Risk Factors Associated With HSV-2 Sero-prevalence and, the Level of Symptom Recognition among Women in Inner City Johannesburg – Implications for Public Health Interventions

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A **CORRECTED** RESEARCH REPORT SUBMITTED TO THE FACULTY OF HEALTH SCIENCES, UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PUBLIC HEALTH (MPH)

JOHANNESBURG, 2009

Declaration

I, Nonkululeko Z Mlaba, declare that this research report is my own work. It is being submitted for the degree of Master of Public Health of the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Dedication

This research report is dedicated to my husband Viwe and our children; I am grateful for your support.

Abstract

Background: Herpes Simplex Virus type 2 (HSV-2) is a common cause of genital ulcers worldwide and has emerged as a co-factor in human immunodeficiency virus (HIV) acquisition and transmission. A study was conducted to determine the prevalence of HSV-2, its correlates, the accuracy of reported history of genital ulcer disease (GUD) to predict HSV-2 infection and the extent of symptom recognition in a clinic population in Johannesburg.

Methods: 210 women aged 18 years or older were interviewed and socio-demographic, sexual behaviour and clinical information collected. Serological testing for HSV-2 and HIV infections was performed, but only where sera were available for the latter. Factors associations with HSV-2 infection were assessed using logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). The sensitivity, specificity, predictive values and likelihood ratios of a history of GUD were calculated.

Results: The estimated sero prevalence of HSV-2 was 73% (95% CI 67% - 79%). Few participants, 13/206 (6%) participants had knowledge of genital herpes. Only 9/203 (4%) participants recognised lesions of genital herpes following education and counselling about HSV-2 infection. HSV-2 infection was associated with older age(>25 years of age) OR 2.6 (95% CI 1.4-5.0), spending more than 2 nights away from home, OR 6.0 (95% CI 1.0-62.7), having more than 2 sexual lifetime partners, OR 2.2 (95% CI 1.1-3.9), a history of an STI in the past 3 months ,OR 3.6 (95% CI 1.2-9.5) and HIV infection, OR3.3(95%CI 1.4-7.9). A history of genital ulceration

performed poorly as a predictor of HSV-2 seropositivity; the sensitivity was 7% and specificity was 96%.

Conclusion: HSV-2 prevalence was high and few participants were aware of their infection. HVS-2 infection was associated with risky sexual behaviour .A history of genital ulcer disease was not sufficient as a diagnostic tool for HSV-2 infection. Public health interventions should focus on behavioural modification and increasing awareness of genital herpes. HSV-2 management should be incorporated into HIV care and STI protocols.

Acknowledgement

My sincere gratitude goes to my supervisor, Dr Sinead Delany for her intellectual input and guiding me from the inception of this project right through to the final report.I would like to thank my colleagues Adam Readhead and Godspower Akpomiemie for their valuable input and lessons given on analysis.

Finally, my heartfelt appreciation goes to the Reproductive Health and HIV Research Unit (RHRU) for granting me the permission to use data for this report.

This report has been partially presented at the 2007 South African AIDS Conference as a poster presentation.

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Acronyms and Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BOM	Burning on micturition
GUD	Genital Ulcer Disease
HSV	Herpes Simplex Virus
HSV-1	Herpes Simplex Virus Type 1
HSV-2	Herpes Simplex Virus Type 2
HIV	Human Immunodeficiency Virus
LAP	Lower abdominal pain
NHANES	National Health and Nutrition Examination Survey
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
STIs	Sexually Transmitted Infections
RCT	Randomised controlled trial
RHRU	Reproductive Health and HIV Research Unit
VCT	Voluntary counseling and testing
WHO	World Health Organization

