change

interventions on transmission of HIV-1 in rural Uganda: a community randomised trial. Lancet. 2003;361(9358):645-52.

78. Wawer MJ, Sewankambo NK, Serwadda D, Quinn TC, Paxton LA, Kiwanuka N, et al. Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. Lancet. 1999;353(9152):525-35.

79. Grosskurth H, Gray R, Hayes R, Mabey D, Wawer M. Control of sexually transmitted diseases for HIV-1 prevention: understanding the implications of the Mwanza and Rakai trials. Lancet. 2000;355(9219):1981-7.

80. White RG, Orroth KK, Korenromp EL, Bakker R, Wambura M, Sewankambo NK, et al. Can population differences explain the contrasting results of the Mwanza, Rakai, and Masaka HIV/sexually transmitted disease intervention trials?: A modeling study. J Acquir Immune Defic Syndr. 2004;37(4):1500-13.

81. HIV Gene. What is CCR5? Available from: http://www.hivgene.com/what-is-ccr5.php. Accessed February 03, 2014.

82. Klein MR, van der Burg SH, Hovenkamp E, Holwerda AM, Drijfhout JW, Melief CJ, et al. Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. J Gen Virol. 1998;79 (Pt 9):2191-201.

83. Itescu S, Mathur-Wagh U, Skovron ML, Brancato LJ, Marmor M, Zeleniuch-Jacquotte A, et al. HLA-B35 is associated with accelerated progression to AIDS. J Acquir Immune Defic Syndr. 1992;5(1):37-45.

84. Sewram S, Singh R, Kormuth E, Werner L, Mlisana K, Abdool Karim SS, et al. Human TRIM5alpha expression levels and reduced susceptibility to HIV-1 infection. J Infect Dis. 2009;199(11):1657-63.

85. Naicker DD, Werner L, Kormuth E, Passmore JA, Mlisana K, Abdool Karim SS, et al. Interleukin-10 promoter polymorphisms influence HIV-1 susceptibility and primary HIV-1 pathogenesis. J Infect Dis. 2009;200(3):448-52.

86. Ramsuran V, Kulkarni H, He W, Mlisana K, Wright EJ, Werner L, et al. Duffy-null-associated low neutrophil counts influence HIV-1 susceptibility in high-risk South African black women. Clin Infect Dis. 2011;52(10):1248-56.

87. Levy JA. Pathogenesis of human immunodeficiency virus infection. Microbiol Rev. 1993;57(1):183-289.

88. Achhra A, Amin J. Race and CD4+ T cell count in HIV prognosis and treatment. Future Virology. 2012;7(2):193-203.

89. van Asten L, Danisman F, Otto SA, Borghans JA, Hazenberg MD, Coutinho RA, et al. Pre-seroconversion immune status predicts the rate of CD4 T cell decline following HIV infection. AIDS. 2004;18(14):1885-93.

90. Mehendale SM, Shepherd ME, Brookmeyer RS, Semba RD, Divekar AD, Gangakhedkar RR, et al. Low carotenoid concentration and the risk of HIV seroconversion in Pune, India. J Acquir Immune Defic Syndr. 2001;26(4):352-9.

91. Szerlip MA, DeSalvo KB, Szerlip HM. Predictors of HIV-infection in older adults. J Aging Health. 2005;17(3):293-304.

92. Moore PS, Allen S, Sowell AL, Van de Perre P, Huff DL, Serufilira A, et al. Role of nutritional status and weight loss in HIV seroconversion among Rwandan women. J Acquir Immune Defic Syndr. 1993;6(6):611-6.

93. MacDonald KS, Malonza I, Chen DK, Nagelkerke NJ, Nasio JM, Ndinya-Achola J, et al. Vitamin A and risk of HIV-1 seroconversion among Kenyan men with genital ulcers. AIDS. 2001;15(5):635-9.

94. Hilber AM, Chersich MF, van de Wijgert JH, Rees H, Temmerman M. Vaginal practices, microbicides and HIV: what do we need to know? Sex Transm Infect. 2007;83(7):505-8.

95. WHO. Preventing HIV among sex workers in sub-Saharan Africa: A literature review. Available from: http://whqlibdoc.who.int/publications/2011/9789241501279_eng.pdf. Accessed January 24, 2014.

96. Mavedzenge SN, Weiss HA, Montgomery ET, Blanchard K, de Bruyn G, Ramjee G, et al. Determinants of differential HIV incidence among women in three southern African locations. J Acquir Immune Defic Syndr. 2011;58(1):89-99.

97. Pettifor A, O'Brien K, Macphail C, Miller WC, Rees H. Early coital debut and associated HIV risk factors among young women and men in South Africa. Int Perspect Sex Reprod Health. 2009;35(2):82-90.

98. Wand H, Ramjee G. The relationship between age of coital debut and HIV seroprevalence among women in Durban, South Africa: a cohort study. BMJ Open. 2012;2:e000285.

99. Akileswaran C, Lurie MN. Understanding age-disparate relationships and HIV/AIDS among young women in sub-Saharan Africa: a sytematic review. AIDS 2006 - XVI International AIDS Conference. Toronto, 2006 [Abstract no. WEPE0713].

100. Pettifor AE, Rees HV, Kleinschmidt I, Steffenson AE, MacPhail C, Hlongwa-Madikizela L, et al. Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. AIDS. 2005;19(14):1525-34.

101. Harling G, Newell ML, Tanser F, Kawachi I, Subramanian SV, Barnighausen T. Do Age-Disparate Relationships Drive HIV Incidence in Young Women? Evidence from a Population Cohort in Rural KwaZulu-Natal, South Africa. J Acquir Immune Defic Syndr. 2014;66(4):443-51.

102. Leclerc-Madlala S. Age-disparate and intergenerational sex in southern Africa: the dynamics of hypervulnerability. AIDS. 2008;22 Suppl 4:S17-25.

103. Chen L, Jha P, Stirling B, Sgaier SK, Daid T, Kaul R, et al. Sexual risk factors for HIV infection in early and advanced HIV epidemics in sub-Saharan Africa: systematic overview of 68 epidemiological studies. PLoS One. 2007;2(10):e1001.

104. Onoya D, Reddy P, Sifunda S, Lang D, Wingood GM, van den Borne B, et al. Transactional sexual relationships, sexually transmitted infection risk, and condom use among young Black Women in peri-urban areas of the Western Cape Province of South Africa. Womens Health Issues. 2012;22(3):e277-82.

105. Dunkle KL, Jewkes RK, Brown HC, Gray GE, McIntryre JA, Harlow SD. Transactional sex among women in Soweto, South Africa: prevalence, risk factors and association with HIV infection. Soc Sci Med. 2004;59(8):1581-92.

106. South African National AIDS Council. National Strategic Plan for HIV Prevention, Care and Treatment for Sex Workers. 2013. Pretoria, South Africa.

107. Dunkle KL, Beksinska ME, Rees VH, Ballard RC, Htun Y, Wilson ML. Risk factors for HIV infection among sex workers in Johannesburg, South Africa. Int J STD AIDS. 2005;16(3):256-61.

108. Varga CA. Coping with HIV/AIDS in Durban's commercial sex industry. AIDS Care. 2001;13(3):351-65.

109. Halperin DT. Why is HIV prevalence so severe in Southern Africa? The Southern African Journal of HIV Medicine. 2007;8(1):19-25.

110. Steffenson AE, Pettifor AE, Seage GR, 3rd, Rees HV, Cleary PD. Concurrent sexual partnerships and human immunodeficiency virus risk among South African youth. Sex Transm Dis. 2011;38(6):459-66.

111. Wilkinson D. Condom effectiveness in reducing heterosexual HIV transmission: RHL commentary (last revised: 11 November 2002). *The WHO Reproductive Health Library*; Geneva: World Health Organisation. Available from: http://apps.who.int/rhl/hiv_aids/dwcom/en/. Accessed March 20, 2013.

112. van Loggerenberg F, Dieter AA, Sobieszczyk ME, Werner L, Grobler A, Mlisana K. HIV prevention in high-risk women in South Africa: condom use and the need for change. PLoS One. 2012;7(2):e30669.

113. Kalichman SC, Simbayi LC, Kaufman M, Cain D, Jooste S. Alcohol use and sexual risks for HIV/AIDS in sub-Saharan Africa: systematic review of empirical findings. Prev Sci. 2007;8(2):141-51.

114. Pithey A, Parry C. Descriptive systematic review of Sub-Saharan African studies on the association between alcohol use and HIV infection. Sahara J. 2009;6(4):155-69.

115. Browne FA, Wechsberg WM. The intersecting risks of substance use and HIV risk among substance-using South African men and women. Curr Opin Psychiatry. 2010;23(3):205-9.

116. Parry C, Petersen P, Carney T, Dewing S, Needle R. Rapid assessment of drug use and sexual HIV risk patterns among vulnerable drug-using populations in Cape Town, Durban and Pretoria, South Africa. Sahara J. 2008;5(3):113-9.

117. Priddy FH, Wakasiaka S, Hoang TD, Smith DJ, Farah B, del Rio C, et al. Anal sex, vaginal practices, and HIV incidence in female sex workers in urban Kenya: implications for the development of intravaginal HIV prevention methods. AIDS Res Hum Retroviruses. 2011;27(10):1067-72.

118. Turner AN, Morrison CS, Munjoma MW, Moyo P, Chipato T, van de Wijgert JH. Vaginal practices of HIV-negative Zimbabwean women. Infect Dis Obstet Gynecol. DOI 10.1155/2010/387671.Epub 24 Aug 2010.

119. Scorgie F, Smit JA, Kunene B, Martin-Hilber A, Beksinska M, Chersich MF. Predictors of vaginal practices for sex and hygiene in KwaZulu-Natal, South Africa: findings of a household survey and qualitative inquiry. Cult Health Sex. 2011;13(4):381-98.

120. Smit J, Chersich MF, Beksinska M, Kunene B, Manzini N, Hilber AM, et al. Prevalence and self-reported health consequences of vaginal practices in KwaZulu-Natal, South Africa: findings from a household survey. Trop Med Int Health. 2011;16(2):245-56.

121. McClelland RS, Lavreys L, Hassan WM, Mandaliya K, Ndinya-Achola JO, Baeten JM. Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study. AIDS. 2006;20(2):269-73.

122. van de Wijgert JH, Morrison CS, Cornelisse PG, Munjoma M, Moncada J, Awio P, et al. Bacterial vaginosis and vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. J Acquir Immune Defic Syndr. 2008;48(2):203-10.

123. Myer L, Denny L, de Souza M, Wright TC, Jr., Kuhn L. Distinguishing the temporal association between women's intravaginal practices and risk of human immunodeficiency virus infection: a prospective study of South African women. Am J Epidemiol. 2006;163(6):552-60.

124. McClelland RS, Richardson BA, Graham SM, Masese LN, Gitau R, Lavreys L, et al. A prospective study of risk factors for bacterial vaginosis in HIV-1-seronegative African women. Sex Transm Dis. 2008;35(6):617-23.

125. Iliyasu Z, Abubakar IS, Kabir M, Aliyu MH. Knowledge of HIV/AIDS and attitude towards voluntary counseling and testing among adults. J Natl Med Assoc. 2006;98(12):1917-22.

126. Hentgen V, Jaureguiberry S, Ramiliarisoa A, Andrianantoandro V, Belec M. Knowledge, attitude and practices of health personnel with regard to HIV/AIDS in Tamatave (Madagascar). Bull Soc Pathol Exot. 2002;95(2):103-8.

127. Ochako R, Ulwodi D, Njagi P, Kimetu S, Onyango A. Trends and determinants of Comprehensive HIV and AIDS knowledge among urban young women in Kenya. AIDS Res Ther. 2011;8:11.