Chapter One Study Background

1.1 Introduction

The sub -Saharan region remains one of the regions hardest hit by the HIV/AIDS epidemic in the world. It is estimated that it contributes 68% (22.5 million) of the people living with HIV and women in particular bear the burden of the disease. [1] South Africa has a much higher HIV /AIDS prevalence in the sub-Saharan region, estimated to be 18.8% compared to 6.1% for the region.[2] The epidemic is fuelled in part by sexually transmitted infections (STIs).[2] Prompt and effective STI case management is one of the cornerstones of STI and HIV control and prevention strategy. Population-based trials assessing the impact of STI treatment on HIV incidence were conducted in Uganda and Tanzania and showed differential outcomes in the two countries. In Mwanza, Tanzania, improved syndromic STI case management (treatment according to presenting clinical STI syndromes such as vaginal discharge) at primary care clinics reduced the HIV incidence by 38% (95%CI 15%-55%).[3] In contrast, trials of mass STI treatment in Rakai, Uganda (at least 3 rounds STI treatment for the community) and of a behavioural intervention in conjunction with STI syndromic treatment in Masaka, Uganda showed no effect.[4,5] An attempt to understand the contrasting results revealed that differences between the study populations (baseline sexual risky behaviour, STI rates and the stage of the HIV epidemic) accounted for the discrepancies.[6] Shorter duration STIs that are amenable to treatment were less prevalent in Rakai and Masaka than in Mwanza whereas, longer duration STIs which are such as Herpes Simples Virus type-2 (HSV-2) were similar across 3 sites. Unpublished data also suggested that most symptomatic ulcers in Mwanza were due to chancroid whilst HSV-2 was the commonest cause of genital ulceration in Rakai and Masaka.[6, 7] The researchers concluded that in areas of early, nascent HIV epidemics such as observed in

Mwanza, curable STIs play a greater role in HIV transmission. By contrast, incurable STIs such as HSV-2 have a larger impact on HIV spread. HSV-2 has emerged as the most prevalent STI pathogen and the most common cause of genital ulcer disease worldwide in the past decade.[8,9,10] Many studies show that HSV-2 infection, even without clinically recognised lesions is a risk factor for HIV infection.[8,9] Modelling studies have estimated that more than a third of all new HIV cases are attributable to HSV-2 infection in sub-Saharan Africa.[11, 12]

In the developed world, the prevalence is higher in the US than Europe; representing 17%-24% of the adult population according to the NHANES III survey in the United States, and in different European countries ranges from 0.1%-11%.[13] The prevalence of HSV-2 in the adult general population in Sub-Saharan Africa ranges from 30%-80% among women and 10%-50% among men.[8] HSV-2 is transmitted sexually, primarily from partners with asymptomatic or unrecognized infection resulting in the epidemic proportions seen worldwide.[14] Recently, another study demonstrated that over half of HSV reactivations last for 12 hours or less and that these short reactivations are usually asymptomatic and associated with rapid appearance and clearance of virus in immunocompetent hosts.[15] At such times, asymptomatic persons shedding virus could unknowingly transmit HSV-2 to their sexual partners.

HSV-2 infection manifests clinically as genital ulcers, a known risk factor for HIV infection. The past decade has seen a shift in the epidemiology of genital ulcer disease in Africa; in the early 1990s, data published identified syphilis and chancroid as the commonest cause of GUD whereas in the past 10 years, studies have been reporting on HSV-2 as the main cause of GUD.[16] In South Africa, the proportion of ulcers due to HSV-2 increased from 3% in 1989 to 17% in 1994 and 36% in 1998.[17] A sexually transmitted infections microbiological surveillance conducted between November 2006 and February

2007 in the Western Cape and Gauteng reported that genital herpes accounted for the majority of ulcers where an infectious aetiology was established; 71% in Cape Town; 87% in Johannesburg.[18] Similarly, neighbouring South African countries such Botswana have also reported similar trends where 24% of GUD among patients with STIs were due to HSV-2 in 1993 increasing to 60% in 2002.[10] In the developed countries such as Europe, the shift in the epidemiology of genital ulcerations has seen an emergence of HSV-1 as a more common aetiological pathogen of primary genital herpes than HSV-2.[19] This observation has been attributed to the decline in the seroprevalence of HSV-1 in the developed world rendering adults without the immunity of HSV-1 who practice oral sex more at risk of primary genital HSV-1 infection. For this reason, it is recommended that both HSV-1 and HSV-2 specific testing must be performed in populations at risk of HSV-1 infection. There have been arguments that this epidemiological shift is due to increased diagnosis of HSV-2 with the sensitive assays such as polymerase chain reaction (PCR), the widespread use of antibiotics to control STIs resulting in low GUD prevalence and the spread of HIV.

The presence of genital ulcers enhances both the acquisition and the transmission of HIV. Paz-Bailey et al reported that several cohort studies have demonstrated that GUD is associated with a 7-11 fold increase in risk of HIV acquisition.[10] HIV acquisition is highest among those with recent or incident HSV-2 infection which may reflect the higher severity of herpetic ulcers with primary HSV-2 infection and frequency of reactivation in the first year of HSV acquisition. In a study conducted in Rakai, Uganda; Gray et al studied the HIV transmission rate per sex act and found that the two most important risk factors for HIV transmission were the presence of genital ulcers and a higher HIV plasma load.[20] HIV transmission was increased four fold (0.0041 per sex act for those with genital ulcers versus 0.0011 for those without genital ulceration; p=0.002) in the presence of GUD. Even

in the absence of genital ulcer disease, epidemiologic evidence suggests that HIV and HSV-2 manifest synergistic bidirectional interactions. Results from randomised controlled trials of herpes suppressive therapy on HIV positive individuals have demonstrated reductions in the genital and plasma levels of HIV over a 3 month period, suggesting that HSV-2 may play a role in HIV transmission.[21,22] In addition, HIV alters the clinical presentation of HSV-2 infections in HIV and HSV-2 co-infected persons with low CD4 counts resulting in extensive and slow healing ulcers and lead to high rates of HIV and HSV-2 genital shedding.

Vertical transmission of HSV infection in pregnancy is also associated with a rare but serious neonatal morbidity and mortality due to neonatal herpes.[23] Clinical HSV infection in neonates presents with primary vireamia that can lead to fulminate liver disease and death. Visible lesions are observed in a minority of disseminated neonatal herpes. The likelihood of vertical transmission is higher in primary HSV infection in the mother. Contact with infected genital secretions at delivery and intrauterine infections account for only 5-8% of all transmissions.[24] Data on the incidence or the prevalence of neonatal herpes in South Africa is limited. A study of mothers and babies with unfavourable outcomes performed in the Department of Obstetric and Gynaecology at Ga-Rankuwa Hospital, Pretoria in 1999, reported that infection with cytomegalovirus was found to be the most prevalent infection compared to HSV-2 infection and the latter played a less significant role.[25]

The control of HSV-2 appears to be an important part of HIV prevention strategy, but the best way to achieve it is still elusive to researchers. Multicentre clinical trials of episodic and suppressive antiherpes therapy for HIV prevention have been undertaken in Africa and the USA. The results have been disappointing for the impact on HIV acquisition. The trials assessing the impact of suppressive antiherpes on HIV transmission are still ongoing and

the results are much anticipated. The aim of this study is to determine predictors of HSV-2 infection using data collected as part of a study validating the performance of two HSV-2 serology assays in a population in the inner city Johannesburg. The validation study was part of formative research in preparation for at least two of the suppressive antiherpes therapy randomised controlled clinical trials mentioned above. The correlates of HSV-2 will provide valuable information to help identify individuals to be targeted should the intervention prove useful.

1.2 Literature Review

1.2.1 HSV-2 Seroprevalence

HSV-2 infection is higher in developing countries compared to the developed countries, and within countries and populations is higher among high risk individuals.[8, 26] In northwest Tanzania, women working in bars and facilities close to truck stops had a HSV-2 prevalence rate of 80%.[27] Another study conducted in Moshi, Tanzania among women at high risk reported an HSV-2 prevalence of 56,9%.[28] The HSV-2 prevalence rate among commercial sex workers in Durban, South Africa was 84%.[29] A population- based study conducted among the youth in Carltonville, South Africa reported a HSV-2 prevalence rate of 53,3% in females and 17% among males peaking at around 89% and 40% for females and males aged 22-24 years.[30] A community-based randomised controlled trial assessing a behavioural intervention among youth aged 15-24 in rural Eastern Cape province in South Africa reported a baseline prevalence of 27.6% and 31.0% in the intervention and control arms respectively among women and 10.3% and 10.0% among men.[31]

1.2.2 Contributors to the spread of HSV-2 infection

The prevalence of HSV-2 infection is increased and sustained by unrecognized and/or atypical and asymptomatic infections, which in turn lead to under diagnosis and asymptomatic shedding of the virus. Sero-epidemiologic studies often reveal a mismatch between the HSV-2 antibody prevalence and recognition of clinical symptoms. Only 10% to 25% of individuals seropositive for HSV-2 report a history of genital herpes.[14] Only 2% of Tanzanian women reported a history of genital herpes despite a prevalence rate of 39% in a cross-sectional study conducted by Msuya et al.[32] Clinically apparent ulcers were rare amongst a cohort of Kenyan female sex workers despite an HSV-2 prevalence of 77.4% .[33] However, when individuals with previously unrecognized HSV-2 infection are educated about the clinical manifestations of genital herpes, about 60% come to recognize typical symptoms.[34] Wald et al reported that 26/42 (62%) female participants and 7/11 (64%) male participants who had previously denied a history of genital herpes, reported experiencing typical ulcers, blisters and crusts in the genital area following an educational session on genital herpes that included viewing photographs.[14] It is worth noting however that the numbers in this study were small and therefore the results should be interpreted with caution.