128. Brewer DD. Knowledge of blood-borne transmission risk is inversely associated with HIV infection in sub-Saharan Africa. J Infect Dev Ctries. 2011;5(3):182-98.

129. Peltzer K, Seoka P, Raphala S. Characteristics of female sex workers and their HIV/AIDS/STI knowledge, attitudes and behaviour in semi-urban areas in South Africa. Curationis. 2004;27(1):4-11.

130. Ramjee G, Kapiga S, Weiss S, Peterson L, Leburg C, Kelly C, et al. The value of site preparedness studies for future implementation of phase 2/IIb/III HIV prevention trials: experience from the HPTN 055 study. J Acquir Immune Defic Syndr. 2008;47(1):93-100.

131. Kharsany AB, Mlotshwa M, Frohlich JA, Yende Zuma N, Samsunder N, Abdool Karim SS, et al. HIV prevalence among high school learners - opportunities for schools-based HIV testing programmes and sexual reproductive health services. BMC Public Health. 2012;12:231.

132. Abdool Karim Q, Kharsany AB, Frohlich JA, Werner L, Mlotshwa M, Madlala BT, et al. HIV incidence in young girls in KwaZulu-Natal, South Africa--public health

imperative for their inclusion in HIV biomedical intervention trials. AIDS Behav. 2012;16(7):1870-6.

133. Nelson RM, Lewis LL, Struble K, Wood SF. Ethical and regulatory considerations for the inclusion of adolescents in HIV biomedical prevention research. J Acquir Immune Defic Syndr. 2010;54 Suppl 1:S18-24.

Chapter 7: Appendices

1. Study Protocol

2. Approvals from the Postgraduate Education and Research Committee and Biomedical Research Ethics Committee

3. CAPRISA 002 Study Participant Information Leaflet

4. CAPRISA 002 Study Informed Consent Documents

5. CAPRISA 002 Study Risk Behaviour Assessment Questionnaire

6. AIDS and Behavior – Instructions for Authors

Protocol:

Predictors of HIV acquisition in high risk women in Durban, South Africa

Student: Dr Nivashnee Naicker

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CAPRISA

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Version 2.1

For Master of Public Health Degree Purposes (50% component)

Contents

Abbreviations and Acronyms

AIDS:	Acquired immunodeficiency syndrome
AHI:	Acute HIV infection
CAPRISA:	Centre for the AIDS Programme of Research in South Africa
CD4+:	Cluster of Differentiation 4
CI:	Confidence interval
CRF:	Case Report Form
FSW:	Female sex worker
HIV:	Human immunodeficiency virus
HSV-2:	Herpes simplex virus type 2
KZN:	KwaZulu-Natal
OR:	Odds ratio
STI:	Sexually transmitted infection

1. Background and Rationale:

In 2010 it was estimated that there were 5, 24 million people living with Human immunodeficiency virus (HIV) in South Africa [1]. Heterosexual transmission is the most common route of transmission in South Africa with HIV-1 subtype C being the most prevalent strain. Globally, subtype C accounts for an estimated 42% of all HIV infections [2]. The Centre for the AIDS Programme of Research (CAPRISA) 002 cohort was established between 2004 and 2005 in Durban with the aim of understanding host and viral factors that influence the course of disease in high risk women who acquire HIV-1 subtype C infection [3, 4]. Demographic and behavioural data including sexual history and knowledge of HIV and AIDS were also collected to determine the socio-behavioral factors associated with HIV infection.

Risk factors for HIV infection at an individual level include biological and behavioural factors. At a structural level, these include the social, cultural, economic, legal and political elements of an individual's environment which impacts HIV risk [5]. In sub-Saharan Africa a combination of individual and structural factors appear to contribute to HIV risk [6, 7, 8]. A study of 4486 women in Cameroon found that a higher number of lifetime sexual partners, ever users of hormonal contraception, urban dwellers, formerly married women and women aged between 25 and 35 years were at higher risk for HIV infection while multiparous women, women with no formal education and women with a lower wealth index had a lower prevalence of HIV [6]. A systematic review of studies done in Africa found that the use of alcohol in association with sexual activity increased risk for HIV acquisition [9]. Sexually transmitted infections (STIs) such as *Trichomonas vaginalis* and herpes simplex virus type II (HSV-2) and genital infections such as bacterial vaginosis (BV) have also been associated with increased risk for HIV infection [10, 11, 12]. In a study of female sex workers in Kenya, the presence of genital ulcer disease suggested an increased risk for heterosexual acquisition of HIV [13].

In sub-Saharan Africa, HIV prevalence differs substantially across regions. In South Africa itself the estimated overall antenatal HIV prevalence was 29.4% (95% Confidence interval

[CI]: 28.7% – 30.2%) for 2009 with KwaZulu-Natal province disproportionately affected by HIV [14]. The antenatal prevalence for KZN in 2009 was 39.5% (95% CI: 38.1% - 41.0%) compared to Western Cape and Gauteng provinces with HIV prevalence of 16. 9% (95% CI: 13.8% – 20.5%) and 29.8% (95% CI: 28.6% -31.1%) respectively [14]. Geographic variation of HIV prevalence within KZN has also been reported and younger women are known to be at higher risk [15, 16]. In Zimbabwe and Tanzania the HIV prevalence among pregnant women was found to be 26% and 7% respectively [17]. Risky sexual behaviour was however more prominent among Tanzanian women, however, differences in HIV prevalence could not be explained by differential risk behaviours [17]. Within South Africa several studies of HIV risk have been undertaken however comparisons across regions have not been made [7, 8].

Together with effective prevention tools to curb the epidemic, innovative behavioural interventions are needed to reduce the number of new HIV infections. Understanding the epidemic is therefore critical in order to inform the development of prevention programmes targeted at women and in particular women who engage in transactional sex. The purpose of this study is to investigate behavioural and biological predictors of HIV acquisition in a cohort of high risk women in KwaZulu-Natal, South Africa.

2. Literature Review

Several studies within the major cities in South Africa have been undertaken to understand the risk behaviours of men and women who are sexually active.

A study of 2523 sexually active women in Durban, KZN found that lack of cohabitation, frequency of sex acts, presence of STIs, pregnancy and unemployment were significantly associated with an increased risk for HIV acquisition [7]. HIV incidence rate of 6.6 per 100 person years in this study was similar to rates observed in urban and rural women in KZN [7, 16]. Participants were recruited from the general population and did not include self-identified sex-workers [7]. Seventy per cent resided in a rural area and 80% were unemployed [7]. In a study of female sex workers in Durban incident herpes simplex virus type 2 (HSV-2) infections posed significantly increased risk for HIV-1 acquisition [18].

Among a sex worker cohort in Johannesburg, Gauteng, increased condom use and age equal to or greater than 29 years were negatively associated with HIV infection [19].Women above this age were still at high risk if gonorrhoea infection was present [19]. In a community-based study in Carletonville, Gauteng HIV prevalence was significantly increased among migrant women when compared to non-migrant women (Odds ratio [OR] 1.61, 95% CI: 1.11, 2.31) [8]. Prevalent HIV infections were highest among women in the 26 to 35 year age group and the risk of HIV infection increased in women reporting two or more lifetime partners (OR 4.88, 95% CI: 3.01, 7.89) [8]. Marital status, alcohol use, syphilis and gonorrhoea were also found to be independent risk factors for HIV infection [8]. Among antenatal attendees in Soweto, Gauteng, 21% reported ever having transactional sex which was a risk factor for HIV infection and was associated with a history of partner violence, substance use, urban dwelling and substandard housing [20].

In a national household survey among 15-24 year old youth, multiple concurrent partners placed women at increased risk of HIV infection (OR 3.4, 95% CI: 1.8, 6.5) and transactional sex and inability to negotiate condom use was more often reported in these women [21]. In a survey among youth in Cape Town, 62% of females engaged in one or more one of seven high risk behaviours which included having more than two sex partners

in 3 months, reporting STIs and engaging in transactional sex [22]. Knowledge about HIV transmission, among males and females, was adequate although misconceptions regarding HIV acquisition and prevention still prevailed [22]. Among high school students in Durban, KZN, 30% of girls reported engaging in sex between 16 and 20 years, more females reported forced sex than males and alcohol use during the last sexual act was more common among boys [23].

From these results risk factors for HIV acquisition appear to be multifactorial. Further evidence is required to establish risk factors for HIV acquisition in high risk sexually active women, particularly those engaging in transactional sex, living in KZN where HIV incidence remains high [16].

3. Study Aim:
The aim of the study is to assess predictors of HIV acquisition in high risk women in Durban, KwaZulu-Natal between 2004 and 2007.

4. Study Objectives:
The objectives of this study are:

1. to assess sexual behaviour characteristics as predictors of HIV infection; and

2. to assess factors which impact the integrity and physiology of the vaginal mucosa such as use of hormonal contraception and pregnancy as predictors of HIV infection; and

3. to assess socio-demographic characteristics, knowledge of HIV and baseline laboratory parameters as predictors of HIV infection.

5. Type of Research:
Epidemiological research

6. Definitions:
High risk: Self-identified female sex workers (FSW) or women reporting more than 3 sexual partners in the 3 months preceding enrolment (see Appendix A).

7. Study Methods:

7.1 Study Overview:
For this study data collected in the CAPRISA 002 study[1] will be analysed (see Appendix A). A secondary analysis of existing data will be undertaken in order to fulfil the objectives of this study. A retrospective review of data collected during study visits will be

[1] **The author of this protocol, Dr Nivashnee Naicker, currently works as the primary clinician on the CAPRISA 002 study. She is involved in collecting data and providing clinical care to participants who are still in follow up.**

done. Risk behaviour, socio-demographic, pregnancy status and laboratory data will be compared between women who remained HIV negative during follow up and those who became HIV infected.

7.2 Study setting:
The CAPRISA 002 study was conducted at a clinical research site in central Durban (see Appendix A).

7.3 Study Design:
Observational analytic prospective cohort study.

7.4 Target Population:
High risk sexually active women in urban KwaZulu-Natal.

7.5 Study Population:
Two hundred and forty five HIV uninfected women enrolled in the CAPRISA 002 study.

8. Eligibility Criteria
All women enrolled in the CAPRISA 002 study will be included in this analysis. Two hundred and forty five women in total were enrolled. Women were recruited from truck stops and venues known to be frequented by female commercial sex workers. A recruitment team visited these sites at night and explained the study to the women at these sites and thereafter invited them to join the study. Women who were interested in joining the study and who fulfilled the eligibility criteria as outlined in the CAPRISA 002 study protocol were enrolled [4]. One of the core inclusion criteria was 'self-reported sex with more than 3 different partners in the 3 months prior to screening'. .(see Appendix A for the complete list of eligibility criteria used for the CAPRISA 002 study).

9. Study sample:
All 245 women enrolled in the CAPRISA 002 study.

10. Data Collection

10.1 Data Sources
Interviewer administered Risk Behaviour Assessment questionnaires were completed at enrolment into the CAPRISA 002 study. The data captured on the Risk Behaviour Assessment questionnaires include socio-demographic data, sexual history and behavioural (including contraception use, alcohol and substance use) data and knowledge about HIV and AIDS. For FSWs, an additional Risk Behaviour Assessment questionnaire was administered with questions pertaining to sex work. Pregnancy data were recorded on the physical examination Case Report Forms (CRF). Laboratory data was also captured on a CRF. These data were stored in the CAPRISA database following verification of data by data management personnel. Data will be accessed from the CAPRISA database with permission from the Principal Investigator of the CAPRISA 002 study.

10.2 Variables to be Analysed
Dependent Variable:

- Time to HIV infection

Independent Variables:

- **Sexual behaviour data:**
  Age of sexual debut
  Number of sexual partners
  Type (vaginal, anal, oral) and frequency of sex
  Contraception use
  Alcohol or substance use during sex
  Vaginal cleansing practices
- **Sexual behaviour data specific to self-reported FSWs**

Time period involved in sex work

Number of days per week engaged in transactional sex

Average number of sex clients per week

Use of male condoms

Number of sites worked at in a year

- **On study pregnancy**
- **Socio-Demographic variables**

  Age

  Relationship status

  Highest Level of education achieved

  Number of dependents
- **Knowledge of HIV and AIDS**

  Ever heard of HIV and AIDS

  Ways in which HIV and AIDS is spread

  Ways to prevent HIV and AIDS

  Impact of concomitant STI in HIV acquisition

  Cure for HIV and AIDS

  Treatment for HIV and AIDS
- **Baseline laboratory parameters**

List of possible confounding variables:

1. Sexually transmitted infections – the effect of STIs on HIV acquisition is explored in a separate study. Information on baseline STIs are available from a previous study [3].
2. Host genetic and immunological factors
3. Viral factors
4. Co-morbidities – a baseline medical history and physical examination was taken at enrolment into the study.

11. Validity

11.1 Internal Validity

11.1.1 Reduction of Bias:

Selection Bias:

- All women enrolled in the CAPRISA 002 cohort, including those who have seroconverted while on study, will be included in the analysis.
- Women were specifically selected for participation because of their employment as female sex workers which place them at high risk for HIV. Failure to randomly select this cohort introduces bias which may affect the results of this study.

Information Bias:

- The use of standardized questionnaires in the collection of data minimised bias.
- Data were coded with participant identification numbers only which makes it difficult for investigators to link data to participants known to them.
- Since questionnaires were interviewer administered social desirability bias is introduced which may impact the results of this study.

11.2 External Validity:

KZN is known to be an area of high HIV prevalence as evidenced by antenatal prevalence surveys and therefore all women living in KZN who are sexually active may be considered to be at high risk. However, results from this analysis may be generalized only to women who participate in transactional sex, women who identify as female sex workers and women reporting multiple concurrent sexual partners in KZN.

Results from this study will be less generalizable to other regions in South Africa and to other countries as socio-demographic background may be dissimilar. Sex work in urban KZN may also differ from other regions in Africa and countries outside Africa. This study will also be undertaken in women with HIV-1 subtype-C infection therefore HIV

seroconversion rates may not reflect that of other countries and other host and viral factors may preclude the ability to generalize results of this study outside South Africa.

12. Statistical Consideration:

- **Descriptive and Analytic statistics**

Data will be summarised using descriptive statistics (appropriate measures of central tendency and dispersion will be used). Time to HIV infection will be calculated from date of enrolment into the CAPRISA 002 cohort until estimated date of HIV infection. Date of infection will be estimated as the midpoint between last negative and first positive antibody test, or 14 days prior to the event of having a positive PCR test with a concurrent negative antibody test. Risk factors for HIV acquisition will be assessed using univariate and multivariate proportional hazards regression analysis, while adjusting for potential covariates. Kaplan-Meier curves will also be constructed. Independent variables to be analysed and assessed for risk are listed in section 10.2 above. Analysis will be performed using SAS software version 9.2 (SAS Institute Inc., Cary).

The data analysis will be done by the author of this protocol, Dr Nivashnee Naicker, under the supervision of a qualified biostatistician (Ms Lise Werner).

13. Permissions

Permission to review data captured in the CAPRISA database for women enrolled in the CAPRISA 002 study has been granted by the Principal Investigator[2] of that study.

14. Ethical Consideration

This study will be using data collected in the CAPRISA 002 study, for which ethical approval was granted in 2004, therefore an expedited review of this protocol will be requested from the Biomedical Research Ethics Committee.

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[2] The supervisor of this study, Professor Salim S. Abdool Karim, is the Principal Investigator of the CAPRISA 002 study.

All participants enrolled in the CAPRISA 002 study have previously signed informed consent at study entry. No further contact with trial participants will be necessary during this study and only existing data will be used therefore no further consent from participants is required. The results of this study will not impact the current health status of individuals therefore future contact with participants is not anticipated.

15. Study Timeframe

Analysis of data will begin as soon as approval is received from the BREC and Postgraduate Education Committee.

Timelines for completion:

Literature review	September 2011
Methods	December 2011
Results	March 2012
Discussion	April 2012
1st Completed draft	May 2012
Submission to supervisor for 1st review	May 2012
Submission to supervisor for final review	June 2012
Submission for marking	July 2012

16. Use of Information and Publications

Presentation and publication of the results of this study will be governed by CAPRISA's publication policy.

Appendix A [3]

The CAPRISA 002 cohort was established between August 2004 and May 2005. This study aimed to establish host and viral factors which predict the course of disease following acute HIV-1 infection. The study was conducted at the Doris Duke medical Research Institute (DDMRI) at the Nelson R. Mandela School of medicine of the University of KwaZulu-Natal in Durban, South Africa. Seven hundred and seventy -five women were screened for participation. Four hundred and sixty -two (59,6%) were found to be ineligible due to being HIV positive. Of the remaining 313 testing HIV negative, 245 were enrolled into the CAPRISA 002 cohort and were followed up monthly for 24 months. Of these women, 193 (78,8%) were self-identified female sex-workers with the remaining women reporting at least three sexual partners in the three months preceding enrolment. Within this period 28 women were diagnosed with acute HIV infection. Women were recruited from the Durban region, up to 45km away from the research site, mainly from truck stops and venues known to be frequented by female commercial sex workers. A recruitment team visited these sites at night and explained the study to the women at these sites and thereafter invited them to join the study.

The following eligibility criteria were used:

Core Inclusion Criteria

- Able and willing to provide adequate locator information for study retention purposes as defined in the SOP;
- Willing to participate in the follow-up phase of the protocol;
- Willing to receive HIV test result;
- Willing and capable of providing documentation of informed consent.

Inclusion criteria Phase I* (female sex worker cohort only, recruitment completed)

- Age $\geq$ 18 years;
- HIV antibody negative or indeterminate on screening; and
- Self-reported sex with more than 3 different partners in the 3 months prior to screening.

Core Exclusion Criteria

Participants who meet any of the following criteria are NOT eligible for this study:

- Pregnant on screening for Phase I;

- Subjects who plan to travel away from the recruitment area for > 3 months during the 24 months following screening; and
- Subjects who have any other condition that, in the opinion of the Principal Investigator or designee would preclude provision of informed consent, make participation in the study unsafe.

***Phase I:** HIV negative women enrolled and monitored monthly for HIV infection

****Phase II- IV:** Women with confirmed acute HIV infection were enrolled and followed up during acute HIV infection (Phase II), early HIV infection (Phase III) and established HIV infection (Phase IV).

The CAPRISA 002 study is currently on-going at the Durban eThekwini Clinical Research Site. Women who were HIV negative and who did not seroconvert during the specified follow-up period were terminated from the study. Women who acquired HIV infection are currently in follow- up.

References

1. Stats SA. Mid-year population estimates 2010. Available at: http://www.statssa.gov.za. [Accessed 21 April 2011]

2. BioAfrica.net – Bio informatics for HIV Research. Available at: http://www.bioafrica.net. [Accessed 05 May 2011]

3. van Loggerenberg F, Mlisana K, Williamson C, Auld SC. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. *PLoS ONE* 3(4): e1954. doi:10.1371/journal.pone.0001954.

4. CAPRISA 002 Viral Set Point and Clinical Disease Progression in HIV-1 Subtype C Infection: The role of immunological, genetic and viral factors over the course of disease and during antiretroviral therapy, acute and early infection Protocol, SS Abdool Karim, PI.

5. Gupta GR, Parkhusrt JO, Ogden JA, Aggleton P, Mahal A. Structural approaches to HIV prevention. *Lancet* 2008; 372:764-75.

6. Kongnyuy EJ, Soskolne V, Adler B. Hormonal contraception, sexual behaviour and HIV prevalence among women in Cameroon. *BMC Womens Health* 2008;8:19.

7. Wand H, Ramjee G. Combined impact of sexual risk behaviours for HIV seroconversion among women in Durban, South Africa: implications for prevention policy and planning. *AIDS Behav* 2011;15:479-486.

8. Zuma K, Gouws E, Williams B, Lurie M. Risk factors for HIV infection among women in Carletonville, South Africa: migration, demography and sexually transmitted diseases. *Int J STD AIDS* 2003; 14(12):814-817.

9. Pithey A, Parry C. Descriptive systematic review of sub-Saharan African studies on the association between alcohol use and HIV infection. *Sahara J* 2009; 6(4):155-169.

10. van der pol B, Kwok C, Pierre-Louis B, Rinaldi A. Trichomonas vaginalis infection and human immunodeficiency virus acquisition in African women. *J Infect Dis* 2008;197 (4): 548-554.

11. Watson- Jones D, Baisely K, Weiss HA, Tanton C .Risk factors for HIV incidence in women participating in an HSV suppressive treatment trial in Tanzania. *AIDS* 2009; 23(3): 415-422.

12. Schwebke JR. Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually transmitted diseases. *J Infect Dis* 2005, Oct 15;192(8) 1372-1380.

13. Hayes JR, Shulz KF, Plummer RA. The co-factor effect of genital ulcers on the per- exposure risk of HIV transmission in sub- Saharan Africa. *J Trop Med Hyg* 1995; 98(1): 1-8.

14. South African Department of Health. Reports 2010. Available at: http://www.doh.gov.za/docs/index.html. [Accessed 01 May 2011]

15. Wand H, Ramjee G. Targeting the hotspots: investigating spatial and demographic variations in HIV infection in small communities in South Africa. *J Int AIDS* Soc 2010; 13:41.

16. Abdool Karim Q, Kharsany AB, Frohlich JA, Werner L. Stabilising HIV prevalence masks high HIV incidence rates among rural and urban women in KwaZulu-Natal, South Africa. *Int. J. Epidemiol.* (2010) doi: 10.1093/ije/dyq176.

17. Mapingure MP, Msuya S, Kurewa NE, Munjoma MW. Sexual behaviour does not reflect HIV-1 prevalence differences: a comparison study of Zimbabwe and Tanzania. *J Int AIDS Soc* 2010;13:45.

18. Ramjee G, Williams B, Gouws E, Van Dyck E. The impact of incident and prevalent herpes simplex virus 2 infection on the incidence of HIV-1 infection among commercial sex workers in South Africa. *J Acquir Immune Defic Syndr* 2005; 39(3):333-339.

19. Dunkle KL, Beksinska ME, Rees VH, Ballard RC. Risk factors for HIV infection among sex workers in Johannesburg, South Africa. *Int J STD AIDS* 2005; 16(3):256-261.

20. Dunkle KL, Jewkes RK, Brown HC, Gray GE. Transactional sex among women in Soweto, South Africa: prevalence, risk, factors and association with HIV infection. *Soc Sci Med* 2004; 59(8): 1581-1592.

21. Steffenson AE, Pettifor AE, Seage GR 3rd, Rees HV, Cleary PD. Concurrent sexual partnerships and Human Immunodeficiency Virus Risk among South African youth. *Sex Transm Dis* 2011 Jan 20. [Epub ahead of print] PubMed PMID: 21258268.

22. Simbayi LC, Kalichman SC, Jooste S, Cherry C. Risk factors for HIV-AIDS among youth in Cape Town, South Africa. *AIDS Behav* 2005;9(1):53-61.

23. Frank S, Esterhuizen T, Jinabhai CC, Sullivan K. Risky sexual behaviours of high- school pupils in an era of HIV AIDS. *S Afr Med J* 2008;98(5): 394-398.

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11 October 2011

Professor S Abdool-Karim
CAPRISA
NRMSM

Dear Professor Abdool-Karim

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa. M-PH, N Naicker SN 983171064

The Postgraduate Education Committee ratified the approval of the abovementioned study on 11 October 2011.