The other reasons genital herpes infection goes unrecognized and under diagnosed is that the lesions may be located in difficult to locate anatomical site for the attending health care provider especially in females and/ or the presentation may be subtle and non specific presenting with dysuria, vaginal discharge and vulval irritation and fissures suggestive of other conditions such as candidiasis.[8, 14] Individuals could still unknowingly transmit HSV-2 virus.

Sub-clinical or asymptomatic shedding refers to the detection of HSV on genital mucosa on days without lesions consistent with genital herpes. Individuals with symptomatic genital herpes have both clinical and asymptomatic reactivations. It is thought that viral shedding in individuals who are unaware that they are infected with HSV-2 is responsible for at least 70% of HSV transmission.[14] The rate of asymptomatic genital herpes shedding is dependent on the virus type, duration of genital herpes infection and co-infection with HIV. Shedding rates are higher with HSV-2 compared to HSV-1 infection. In immunocompetent persons the rate of asymptomatic shedding appears to decrease over time but does persist for life just as symptomatic reactivation of the virus. The likelihood of asymptomatic shedding of the virus is more common in HIV positive individuals than HIV negative individuals and correlates with plasma CD4 level. In a cross sectional study of HIV positive women, the occurrence of HSV-2 shedding was four fold more common among women co-infected with HSV-2 and HIV compared with HIV negative women (OR4.1, 95% CI 1.0-27.4). The rate of total shedding also increased as the CD4+ cell count declined (p=0.025).[35]

1.2.3 Risk factors for HSV-2 infection

Epidemiologic studies in both the developing and developed countries have consistently identified correlates of HSV-2 that can be attributed to frequent sexual activity and cumulative exposure. However published data on risk factors for HSV-2 infection is limited in South Africa. These risk factors can be categorized as socio-demographic [older age, female gender, low education status, socio-economic status (low income among females and high income among men)], behavioural (number and choice of sexual partners, duration of relationships, sexual frequency, knowledge of partner's serostatus and condom use).

Socio-demographic factors

Most epidemiological studies have demonstrated that HSV-2 infection increases with age, increasing from negligible levels in children younger than 12 years to as high as 80% in sexually active years thereafter leveling off after 40 years of age (see table 1). This trend of increasing HSV-2 infection with age is due to the cumulative increase in the number of sexual partners and the chronic nature of HSV-2 infection. HSV-2 has also been consistently found to be higher in women than in men. The reason for this difference can be explained by biological factors such as the large female genital mucosal surface area that increases the risk of acquisition and the tendency for young women to choose sexual partners who are older than them.

Education, socio-economic status and religion have commonly not been associated with HSV-2. However, a study conducted in Tanzania reported a high prevalence of HSV-2 infection among men with low level of education but not in women.[36] Low level of education represents lack of access to information and resources to help control STIs including HIV resulting in riskier sexual behaviour.

Behavioural factors

HSV-2 is sexually acquired and acquisition indicates sexual activity. A higher number of lifetime sexual partners have consistently been associated with HSV-2 infection in both the developing and developing countries (see table 1), which is consistent with most STIs and indicates that the risk of HSV-2 increases with every sexual exposure. In a cross-sectional study conducted between Brazilian and Filipino women participating in a cervical cancer trial, HSV-2 prevalence was higher among Brazilian women compared to Filipino women and was largely explained by the differences in sexual behaviour of women and their husbands. Brazilian women who reported 2 lifetime sexual partners and a husband who

occasionally had other sexual partners had a higher risk of HSV-2 infection [OR 2.7 (1.0-7.20)] and [OR 4.8(2.0-11.4)] respectively.[37]