Please note:

- The Postgraduate Education Committee must review any changes made to this study.
- The study may not begin without the approval of the Biomedical Research Ethics Committee.

Yours sincerely

Professor SJ Botha
Chair Postgraduate Education and Research Committee

CC. N Naicker

Biomedical Research Ethics Committee
Westville Campus

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13 June 2011

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF:BE092/11

EXPEDITED APPLICATION
ACKNOWLEDGEMENT OF RECEIPT

Dear Dr Naicker

We acknowledge receipt of the above EXPEDITED APPLICATION received on 01 June 2011. The application will be sent to reviewers for consideration. Should you not hear from the Biomedical Research Ethics Committee within four weeks from receipt of this letter, please contact this office on **extension 1074** to query the status of your application.

Where queries are raised, you will be expected to respond within 20 days of receipt of the communication to you. Where you anticipate failure to comply, please request for an extension of time, otherwise your file will be closed.

Yours sincerely

Prof. D Wassenaar
Chair: Biomedical Research Ethics Committee
DW/pn

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06 September 2011

Dr. N Naicker
CAPRISA
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University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

PROVISIONAL APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 01 June 2011.

The study is given **PROVISIONAL APPROVAL** pending receipt of:

1. Postgraduate Education Committee Approval

Only when full ethical approval is given, may the study begin. **Full ethics approval has not been given at this stage.**

PLEASE NOTE: Provisional approval is valid for 6 months only - should we not hear from you during this time - the study will be closed and reapplication will need to be made.

Your acceptance of this provisional approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

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09 September 2011

Dr. N Naicker
CAPRISA
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Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

I wish to advise you that your letter dated 01 September 2011 forwarding BREC an amended copy of your protocol has been noted and reviewed by a sub-committee of the Biomedical Research Ethics Committee.

The following document has been noted and approved:

- Protocol Version 2.1

This approval will be ratified by a full Committee at its next meeting taking place on 11 October 2011.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

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RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

30 November 2011

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 01 June 2011.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 16 October 2011 to queries raised on 06 September 2011 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 30 November 2011.

This approval is valid for one year from **30 November 2011.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC

Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **14 February 2012.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

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26 October 2012

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 30 November 2012
Expiration of Ethical Approval: 29 November 2013

I wish to advise you that your application for Recertification dated 03 October 2012 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its **next** meeting taking place on **14 November 2012.**

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

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15 November 2013

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	30 November 2013
Expiration of Ethical Approval:	29 November 2014

I wish to advise you that your application for Recertification dated 08 November 2013 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its next meeting taking place on **10 December 2013.**

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

05 December 2013

The Postgraduate Education Committee

RE: Request for change in supervisor - Predictors of HIV acquisition in high risk women in Durban, South Africa

I, Dr Nivashnee Naicker, would like to request a change in supervisor for the protocol 'Predictors of HIV acquisition in high risk women in Durban, South Africa'.

Version 2.1 of the protocol, which was approved by the Postgraduate Education Committee on 01 September 2011, lists Professor Salim Abdool Karim as supervisor and Professor Ayesha BM Kharsany as co-supervisor. I would like to request that Professor Kharsany now be listed as primary supervisor of the protocol. This change has been approved by Professor Abdool Karim and Professor Kharsany.

Yours sincerely,

Dr Nivashnee Naicker

(Student Number 983171064)

Prof Ayesha Kharsany 6 December 2013

Prof Salim S Abdool Karim 6 December 2013

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 January 2014

Dr N Naicker
983171064

APPROVAL FOR CHANGE IN SUPERVISOR

Dear Dr Naicker

We have pleasure in advising that your request to change your supervisor has been approved.

Yours sincerely

Mrs Devi Arumugam
Postgraduate Administration
School of Nursing & Public Health

Postgraduate Administration
School of Nursing and Public Health
University of KwaZulu-Natal
Postal Address: University of KZN, Durban, 4041, South Africa
Telephone: +27 (0) 31 260 2499
Facsimile: +27 (0) 31 260 1543

16 May 2014

Chair: Biomedical Research Ethics Committee

Dear Professor Wassenaar,

RE: Change in Supervisor (Protocol: Predictors of HIV acquisition in high risk women in Durban, South Africa. REF: BE092/11)

This letter serves to inform you that Professor Salim Abdool Karim, previously listed as supervisor of the above protocol is now listed as co-supervisor. Professor Ayesha BM Kharsany is now primary supervisor of the protocol. An application for this change has been approved by the Postgraduate office. Please see correspondence attached.

Yours Sincerely,

Dr Nivashnee Naicker

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06 June 2014

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

I wish to advise you that your letter dated 16 May 2014 informing BREC of change in supervisor for the above approved study has been noted by a sub-committee of the Biomedical Research Ethics Committee. Postgraduate approval letter has been noted by BREC.

The Committee will be advised of the above by a full Committee taking place on 08 July 2014.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

Student Name : Nivashnee Naicker
Student Number : 983171064
Year : 2014
Qualification : Master of Public Health
Study Period : 1
Offering Type : Med, Full-Time, Contact

TO WHOM IT MAY CONCERN

This is to certify that the above student has registered at the UNIVERSITY OF KWAZULU-NATAL for the current academic year.

MODULE CODE	NAME OF MODULE	FEE
Semester One		
PBHL89S	Research Project in Public Health Subseq Yr	960.00
Semester Two		
PBHL89S	Research Project in Public Health Subseq Yr	960.00
TOTAL FOR 2014 :		1920.00

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI
School of Nursing & Public Health
2014 -07- 11
POSTGRAD OFFICE
CAROLINE DHANRAJ
RECEIVED

11 Jul 2014

Note : This certificate represents the registration status of the student at the date of production of the certificate and is not valid unless stamped and signed by the relevent authority.

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19 November 2014

Dr. N Naicker
CAPRISA
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Naicker

PROTOCOL: Predictors of HIV acquisition in high risk women in Durban, South Africa.
REF: BE092/11

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 30 November 2014
Expiration of Ethical Approval: 29 November 2015

I wish to advise you that your application for Recertification dated 03 November 2014 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be ratified by a full Committee at its next meeting taking place on **09 December 2014.**

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

CAPRISA 002

VIRAL SET POINT AND CLINICAL PROGRESSION IN HIV-1 SUBTYPE C INFECTION: THE ROLE OF IMMUNOLOGICAL AND VIRAL FACTORS DURING ACUTE AND EARLY INFECTION

Short-form Information to patients: To be used during recruitment to advertise the study, and during the study to provide a summary of the overall study.

NOTE: This version corresponds to Version 2.00 of the English Informed Consent Forms.

Study Information
The acute infection study will look at several important elements of the spread of HIV in South Africa. HIV is the virus that causes AIDS. We will be recruiting HIV negative participants and also participants who have been known to have been infected recently (within the previous three months).

After initial infection, the level of virus measured in the blood is usually high, then it drops down over time often reaching a point where it does not change much over time. The amount of virus that can be measured in the blood is referred to as the viral load. This study aims to better understand the relationship between the viral load over time and how soon people might become sick after HIV infection. That is, we are trying to find out whether the amount of virus that can be measured at certain times (at 6, 12 and 18 months) is related to how quickly someone with HIV infection develops AIDS illnesses. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people's thoughts, feelings and behaviors.

Since the point at which the viral load levels out is currently the best indication of progression to illness and AIDS, the results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV disease and AIDS. This study will also provide important information for future treatment interventions, that is interventions aimed at preventing AIDS after HIV has occurred.

By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it will not provide a cure or alter the course of your illness.

Who will be taking part in this study?
We will be recruiting 200 HIV negative participants who we will see at the clinic on a monthly basis. Some of these participants will become infected with HIV, and others not. In addition to this, we will be recruiting 98 participants who are known to have become infected with HIV in the previous 3 months.

Who will not be able to take part in this study?
If you are younger than 18, or will not be able to take part because you feel that the clinic visits are too many or you plan to move away from the area of the clinic for more than 3 months you will not be able to take part.

In order to take part in the study, you will need to receive counseling and be tested for HIV. If you are HIV positive, you will not be able to take part and will receive counseling and be referred to your nearest VCT/HIV clinic. If you are a woman, you will also be tested for pregnancy. If you are pregnant, you will not be able to take part in this study.

How long will I participate in this study?
Participants who remain HIV negative during this study will be followed up for 2 years. Participants who have been recently infected, or who become infected while on the study, will be stay in the study for at least 42 months after infection. The longest that someone will be in this study is five and a half years.

If I become HIV positive on this study will I receive antiretroviral drugs on this study?
No, we cannot provide HIV drugs to you on this study. However, we will refer you to the Caprisa Antiretroviral Treatment programme which provides HIV treatment to research participants. There you would be assessed to see if you require special HIV treatment.

What will happen to me on this study?
If you are HIV negative, you will be asked to come to the clinic once a month to be tested for HIV infection and to receive HIV counseling. In this phase of the study, you will receive condoms and counseling and education on how to protect yourself from sexually transmitted

infections, especially HIV. You will also have a physical exam at these visits. If we find that you have any illness and infection at these visits, you will be referred to the Family Clinic at King Edward Hospital for the appropriate treatment and care. This clinic may refer you to other clinics or services at the hospital should you require this. Participation in this study should not increase your risk of infection.

If at one of these visits it is learned that you have been infected with HIV you will be offered an opportunity to be enrolled onto Phase II of this study. This phase looks very closely at what happens in the body during the early stages of infection with HIV. For the first 3 weeks you will be asked to come to the clinic once a week and then every other week until 3 months after your diagnosis (5 visits). The next 9 months is called Phase III and will require you to come to the clinic once a month. On Phase IV of the study you will be asked to come to the clinic once every three months until the end of the study.

At these visits, you will also be given a medical examination by one of the study doctors. You will be asked to give tubes of blood at each visit. The amount of blood varies between 8 and 15 tubes (each tube is between 1 and 2 teaspoons of blood). The blood will be used to see how your body is responding to HIV infection, and to check on the type of virus that you have been infected with. The virus that infects you, changes over time, and we want to study the way in which the virus changes.

Every six months you will be tested for any sexually transmitted infections. This will be done by testing your blood, and by using a vaginal swab to collect samples if you are a woman or urine if you are a man. You will be referred for treatment for any sexually transmitted infections that you have. You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle.

What benefits are there to me for participating in the study?
You will get no direct benefit from taking part in the study. However, you will get counseling and testing for HIV. If you are infected with HIV, you will be referred for medical care, counseling and other services that are available to help you. If you are a woman who is pregnant, you will be referred to the nearest public health clinic that can provide care and management of your pregnancy.

If you are enrolled onto the Acute Infection Study, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be referred to KEH VIII hospital for treatment. You will receive free condoms and information on how to use condoms properly to reduce risk.

What are the risks of taking part in this study?
You may feel some pain or discomfort during the blood collection. A few people feel faint when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the blood draw.

You may be embarrassed, worried, or anxious when you are discussing your sexual practices, ways to protect yourself and others from infections passed on during sex. You may also feel worried or anxious while waiting for and finding out your test results. If you find that you have HIV or any other infection, this could also cause you anxiety and problems at home or with your friends. A trained counselor will help you deal with any feelings that you may have. You will also be referred for medical and support services available that will be able to help you live in a more healthy and positive way.

Who to contact:
If you would like to know more about this study or to see if you are eligible to take part, please contact Lucky Barnabas (0834796156) or at 031-2604555.