However, other studies have shown an association between HSV-2 and a lower number of reported lifetime sexual partners (see table 1). This variation is likely due to dynamics in sexual mixing patterns, age at sexual debut, condom use and the presence of other STIs in different population groups. It is also probable that in a background of high HSV-2 seroprevalence in the community, the individual risk behaviour is not a risk factor given that the likelihood of having an infected partner is high even among those with fewer partners. The earlier age of sexual debut is a marker of a longer period of sexual activity and therefore exposure to HSV-2. Similarly, factors such as duration of sexual relationship, increased sexual frequency and being in a HSV-2 serodiscordant relationship, all of which are markers of sexual activity and exposure are associated with HSV-2 infection. Consistent and correct condom use was found to be protective in susceptible women in HSV-2 sero-incident study. In an ineffective candidate vaccine study of HSV-2 among HSV-2 serodiscordant couples, use of condoms for more than 25% of sex acts was protective for women (adjusted hazards ratios (HR), 0.085; 95%CI 0.011-0.67).[38] Another sero-incident study among monogamous heterosexual couples on suppressive antiviral therapy showed that there was a beneficial effect of condom use irrespective of the level of condom usage.[39] In this study, the couples who used condoms for 1%-90% of sex acts and those who used them for more than 90% of the time had lower HSV-2 transmission rates.

Sexually transmitted infections and HIV

The association between HSV-2 infection and a history of STIs is a marker of risky sexual behaviour and reflects the risk of acquiring multiple STIs during high-risk exposure. In a

cross sectional study conducted among urban Tanzanian women aged between 15-49 years, a history of syphilis was associated with a higher prevalence of HSV-2 infection [OR 4.7 95%CI (1.4-4.7)].[32] Since both STIs cause genital ulcers, it is biologically plausible that syphillis predisposes to genital herpes and vice versa; however temporality is a limitation of cross-sectional studies and in the above study it was impossible to ascertain which infection was antecedent. Kaul et al were able to establish temporality between HSV-2 infection and other STIs; they demonstrated that prevalent HSV-2 increased the risk of acquisition of other STIs in a study conducted among female sex workers in Kenya. HSV-2 infection increased the risk of Nesseria Gonorrheoa (NG) and syphillis acquisition four fold, [Incident risk ratio (IRR) 4.3 95%CI (1.5-12.2)] and [IRR 4.7 95%CI (1.1-19.90] and of Trichomona Vaginalis (TV) two fold, IRR 2.3 95%CI 1.3-4. 20].[33] They postulated that the immunological changes in the genital tract associated with HSV-2 trigger a cascade of pro-inflammatory cells that increase the susceptibility to other STIs particularly NG. Both HIV and HSV-2 infections fuel one another by increasing transmissibility and susceptibility.[46] [see figure 1.1below]. One of the biological mechanisms by which HSV-2 increases the risk of HIV acquisition is through genital ulceration (macro and micro-ulceration) caused by HSV-2 that creates a portal of entry for HIV.

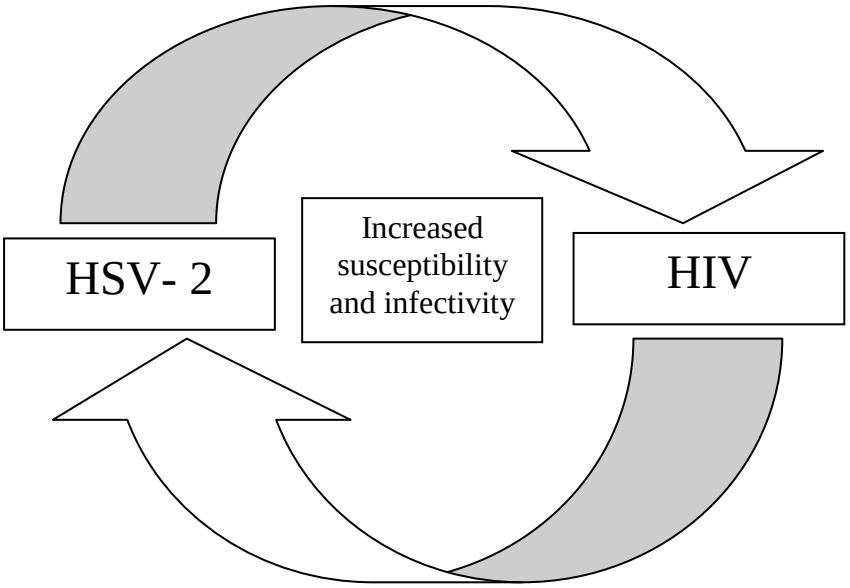

Figure 1.1 HSV-2 and HIV interaction (*Courtesy of Delany*)

HSV also increases HIV susceptibility in the absence of genital ulcer disease. Recent studies show that HSV-2 infection -induced genital inflammation increases the number of HIV susceptible target cells in the genital tract mucosa, (CD4 T cells expressing HIV co-receptor CCR-5 and the cervical immature dendritic cells iDCs expressing co-receptor DC-SIGN) even in the absence of genital ulceration or HSV-2 reactivation.[47] Furthermore, the risk of HIV acquisition is greater with recent (incident) HSV-2 infection than with prevalent HSV-2 infection.[20, 40, 48] (see figure 2.2 below). The higher risk of HIV acquisition with incident HSV-2 infection is partly due to the severity of primary genital herpes infection and more frequent recurrences in the first year of life compared to prevalent HSV-2 infection. A study was conducted in India in a cohort of 2732 HIV negative participant who were mostly men and had an STI (males) or whose partners had an STI (female) in order to assess the impact of prevalent and incident HSV-2 infection on the acquisition of HIV. Incident HSV-2 cases were categorized as either recent or remote HSV-2 infection. Recent incident infection was defined as HSV-2 acquired in the past 6 months following a negative HSV-2 serology test and remote HSV-2 infection as HSV-2

infection acquired after study entry but with more than 6 months since a negative HSV-2 serology test. The adjusted hazards ratios increased with relative timing of HSV-2 infection from 1.67 (95%CI 1.22-2.30) for prevalent HSV-2 infection to 1.92 (95%CI 1.15-3.21) for remote incident infection and 3.81(95%CI 1.81-8.03) for recent incident infection. [48]

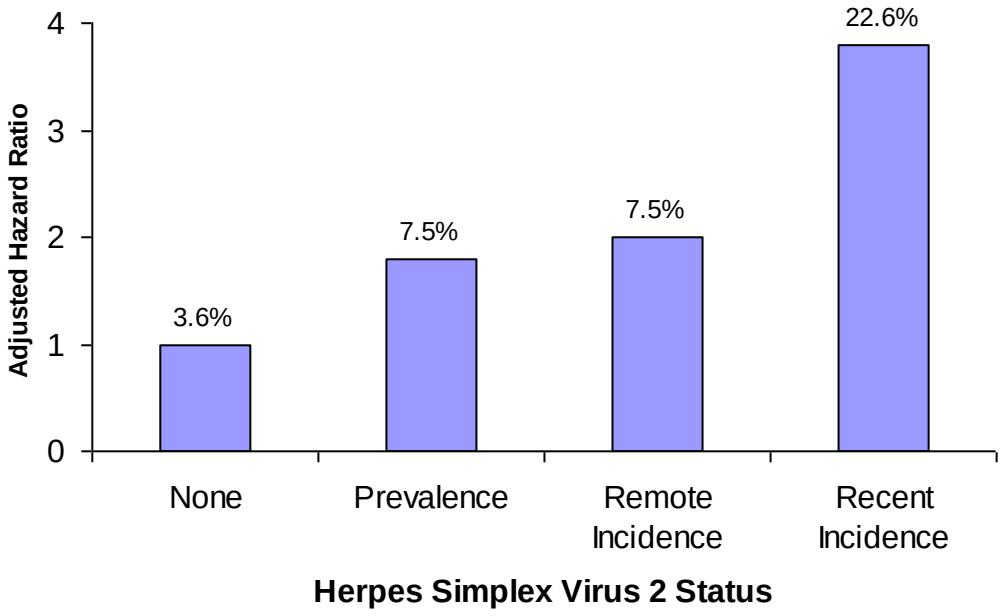

Figure 1.2 Risk of acquiring HIV by HSV-2 infection status (*courtesy of Reynolds et al J Infect Dis, 2003)*

A study investigating the impact of prevalent and incident HSV-2 infection on HIV incidence was conducted in Durban, South Africa amongst a cohort of 416 female sex workers. When HSV-2 was analysed as a time dependent covariate, the risk of HIV acquisition was greater immediately after HSV-2 seroconversion, HR 6.0 (95%CI 2.6-14.0), compared to those who remained HSV-2 positive throughout the study. However, the data did not provide conclusive evidence for the differences between HIV hazard ratios for prevalent and incident HSV-2 infections, i.e. the temporal sequence of events could not

be established. [29] The authors concluded that while prevalent and incident HSV-2 infection has been shown to increase the incidence of HIV in men, the evidence is less convincing for women.[29]

The mechanism by which HSV-2 increases the risk HIV transmission is thought to be due to clinical episodes of genital herpes, which create a direct portal exit for HIV. HIV and HSV can co-infect lymphocytes and regulatory proteins resulting in up regulation of HIV replication, which in turn increases the shedding of both HSV-2 and HIV and increases infectiousness.[46] Ex vivo studies aiming to elucidate the mucosal immune interactions of HIV and HSV-2 in the genital female tract established that HIV-1 infection was associated with the depletion of cervical iDCs in persons co-infected with HSV-2 and HIV which was independent of the HIV disease stage.[47] The cervical iDCs are an important local immune control of HSV-2 infection. This depletion was associated with an increase in HSV-2 genital tract reactivation which in turn was associated with high HIV shedding levels.