1. Informed Consent form (Screening: Monitoring Phase Screening) - V2.00

Title of the Research: **VIRAL SET POINT AND CLINICAL PROGRESSION IN HIV-1 SUBTYPE C INFECTION: THE ROLE OF IMMUNOLOGICAL AND VIRAL FACTORS DURING ACUTE AND EARLY INFECTION**

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 - 2604564 (Francois van Loggerenberg)

INTRODUCTION

You are being asked to take part in a research study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. Over the period of two years, we hope to enroll approximately 298 subjects, from three different sites. You will be asked to take part for as long as you are willing and able, up to a maximum period of five and a half years. The follow-up visits for this study will take place at the Medical Research Institute in Durban.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic. HIV is the virus that causes AIDS.

PURPOSE OF THE STUDY

You are being screened for potential enrollment into study. This study will look at the very early stages of HIV infection by following up HIV negative people who may become infected as well as people who have been found to have been recently infected. We will be screening participants for 24 months. All participants who remain HIV negative at the end of this 24-month period will finish the study at that time. If individuals become infected, they will be followed up for at least 42 months post-infection. The longest time that anyone can take part in the study is five and a half years. Researchers are interested in what happens in a person's body when they become infected with the virus and what determines how well their body is able to cope with HIV infection. Researchers are also interested in determining what factors influence the progression of HIV disease in South Africa. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. This study is an observational study. That is, participants will not be provided with treatment on this study, but will be referred for the treatment that is currently used at the government health facilities.

INFORMED CONSENT

You are being asked to volunteer for screening tests to find out if you are eligible for research in the study named above. The research is for people who are at risk for HIV infection. The screening tests will include a set of questions and you will also be screened for HIV.

YOUR PARTICIPATION IS VOLUNTARY

The information on this form will be discussed with you. Once you understand the screening process and the tests that will be used, and if you agree to take part, you will be asked to sign your name or make your mark on this form. You will be given a copy to keep.

Before you learn more about the screening tests, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to have the screening tests, or to withdraw from the screening tests at any time, without losing the benefits of your routine medical care.
- If you decide not to have the screening tests, you can still join another research study later, if one is available and you qualify.
- You are only being asked to have the screening tests at this time. Even if you agree to have the screening tests, you do not have to join the research study.

PURPOSE OF THE SCREENING TESTS

The research that you will be screened for involves the study of HIV virus. The first phase of the study will be monitoring people who are not infected. The screening tests are therefore looking for people who are HIV negative. For this reason, if you have already been infected with HIV you will not be able to join this study and you will be referred to the nearest appropriate public health clinic. Women who are pregnant at screening will also not be able to take part in the study. If you are HIV negative, a sample of your urine will be tested for signs of pregnancy. If you are pregnant, you will be referred to your local public health care clinic for management of your pregnancy.

Some individuals will not be able to take part in the study based on the information that is found during the screening tests. The screening tests are done on site, and you will be able to get the results the same day.

PROCEDURES

At this visit, you will be able to read, discuss and sign this screening consent form. The study staff will ask you where you live and other questions about you, your health and your sexual practices. If your answers to these questions indicate that you may qualify (be eligible) for this study, you will then undergo a rapid test for HIV.

The study staff will talk to you about the HIV test, and what it may mean for you to know the results of this test. You will be asked to talk about how you would feel about knowing your HIV status, and whether you would be prepared to be tested for HIV. You will also receive information about how to prevent yourself from being infected with the virus and other sexually transmitted infections.

Finding out the result of an HIV test can be very difficult. You will talk to the study staff about the meaning of your result, and how you feel about it. You will need to receive your HIV test result in order to be enrolled and stay in the study.

If you are prepared to have the test, one tube of blood will be drawn. If you test positive or the test results are inconclusive, you will be referred to the nearest public health clinic or Voluntary Counseling and Testing (VCT) centre for more tests, counseling and related health care. You will also receive counseling on how to stay healthy, and assistance with obtaining any services for which you may be eligible. You will be told of any other research projects that you might be able to take part in, if there are any.

If your results show that you have not been infected with HIV, you will be tested for pregnancy using a urine sample.

If you are HIV negative and not pregnant, you could be able to take part in the study. The study staff will explain the purpose of the study to you, and you will be asked to sign another consent form to take part in the study.

All of these screening procedures should be completed within one to two hours.

RISKS AND/OR DISCOMFORTS

You may feel some pain or discomfort during the blood collection. A few people feel faint when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the blood draw.

You may be embarrassed, worried, or anxious when you are discussing your sexual practices, ways to protect yourself and others from infections passed on during sex. You may also feel worried or anxious while waiting for and finding out your test results. If you find that you have HIV or any other infection, this could also cause you anxiety and problems at home or with your friends. A trained counselor will help you deal with any feelings that you may have. You will also be referred for medical and support services available that will be able to help you live in a more healthy and positive way.

BENEFITS

You will get no direct benefit from taking part in the screening tests. However, you will get counseling and testing for HIV. If you are infected with HIV, you will be referred for medical care, counseling and other services that are available to help you. If you are pregnant, you will be referred to the nearest public health clinic that can provide care and management of your pregnancy.

If you are enrolled onto the Acute Infection Study, you will receive regular monitoring of your health. This would allow you to be referred for treatment for many conditions that you may develop; perhaps earlier than if you were not receiving regular checkups.

CONFIDENTIALITY

Research records of your participation in the study will be kept confidential and will not be released without your permission, unless we are required by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However, it is possible that others may learn that you have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by representatives of the agency that is funding this study and by the research team who is responsible for keeping information for this study.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE SCREENING TESTS WITHOUT YOUR CONSENT

You may be removed from the screening tests without your consent for the following reasons:

- The research study is stopped or cancelled.
- The study staff feels that having the screening tests may be harmful to you.
- You are not willing to receive your HIV test result.

COSTS OF THE STUDY

There is no cost to you for the tests that you will be done during the course of this study.

COMPENSATION

You will receive compensation for your time and effort at the scheduled screening visit. You will also receive a refund for the costs incurred by you in order to attend the scheduled clinic (for example, transport costs). You need to find out from the study nurse what the amount of this compensation is before agreeing to take part in the screening tests.

RESEARCH RELATED INJURIES

It is very unlikely that you could be injured as a result of the screening tests. In the case of a research related injury, you will be referred to King Edward VIII Hospital for treatment. The cost of this treatment will be borne by the researchers. There is, however, no compensation provided for research related injuries. In the event of a research related injury, contact:

Mr. Francois van Loggerenberg (study coordinator) 072-219-3122; or
Dr. Koleka Mlisana (study director) 083-560-2036.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following:

Project Co-ordinator: Mr. Francois van Loggerenberg 031-260 4564
Principal Investigator: Professor SS Abdool Karim 031-260 2381 (**Helen**)

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may call:
The Postgraduate Administration Office: 031-260 4495 (Ms Cheryl Borresen)
E-mail: postgrad@med.nu.ac.za

Or, if you have difficulties contacting the above:

Professor A Dhai, Chairman of the Ethics Committee: 031-260 4604

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff concerning the possible advantages and possible adverse effects that may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Iriogbe and Professor SS Abdool Karim.
- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.

- I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:_____ Date:_____
Subject/Parent/Guardian

Signed:_____ Date:_____

Witness

Signed:_____ Date:_____

Researcher

For illiterate subjects:

Mark with an 'X':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

2. Informed Consent form (Enrollment: Phase I - HIV Negative) - V2.00

Title of the Research: **VIRAL SET POINT AND CLINICAL PROGRESSION IN HIV-1 SUBTYPE C INFECTION: THE ROLE OF IMMUNOLOGICAL AND VIRAL FACTORS DURING ACUTE AND EARLY INFECTION**

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 - 2604564 (Francois van Loggerenberg)

INTRODUCTION

You are being asked to take part in a research study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. Over the period of two years, we hope to enroll approximately 298 subjects, from three different sites. You will be asked to take part for as long as you are willing and able, up to a maximum period of five and a half years, as this is how long the whole study will continue. The follow-up visits for this study will take place at the Medical Research Institute in Durban.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic. HIV is the virus that causes AIDS. If you do become infected with HIV during the study, we will continue to do tests that will tell us how your body is responding to the infection. This is an observational study and no treatment will be given to you by the study staff. Should you become ill or reach a point where you require treatment, you will be referred to the Family Clinic at King Edward VIIIth Hospital for the standard treatment that is currently offered to patients in South Africa. You may then also be offered an opportunity to take part on other available studies offering drugs that treat HIV infection, should you be eligible.

INFORMED CONSENT

You are being asked to volunteer for the research study named above. This is a study for people who have recently been screened for HIV infection and have tested negative. Before you decide whether or not you would like to take part in the study, we would like to explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to you, and what is expected of you as a research participant.

YOUR PARTICIPATION IS VOLUNTARY

Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.

PURPOSE OF THE STUDY

Overall, this study will look at the very early and later stages of HIV infection by following up people who have been found to have been infected very recently. These individuals will be followed up for at least 42 months after they have been informed of the diagnosis of HIV infection and have been enrolled onto the study, and up to a maximum of 66 months (five and a half years).

Researchers are interested in what happens in a person's body to determine how well the body of people who have become infected is able to cope with HIV infection. Researchers are also interested in determining what factors influence the progression of HIV disease in South Africa. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people's thoughts, feelings and behaviors.

You have been recruited into the HIV negative phase (called Phase I) of this study as you recently tested HIV negative. You will be asked to come to the clinic once a month to be tested for HIV infection and to receive HIV counseling. In this phase of the study, you will receive condoms and counseling and education on how to protect yourself from sexually transmitted infections, especially HIV. You will also have a physical exam at these visits. If we find that you have any illness and infection at these visits, you will be referred to the Family Clinic at King Edward Hospital for the appropriate treatment and care. This clinic may refer you to other clinics or services at the hospital should you require this. Participation in this study should not increase your risk of infection. All those participants who remain HIV negative will be followed up for 2 years only.

If at one of these visits it is learned that you have been infected with HIV, the virus that causes AIDS, you will be offered an opportunity to be enrolled onto Phase II of this study. This phase looks very closely at what happens in the body during the early stages of infection with HIV. Participants will be asked to come to the clinic on a regular basis for a check-up. For the first 3 weeks you will be asked to come to the clinic once a week and then every other week until 3 months after your diagnosis (5 visits). The next 9 months is called Phase III and will require you to come to the clinic once a month. On Phase IV of the study you will be asked to come to the clinic once every three months until the end of the study, which would be a maximum of five and a half years after your diagnosis.

We will also be providing participants with regular medical check-ups and referral for treatment of any infections or illnesses that they may have. If you become eligible for the second and third phases of this study, you will be provided with more information about those phases of the project. You will be asked to sign an informed consent form to take part in those phases. By agreeing to take part in this phase of the study, you are not compelled to take part in later phases if you become eligible for those. You will always have the right to withdraw from the study at any time.

If, at any time, during this study, the study doctor or you think that you might have fallen pregnant, a sample of your urine will be tested. If you are pregnant, you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services that you and your baby may need.

By taking part in this study, you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic.

PROCEDURES

At this visit, you will be able to read, discuss and sign this consent form. If you decide to consent, the study staff will ask you where you live and other questions about you, your health and your sexual practices. You will also undergo medical tests.

You will also have a physical examination done, which will include looking for clinical evidence of sexually transmitted infections. Specimens to test for sexually transmitted infections, including HIV, will be collected from you. This will include a wash and a swab from your vagina. The study staff will talk to you about the infections that are passed on during sex. If you are having problems with one of these sexually transmitted infections, the study staff will refer you to Kind Edward Hospital where you will be given medicine to treat the infection.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give just less than 50 mls of blood at each visit. This blood will be drawn in around 9 tubes. The blood will be used to test you for signs of early HIV infection, and some samples will be used to check on your general health. Every six months you will be asked to give samples for testing for other sexually transmitted infections. Urine samples will be taken every 6 months to check on your health. If you or the study doctor think you may be pregnant, a sample of your urine will be tested for signs of pregnancy. Some of the specimens that are collected from you may be stored. You will be asked to sign a consent form to indicate whether you agree to allow the team to store some of your samples.

Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

BENEFITS

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be referred to KEH VIII hospital for treatment. You will receive free condoms, which you can use to reduce your risk of HIV infection. You will receive information on how to use condoms properly to reduce risk.

PARTICIPANT RESPONSIBILITY

By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinics. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study, please tell this to the study nurse or doctor. We will encourage you to come to the clinic for a final exit interview and to receive any test results that you may not have received yet.

COMPENSATION

You will be compensated for your transport costs when you come in to the scheduled clinics. If your clinic visit takes up most of your day, you will be provided with food and refreshments for the time that you are at the clinic.

RISKS/DISCOMFORT

Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There

is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital secretions (wash and swab from your vagina) are pain and discomfort.

You may learn that you have become HIV positive while on this study. Learning that you are HIV positive can be a very stressful experience. In addition to this, you may be treated badly by friends and family if your HIV status becomes known to others. You may find it difficult to find work should others learn of your HIV status, and you may find that members of your family or community discriminate against you.

Some of the tests being used for this study are very sensitive and can pick up an infection much earlier than the tests used at public health facilities. This means that we will sometimes be able to detect infection before we can actually tell you for sure that you have been infected. We will be able to tell you that you have been exposed, but will have to wait for the standard test to come back positive before we can confirm whether you have been infected. This period is likely to be very stressful while you wait for the test result.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

NEW FINDINGS

You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to gain access to them.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION

There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about. There may also be other places where you can go for HIV counseling and testing. We will also tell you about those places if you wish.

COSTS OF THE STUDY

There is no cost to you for the physical examination or laboratory tests that you will receive during the course of this study. You will receive treatment for all sexually transmitted infections that are detected while you take part in this study. You will be referred to King Edward VIIIth Hospital for any other non-study related illnesses that may be detected at the clinic.

CONFIDENTIALITY

Research records of your participation in the study will be kept confidential and will not be released without your permission, unless we are required by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However, it is possible that others may learn that you

have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by the agency who are funding this study and by the research team who are responsible for keeping information for this study.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission. No publications based on this research will contain your name. Only the project and clinic co-ordinators will know both your name and your identification number. This is necessary so that the co-ordinators can ensure that you will receive the correct tests and that you are called in to the correct clinics. The co-ordinators will not release your number and name to anyone else on the research team.

RESEARCH RELATED INJURIES

It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred to the King Edward hospital for treatment. The cost of this treatment will be borne by the research team. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact:

Mr. Francois van Loggerenberg (study coordinator) 072-219-3122; or
Dr. Koleka Mlisana (study director) 083-560-2036.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following:

Project Co-ordinator: Mr. Francois van Loggerenberg 031-260 4564
Principal Investigator: Professor SS Abdool Karim 031-260 2381 (Helen)

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research subject, you may call:
The Postgraduate Administration Office: 031-260 4495 (Ms Cheryl Borresen)
E-mail: postgrad@med.nu.ac.za

Or, if you have difficulties contacting the above:

Professor A Dhai, Chairman of the Ethics Committee: 031-260 4604

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.

- I acknowledge that I have been informed by the clinic staff and doctor (Dr Iriogbe) concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.

- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Mlisana and Professor SS Abdool Karim.
- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
- I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:_____ Date:_____
Subject/Parent/Guardian

Signed:_____ Date:_____
Witness

Signed:_____ Date:_____
Researcher

For illiterate subjects:

Mark with an 'X':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

3. Informed Consent form (Enrollment: Phase II-IV - Acute Infection) - V2.00

Title of the Research: **VIRAL SET POINT AND CLINICAL PROGRESSION IN HIV-1 SUBTYPE C INFECTION: THE ROLE OF IMMUNOLOGICAL AND VIRAL FACTORS DURING ACUTE AND EARLY INFECTION**

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 - 2604564 (Francois van Loggerenberg)

INTRODUCTION

You are being asked to take part in a research study. This document gives you information about the study that will be discussed with you by the study doctor or nurses. Once you understand the study and if you agree to take part, you will be asked to sign the informed consent sheet, or make a mark in front of a witness. You will be given a copy to keep. Please read this carefully and do not hesitate to ask the doctor or nurse any questions. You should not agree to take part unless you are completely happy with the study, and you feel that you understand the information that has been given to you.

Participation in this study is completely voluntary.

Scientists associated with the University of KwaZulu-Natal's Nelson Mandela School of Medicine are doing this study. Over the period of two years, we hope to enroll approximately 298 subjects, from three different sites. You will be asked to take part for as long as you are willing and able, up to a maximum period of 5 and a half years. The follow-up visits for this study will take place at the Medical Research Institute in Durban, and at the CAPRISA Vulindlela Research Facility.

By taking part in this study, you are contributing to a greater understanding of the Human Immunodeficiency Virus (HIV) infection in South Africa, which is an important step towards attempting to reduce this epidemic.

INFORMED CONSENT

You are being asked to volunteer for the research study named above. This is a study for people who have recently been infected with HIV. Before you decide whether or not you would like to take part in the study, we would like to explain the study to you. You will learn what the study is about, what procedures will form a part of the study, what the risks and benefits of the study are to you, and what is expected of you as a research participant.

HPTN035 AND VULINDLELA

If you have been referred from the HPTN035 or a Vulindlela research cohort, it will be necessary for us to look at your HIV test results from your previous study participation. By signing this form, you are agreeing to your HIV test records being reviewed and information collected about your previous HIV test results.

YOUR PARTICIPATION IS VOLUNTARY

Before you learn more about this study and decide whether you want to take part, it is important that you know the following:

- Your participation is entirely voluntary.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your routine medical care.
- If you decide not to take part in this study, you can still join another study later, if one is available and you qualify.

PURPOSE OF THE STUDY

This study will look at several important elements of the spread of HIV in South Africa. HIV is the virus that causes AIDS. After initial infection, the level of virus measured in the blood is usually high, then it drops down over time often reaching a point where it does not change much over time. The amount of virus that can be measured in the blood is referred to as the viral load. This study aims to better understand the relationship between the viral load over time and disease progression in HIV infection in South Africa. That is, we are trying to find out whether the amount of virus that can be measured at certain times (at 6, 12 and 18 months) is related to how quickly someone with HIV infection develops AIDS illnesses. The researchers are going to be looking at events in the body very early in infection to see if they impact on the course of the illness over time. The researchers are also interested in the way in which HIV infection may affect people's thoughts, feelings and behaviors.

We know that across the world there are different types of the HIV virus. These viruses are known as subtypes. In South Africa, subtype 'C' is the most common HIV infection between males and females. Although there is extensive information about disease resulting from subtype B infections in the developed world, there is limited information available to determine what influences the viral load in subtype C infections.

Since the point at which the viral load stabilizes is currently the best indication of progression to illness and AIDS, the results from this study could have an impact on future treatment and prevention strategies and research. This study will provide important information for vaccine development and more effective treatment of patients with HIV disease and AIDS. This study will also provide important information for future treatment interventions, i.e. interventions aimed at preventing AIDS after HIV has occurred.

You have been referred to this study because you are known to have been recently infected with HIV.

By taking part in this study you are contributing to a greater understanding of HIV infection in South Africa, which is an important step towards attempting to reduce this epidemic. This study may not affect your own situation directly. That is, it will not provide a cure or alter the course of your illness. However, you will be monitored closely and referred for care and treatment relating to any HIV-related illness that you may develop.

As people become ill after HIV infection, their blood cells know as CD4+ T cells (protective cells) become reduced. This is an indication of the immune system's failure to control infection. Most infections occur when the level of these cells drop below 200 copies/ml. At this time people can become sick very easily.

PROCEDURES

Entering the Study

At this visit, you will be able to read, discuss and sign this consent form. If you decide to sign, the study staff will ask you where you live and other questions about you, your health and your sexual practices.

If your previous HIV test was inconclusive you will be asked to have HIV tests and counseling every two weeks until a conclusive test result is achieved.

You will also have a physical examination done, which will include looking for evidence of sexually transmitted infections and the presence of any AIDS related illnesses. You will be asked to give specimens for the testing of sexually transmitted infections. If you are a women this could include a wash and a swab from you vagina. The study staff will talk to you about the infections that are passed on during sex. If you have clinical signs and symptoms of sexually transmitted

infections, you will be referred to the Family Clinic where you will be given medicine to treat those infections.

You will be asked to give tubes of blood. Each tube is between 5 and 10 mls of blood (1 to 2 teaspoons). You will be asked to give around 90 mls of blood (around 15 tubes) at your first visit. The blood will be used for tests that look at the way in which your body is responding to HIV infection. It will also be used to look at the virus that you have been infected with. Some of this blood together with a urine specimen, will be used to check on your general health. If you are a woman and you or the study doctor suspect that you are pregnant, this sample will be checked for signs of pregnancy. If you are pregnant, you will be encouraged to stay in the study. You will also be referred to your nearest public health clinic for care relating to your pregnancy.

You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle. You will also be evaluated for the presence of any AIDS related illnesses.

During the Study

Community liaison persons or the study nurse will notify you of your scheduled clinic visits. The number of times that you will be called in to the clinic will vary over time. You may leave the study at any time, but we would like to follow you for at least 42 months (3 and a half years). You may be able to continue in the study for longer than this, depending on when you enroll into the study, up to 5 and a half years.

At first you will be asked to come in once a week for three weeks, then every other week for five visits. After this you will come in once a month until one year after starting, and then once every three months until the end of the study.

At these visits, you will also be given a medical examination by one of the study doctors. You will be asked to give tubes of blood at each visit. The amount of blood varies between 8 and 15 tubes. The blood will be used to see how your body is responding to HIV infection, and to check on the type of virus that you have been infected with. The virus that infects you, changes over time, and we want to study the way in which the virus changes.

Every six months you will be tested for any sexually transmitted infections. This will be done by testing your blood, and by using a vaginal swab to collect samples if you are a woman or urine if you are a man. You will be referred for treatment for any sexually transmitted infections that you have. You will be asked questions about your sexual behavior, general health as well as other questions related to your lifestyle.

People who are HIV positive tend to get sick more often with different kinds of infections because of the reduced level of immunity. These are known as 'opportunistic infections'. This is an observation study and you will not receive treatment from the study staff. Any opportunistic infections that you may develop will be detected at the scheduled clinic visits and you will then be referred to the Family Clinic at King Edward Hospital where you will receive treatment for any treatable opportunistic infections. Your body has cells in it that help you fight off infection and these helper cells are called CD4+ T cells. The level of CD4+ cells in your body can be an indication of severity of disease. When your CD 4+ cell count drops below a certain level (a CD4 count of 350) or you develop an opportunistic infection, you will no longer be able to take part in this study. You will be referred to the Family Clinic at King Edward where you will be treated for any opportunistic infections and be given the opportunity to participate (if eligible) in any antiretroviral drug therapy trial available. However your participation in our study, is no guarantee that you will participate in such trials. Antiretroviral therapy can help people's immune system fight HIV. The government is also setting up plans to provide treatment to those who need it. If you are referred to the King Edward Hospital Family Clinic, you will be told about the care and treatment that you will receive at the clinic by the clinic staff. You will also be able to get

counseling on how to stay as healthy as possible and how to get any services that you may be eligible for.

You will be asked to tell the study staff about any medical problems you may have during the study, especially those related to your genitals or to sexually transmitted infections. You must contact the study staff between your regularly scheduled visits if you need to report these problems. The study staff will examine you and they will refer you to a clinic for medical care, if needed.

If you are a woman and you become pregnant during this study you will be encouraged to stay in the study. The study staff will be able to refer you to available sources of medical care and other services you and your baby may need.

At each visit the study staff will update information on where you live and how to keep in contact with you. They will use this information to remind you of your scheduled visits. If you miss a visit, the study staff will try to contact you to find out why you missed a visit. They may also visit your home to try and find you. They will try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you. If you have a cellular phone, study staff may ask if they can use this number to contact you.

Your visit should not last more than three hours. You will usually receive your results at your next scheduled visit. Should the study team feel that you need to receive results sooner, you will be contacted to come to the clinic. For this reason, it is important to make sure that your contact details are up-to-date and that you have indicated how you would prefer to be contacted.

Some of the specimens collected may be stored. You will be asked to sign a separate consent form to indicate whether you agree to allow the team to store you specimens.

BENEFITS

There may be no direct benefits to participation in this study. Participants and others may benefit in the future from the information learned in this study. Specifically, information learned in this study will inform the design of future treatment and prevention interventions like microbicides and vaccine trials, and the effectiveness of HIV risk reduction and prevention messages.

However, you may benefit from the advice of the research team and/or the test results you will receive as a result of your participation in the study. For example, you will receive HIV/STI prevention education. This may help you protect yourself and others from sexually transmitted illnesses and HIV infection. If you contract a treatable STI during the course of this study, you will be referred to KEH VIII hospital for treatment. You will receive free condoms, which you can use to reduce your risk of HIV infection. You will receive information on how to use condoms properly to reduce risk.

PARTICIPANT RESPONSIBILITY

By signing this informed consent form, you are undertaking to make yourself available to attend the scheduled clinics. You are not, however, giving up your right to freely withdraw from this study at any time. If you do decide to withdraw from the study please tell the study nurse or doctor. We will encourage you to come to the clinic for a final exit interview and to receive any test results that you may not have received yet.

COMPENSATION

You will be compensated for your transport costs. Clinic visits may take up most of your day. If you have to be at the clinic for the day you will be provided with food and refreshments for the time that you are at the clinic.

RISKS/DISCOMFORT

Your participation in this study is for research purposes. Your involvement does not mean that you will receive special medicines or treatments that other people cannot get from clinics or hospitals. Although you may choose to be involved in a study of HIV/AIDS medicines, these studies are also researching the effectiveness of medicines and taking those medicines would have their own risks.

You may feel some pain or discomfort during biological sample collection. A few people feel faint or lightheaded when they see blood. You may have bruising at the site of the blood draw. There is a small chance that you may get an infection in the site of the specimen draws. The risks of collection of genital secretions (wash and swab from your vagina) are pain and discomfort.

Being HIV positive can be a very stressful experience. In addition to this, you may be treated badly by friends and family if your HIV status becomes known to others. You may find it difficult to find work should others learn of your HIV status, and you may find that members of your family or community discriminate against you.

Some genetic testing may be done on your samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

NEW FINDINGS

You will be told of any new information learned during this study that might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from the study without your consent for the following reasons:

- The study is stopped or cancelled.
- The study staff feels that staying in the study may be harmful to you.
- You are not able or willing to attend study visits or to complete the study procedures.

ALTERNATIVES TO PARTICIPATION

There may be other HIV research studies going on at the clinic or in your community that you may be eligible for. We will tell you about the other studies that we know about. There may also be other places where you can go for HIV counseling and testing. We will also tell you about those places.

COSTS OF THE STUDY

There will be no financial cost to you for participation in this study. You will be referred for treatment for all sexually transmitted infections that are detected while you take part in this study.

CONFIDENTIALITY

Research records of your participation in the study will be kept confidential and we will not give this information to anyone, unless require by law to do so.

We will make every effort to protect your privacy and confidentiality during the screening procedures. Your visit will take place in private. Only your doctor and study staff will know the outcome of your screening procedures. However it is possible that others may learn that you have been here and assume that you are at high risk for HIV infection. Because of this, others may treat you differently or discriminate against you.

Medical records that identify you by name may be inspected by the agency who are funding this study and by the research team who are responsible for keeping information for this study.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission. No publications based on this research will contain your name. Only the project and clinic co-ordinators will know both your name and your identification number. This is necessary so that the co-ordinators can ensure that you will receive the correct tests and that you are called in to the correct clinics. The co-ordinators will not release your number and name to anyone else on the research team.

RESEARCH RELATED INJURIES

It is very unlikely that you will become injured as a result of involvement in this study. However, in the case of a research related injury, you will be referred to the King Edward hospital for treatment. The cost of this treatment will be free. There is, however, no compensation provided for research related injuries. You do not give up any legal rights by signing this consent form. In the event of a research related injury, contact:

Mr. Francois van Loggerenberg (study coordinator) 072-219-3122; or
Dr. Koleka Mlisana (study director) 083-560-2036.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about your involvement in this study, or would like to know more about the study, please call either of the following:

Project Co-ordinator: Mr. Francois van Loggerenberg 031-260 4564
Principal Investigator: Professor SS Abdool Karim 031-260 2381 (Helen)

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research subject, you may call:
The Postgraduate Administration Office: 031-260 4495 (Ms Cheryl Borresen)
E-mail: postgrad@med.nu.ac.za

Or, if you have difficulties contacting the above:

Professor A Dhai, Chairman of the Ethics Committee: 031-260 4604

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree to have the screening tests, please sign your name or make your mark after reading the statements below:

- I hereby consent to the procedures as outlined in the Informed Consent document being conducted on myself.
- I acknowledge that I have been informed by the clinic staff and doctor (Dr Iriogbe) concerning the possible advantages and possible adverse effects which may result from my involvement in the abovementioned study.
- I acknowledge that I understand and accept that this study involves research and that a copy of this form has been offered to me to keep.
- I agree that the study will be conducted under the supervision of: Dr Mlisana and Professor SS Abdool Karim.

- I acknowledge that I understand the contents of this form, including all the information regarding the procedures and purpose of the study.
- I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:_____ Date:_____
Subject/Parent/Guardian

Signed:_____ Date:_____
Witness

Signed:_____ Date:_____
Informant

Signed:_____ Date:_____
Researcher

For illiterate subjects:

Mark with an 'X':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

4. Informed Consent: Specimen Storage- V2.00

Title of the Research: **VIRAL SET POINT AND CLINICAL PROGRESSION IN HIV-1 SUBTYPE C INFECTION: THE ROLE OF IMMUNOLOGICAL AND VIRAL FACTORS DURING ACUTE AND EARLY INFECTION**

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 - 2604564 (Francois van Loggerenberg)

INTRODUCTION

You have been enrolled into an HIV acute infection research study. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This consent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to sign this consent form. You will be given a copy to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

BLOOD AND BIOLOGICAL SAMPLES

At each of your clinic visits, blood and other biological samples (vaginal wash, urine) will be taken from you. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES

The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the Nelson R. Mandela School of Medicine.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

STORAGE OF SAMPLES

Your samples will be stored at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is no time limit on how long your samples may be stored.

BENEFITS

There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

RISKS

There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a study number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

CONFIDENTIALITY

The results of future tests of your samples will not go onto your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your personal information may be disclosed.

Medical records that identify you by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

PARTICIPANT RIGHTS

The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, "Withdrawal of Consent" so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, please call either of the following:

Project Co-ordinator:	Mr. Francois van Loggerenberg	031-260 4564
Principal Investigator:	Professor SS Abdool Karim	031-260 2381 (Helen)

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research subject, you may call:
The Postgraduate Administration Office: 031-260 4495 (Ms Cheryl Borresen)
E-mail: postgrad@med.nu.ac.za

Or, if you have difficulties contacting the above:

Professor A Dhai, Chairman of the Ethics Committee: 031-260 4604

SIGNATURES
Please read the statement below and think about your choice. No matter what you decide, it will not affect your care.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_____ Yes

_____ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed:_____ Date:_____
Subject/Parent/Guardian

Signed:_____ Date:_____
Witness

Signed:_____ Date:_____
Researcher

For illiterate subjects:

Mark with an 'X':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

Withdrawal of Consent

I hereby withdraw my consent for the storage of my biological samples. It is my wish that (please tick only one):

_____ Samples that have already been stored may continue to be stored and used.

_____ All samples that have already been stored must be destroyed.

Signed:_____ Date:_____
Subject/Parent/Guardian

Signed:_____ Date:_____

Witness

Signed:_____ Date:_____

Researcher

For illiterate subjects:

Mark with an 'X':_____ Date:_____

Independent Witness:_____ Date:_____

Title and Name:_____

Telephone Number:_____

CAPRISA 002 Plate 009

Visit Code ☐ . ☐☐ [0]
Phase *Visit* *Interim #*

Participant ID ☐☐ ☐☐☐☐
Site *Participant Number*

Visit Date ☐☐ ☐☐☐ ☐☐
dd *MMM* *yy*

Risk Behaviour Assessment

A. SOCIO-DEMOGRAPHIC INFORMATION

We need to collect information about who you are.

1. What is your date of birth (preferable, if available)? ☐☐ ☐☐☐ ☐☐ *dd MMM yy*

   or

   How old are you (completed years)? ☐☐ *Years*

2. Gender ☐ *Male* ☐ *Female*

3. What is your marital status?
   ☐ *Single, no partner* ☐ *Divorced*
   ☐ *Married* ☐ *Widowed*
   ☐ *Stable partner* ☐ *Many partners*
   ☐ *Separated* ☐ *Refused*

4. *Observed race?*
   ☐ *White* ☐ *Black*
   ☐ *Coloured* ☐ *Indian*
   ☐ *Other, specify:* _____

Race is recorded to assess the representation of the population in the sample.

5. Highest level of school passed? ☐☐ *Grade* ***(see notes for Grade table)***

6. Does anyone depend on you financially? ☐ *Yes* ☐ *No* ☐ *Refused*

   If yes, please indicate number

   6a. ☐☐ *Adults* 6b. ☐☐ *Children*

Date: ☐☐ ☐☐☐ ☐☐ *dd MMM yy*

Completed by: ☐☐☐

Signature: _____

CAPRISA 002 Plate 010 **Visit Code** ☐ . ☐☐ [0]
Phase Visit Interim #

Participant ID ☐☐ ☐☐☐☐ **Visit Date** ☐☐ ☐☐☐ ☐☐
Site Participant Number *dd MMM yy*

Risk Behaviour Assessment

B. SEXUAL HISTORY AND BEHAVIOURAL RISK SECTION

We would like to ask you about your life and your sexual behaviour in the recent past.

7. Age at first sexual intercourse? ☐☐ *Years old*

8. Last sexual contact? ☐☐☐ *Days ago*

9. Number of sexual partners in the last 3 months. A steady partner is one that you see most of the time, often over a period of time. A casual partner is one that you may see only occasionally or even only once *[sex worker cohort - include only those who do not pay you for sex].*

   a. Steady ☐☐☐ ☐ *Too many to remember* ☐ *Refused*

   b. Casual ☐☐☐ ☐ *Too many to remember* ☐ *Refused*

10. Total lifetime sexual partners *[sex worker cohort - include only those who do not pay you for sex].*

    a. Steady ☐☐☐ ☐ *Too many to remember* ☐ *Refused*

    b. Casual ☐☐☐ ☐ *Too many to remember* ☐ *Refused*

For all partners:

11. Have you ever had peno-vaginal sex? ☐ *Yes* ☐ *No* ☐ *Refused*
    → **go to 12.**

    If yes, on average how many times per month?

    ☐☐☐ *per month, or* ☐ *Less than once a month* ☐ *Less than once a year*
    ☐ *Only once* ☐ *Refused*

12. Have you ever had anal sex? ☐ *Yes* ☐ *No* ☐ *Refused*
    → **go to 13.**

    If yes, on average how many times per month?

    ☐☐☐ *per month, or* ☐ *Less than once a month* ☐ *Less than once a year*
    ☐ *Only once* ☐ *Refused*

Date: ☐☐ ☐☐☐ ☐☐ Completed by: ☐☐☐
dd MMM yy

Signature: _____

CAPRISA 002 Plate 011

Visit Code ☐ . ☐☐ 0
Phase Visit Interim #

Participant ID ☐☐ ☐☐☐☐ Visit Date ☐☐ ☐☐☐ ☐☐
Site Participant Number dd MMM yy

Risk Behaviour Assessment

13. How safe do you think anal sex is compared to peno-vaginal sex when it comes to HIV infections? ☐ The same risk ☐ More risk ☐ Less risk ☐ Don't know

14. Have you ever had oral sex? ☐ Yes ☐ No ☐ Refused → go to 15.

If yes, on average how many times per month?

☐☐☐ per month, or ☐ Less than once a month ☐ Less than once a year ☐ Only once ☐ Refused

15. How safe do you think oral sex is compared to peno-vaginal sex when it comes to HIV infections? ☐ The same risk ☐ More risk ☐ Less risk ☐ Don't know

16. Do you do anything to prevent yourself from falling pregnant? ☐ Yes ☐ No ☐ Refused → go to 17.

a. If yes, how do you prevent yourself from becoming pregnant?

	Yes	No	Don't know	Refused
1. Condom	☐	☐	☐	☐
2. Pill	☐	☐	☐	☐
3. IUD	☐	☐	☐	☐
4. Injections	☐	☐	☐	☐
5. Diaphragm / foam / jelly	☐	☐	☐	☐
6. Female sterilisation	☐	☐	☐	☐
7. Rhythm / Calendar method	☐	☐	☐	☐
8. Withdrawal	☐	☐	☐	☐
9. Herb / Traditional remedies	☐	☐	☐	☐
10. Quinine	☐	☐	☐	☐
11. Thigh sex	☐	☐	☐	☐
12. Anal sex	☐	☐	☐	☐
13. Other, specify _____	☐	☐	☐	☐

Date: ☐☐ ☐☐☐ ☐☐ Completed by: ☐☐☐
dd MMM yy

Signature: _____

CAPRISA 002 Plate 012

Visit Code ☐ . ☐☐ 0
Phase Visit Interim #

Participant ID ☐☐ ☐☐☐☐
Site Participant Number

Visit Date ☐☐ ☐☐☐ ☐☐
dd MMM yy

Risk Behaviour Assessment

17. Some women report that they use substances to clean their vaginas after and between sexual encounters (douching). Do you use any substance to do this? ☐ Yes ☐ No ☐ Refused → go to 18.

If yes, please describe what you use and how often:

	More than once a day	Almost every day	About once a week	Once a month
1. _____	☐	☐	☐	☐
2. _____	☐	☐	☐	☐
3. _____	☐	☐	☐	☐

18. Have you ever had sex while drunk from alcohol or taking any other drugs/substances? ☐ Yes ☐ No ☐ Don't know ☐ Refused → go to 19.

18a. If yes, how often?

☐☐☐ per month, or ☐ Less than once a month ☐ Refused

18b. How often was a condom used in this situation?
☐ Every time ☐ More than half the time
☐ Less than half the time ☐ Never
☐ Don't know ☐ Refused

19. How often can you insist on using a condom if you want to? *(sex workers - not clients)*

	Never	Occasionaly	Less than half the time	More than half the time	Every sexual act	No partners
a. With a ***steady*** partner?	☐	☐	☐	☐	☐	☐
b. With ***casual*** partners?	☐	☐	☐	☐	☐	☐

20. Was your last sexual encounter with a steady or casual partner/client? ☐ Steady ☐ Casual

a. Was a condom used? ☐ Yes ☐ No

b. Why? _____

11-AUG-04 v1.3 FINAL

Date: ☐☐ ☐☐☐ ☐☐
dd MMM yy

Completed by: ☐☐☐

Signature: _____

CAPRISA 002 Plate 013 Visit Code ☐.☐☐ 0
Phase Visit Interim #

Participant ID ☐☐ ☐☐☐☐ Visit Date ☐☐ ☐☐☐ ☐☐
Site Participant Number dd MMM yy

Risk Behaviour Assessment

C. HIV/AIDS SECTION

21. Have you ever heard about HIV/AIDS? ☐ *Yes* ☐ *No* → **End of Form**

22. What are all the ways that you think HIV/AIDS is spread ? *(tick appropriate, without prompting)*

☐ *Vaginal sex (no condom)* ☐ *Oral sex (no condom)* ☐ *Anal sex (no condom)*
☐ *Breastfeeding* ☐ *Touching infected person* ☐ *Contact with infected blood*
☐ *Using public toilets* ☐ *Mosquito bites* ☐ *From mother to child during pregnancy*
☐ *Sharing the same cup or eating utensils with someone who is infected*
☐ *Other, specify:* _____

23. What are all the ways that you think we can prevent the spread of HIV/AIDS? *(tick appropriate, without prompting)*

☐ *Having a good diet* ☐ *Using condoms* ☐ *Staying faithful to one partner*
☐ *Abstaining from sex* ☐ *Making sure all injections are done with clean needles*
☐ *Avoid sharing razors* ☐ *Other, specify:* _____

24. If you have any other sexually transmitted infection, do you think you are more likely to get HIV/AIDS than someone who does not have an infection?

☐ *Yes* ☐ *No* ☐ *Only sometimes* ☐ *Unsure*

Why? _____

25. Do you think that HIV/AIDS can be ***cured***? That is, is there something you can do to get rid of the HIV infection once you have it?

☐ *Yes* ☐ *No* ☐ *Unsure*

If yes, how? _____

_____

26. Do you think that HIV/AIDS can be ***treated***? That is, is there something you can do to keep well even though you have been infected with HIV?

☐ *Yes* ☐ *No* ☐ *Unsure*

If yes, how? _____

_____

Date: ☐☐ ☐☐☐ ☐☐ Completed by: ☐☐☐
dd MMM yy

Signature: _____

CAPRISA 002 Plate 014

Visit Code ☐ . ☐☐ 0
Phase Visit Interim #

Participant ID ☐☐ ☐☐☐☐
Site Participant Number

Visit Date ☐☐ ☐☐☐ ☐☐
dd MMM yy

Risk Behaviour Assessment Phase 1 cohort

Phase I cohort only

This section refers only to sex for compensation

1. How long have you **been** in sex work? ☐☐ *Years* ☐☐ *Months* ☐ *Refused*

2. At what age did you start sex work? ☐☐ *Years old*

3. How many days a week do you perform sex work? ☐☐ *Days*

4. At how many sites do you work over a year? ☐☐ *Sites / year*

5. On an average working day, how many clients do you see? ☐☐ *Clients*

6. In the last week, how many clients did you have? ☐☐ *Clients*

7. Per week, how many of your sessions with clients are

   a. Short sessions/jobs ☐☐ *b. Overnight stays* ☐

8. In an overnight stay, on average how many times do you have sexual contact with the client? ☐☐ *Times*

9. How many condoms were used during last week with your clients? ☐☐☐ *condoms*

10. How often was a condom used with your clients during the last ***month***?

    ☐ *Never* ☐ *Sometimes, less than half* ☐ *Often, more than half*
    ☐ *Always (100%)* ☐ *No clients last month*

11. If always, was a new condom used for every act if you had more than one sex act with the same client? ☐ *Yes* ☐ *No*

Date: ☐☐ ☐☐☐ ☐☐ Completed by: ☐☐☐
dd MMM yy

Signature: _____

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Instructions for Authors

Instructions for Authors

AIDS and Behavior

Manuscript Submission

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- Type double-spaced on one side of 8 ½ × 11-inch white paper using generous margins on all sides, (including copies of all illustrations and tables).
- A title page is to be provided and should include the title of the article, authors name (no degrees), authors affiliation, and suggested running head. The affiliation should comprise the department, institution (usually university or company), city, and state (or nation) and should be typed as a footnote to the authors name. The suggested running head should be less than 80 characters (including spaces) and should comprise the article title or an abbreviated version thereof. For office purposes, the title page should include the complete mailing address, telephone number, fax number, and email address of the one author designated to review proofs.
- With the exception of Brief Reports and Behavioral Surveillance Reports, initial submissions to AIDS and Behavior do not have word or page limits. Briefer and more succinct papers tend to review better and papers may be reduced in length as part of the review process. However, the length of the original submission is left to author discretion.
- An abstract is to be provided, preferably no longer than 150 words.
- A list of 4-5 key words is to be provided directly below the abstract. Key words should express the precise content of the manuscript, as they are used for indexing purposes.
- All sections should carry headings (such as INTRODUCTION, METHODS, RESULTS, DISCUSSION, CONCLUSIONS, etc.), typed flush left. All acknowledgments (including those for grant and financial support) should be typed in one paragraph (so-headed) on a separate page, that directly precedes the References section.
- Illustrations (photographs, drawings, diagrams, and charts) are to be numbered in one consecutive series of Arabic numerals. The captions for illustrations should be typed on a separate sheet of paper. All illustrations must be complete and final, i.e., camera-ready. Photographs should be large, glossy prints, showing high contrast. Drawings should be high quality laser prints or should be prepared with india ink. Either the original drawings or good-quality photographic prints are acceptable. Artwork for each figure should be provided on a separate sheet of paper. Identify figures on the back with authors name and number of the illustration. Electronic artwork submitted on disk should be in the TIFF or EPS format (1200 dpi for line and 300 dpi for halftones and grayscale art). Color art should be in the CYMK color space. Artwork should be on a separate disk from the text, and hard copy must accompany the disk.
- Tables should be numbered (with Roman numerals) and referred to by number in the text. Each table should be typed on a separate sheet of paper. Center the title above the table, and type explanatory footnotes (indicated by superscript lowercase letters) below the table.

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A reference number is allocated to a source in the order in which it is cited in the text. In text, identify references as Arabic numerals in brackets (1). If the source is referred to again, the same number is used. References are listed in numerical order in the Reference List at the end of the paper. Do not alphabetize. Use abbreviated names of journals according to the journal list in PubMed. List all authors and/or editors up to 6; if more than 6, list the first 3 followed by "et al." The following are examples.

1) McKirnan DJ, Vanable PA, Ostrow DG, Hope B. Expectancies of sexual "escape" and sexual risk among drug and alcohol-involved gay and bisexual men. J Subst Abuse. 2001;13(1-2):137-54.

2) van der Straten A, Cheng H, Moore, J et al. The use of the diaphragm instead of condoms in a phase III diaphragm trial. AIDS Behav. 2009; 13(3):564-72.

3) Eaton LA, Kalichman SC. Changes in transmission risk behaviors across stages of HIV disease among people living with HIV. J Assoc Nurses AIDS Care. 2009 Jan-Feb;20(1):39-49.

4) Bangsberg D, Hecht F, Charlebois E, Chesney M, Moss A. Comparing objectives measures of adherence to HIV antiretroviral therapy: electronic medication monitors and unannounced pill counts. AIDS Behav 2001, 5:275–281.

5) Richman D, Bozzette S, Morton S, et al. The prevalence of antiretroviral drug resistance in the US. Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, 2001 [abstract LB-17].

6) Hirsch MS, D'Aquila RT, Kaplan JC. Antiretroviral therapy. In: DeVita VT, Hellman S, Rosenberg SA, eds. AIDS: Biology, Diagnosis, Treatment and Prevention. 4th ed. Philadelphia, PA: Lippincott-Raven; 1997.

7) Ray SC. Simplot for Windows, version 2.5. Available at: http://www.med.jhu.edu/deptmed/sray/download/. Accessed November 7, 2001.

Verify that every instance of a number in text corresponds to the numbered reference.

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