HIV changes the natural history of HSV-2 infection.[46] Persistent anogenital lesions were among the first opportunistic infections described among those with AIDS. The natural history of HSV-2 infection will be explored further below.

1.2.4 Natural History of Genital Herpes

Herpes simplex viruses (HSVs) are enveloped, linear, double stranded DNA viruses whose only hosts are humans. There are two types of the virus; type 1 (HSV-1) normally acquired in childhood and adolescence and type 2 (HSV-2) which is sexually transmitted, but either type can infect a person anywhere on the skin. Herpes simplex viruses are transmitted via close contact with infected bodily fluids such as saliva, genital fluids or fluid from active lesions. Exposure of the virus at mucosal surfaces or abraded skin facilitates entry and replication of the virus. They establish latency in sensory ganglia following initial

acquisition causing an infection that persists for life. Activation of the viral genome may occur resulting in viral replication and redevelopment of herpetic lesions in some instances, a process called reactivation. Most genital herpes is caused by HSV-2 although genital infections with HSV-1 are increasingly recognized; half of new cases in the developed countries are due to HSV-1.[19] The primary stage of genital herpes infection is characterized by severe ulceration often presenting as multiple painful ulcers.

Not all cases of newly acquired HSV-2 infection present with clinically apparent lesions. In a study of a HSV-2 candidate vaccine conducted in the USA, of the 155 cases of newly acquired HSV-2 infection, only 57(37%) were symptomatic and the rest 98 (63%) were asymptomatic.[49] Eighty five of the 98 asymptomatic cases were followed up for 45 days and 13 of the individuals (15%) had clinical reactivation of the disease. Thirteen percent of those that were symptomatic and 30% of cases with reactivated genital herpes presented with atypical symptoms that were not immediately suggestive of genital herpes. In this cohort, previous HSV-1 increased the frequency asymptomatic HSV-2 seroconversion but did not reduce the frequency of acquisition of HSV-2 infection. It is likely that the presence of HSV-1 antibodies mask the symptoms of HSV-2 infection resulting in poor recognition of infection. This observation is probably due to the fact that antibodies for one type of HSV seem to provide partial protection against infection with other serotype. The recurrence of ulcers is frequent in the first year following the acquisition of HSV infection and may be milder than the initial episode and may become less frequent over time.

The clinical manifestation of genital herpes often does not follow the described typical presentation of a prodromal illness that precedes the development of vesicles and later ulcers. Atypical presentations are common, vesicles may not form and instead fissures, cracks and crusts manifest. Clinical diagnosis of genital herpes is neither sensitive nor specific even for the most experienced clinician, leaving many patients unaware of their

affliction.[50, 51] In the candidate vaccine study mentioned above, only 39% of new genital herpes were accurately diagnosed on clinical evidence by experienced physicians. A further 20% were inaccurately diagnosed as genital herpes in people who did not have genital herpes.

1.2.5 Laboratory diagnosis of HSV infection

Because clinical diagnosis is often inaccurate, it is important to have HSV-2 diagnostic tests that are highly sensitive and specific. Viral culture is widely available and has been the gold standard for the diagnosis of HSV infection in patients who present with ulcers or other mucocutaneous lesions. Viral culture is highly sensitive for differentiating between HSV-1 and HSV-2 but insensitive for the detection of HSV from genital ulcers. Isolation of the virus is easier in the vesicular or early ulcerative stages but sensitivity declines rapidly as the lesions begin to heal. Polymerase chain reaction (PCR) testing for HSV DNA has greater sensitivity and relative affordability when compared to HSV culture and has overtaken as test of choice.[52] It has a sensitivity of >95% compared with 75% for culture. However the limitation for both tests is that they only focus on those with lesions, which is only the tip of the iceberg in genital herpes infection.

Sero-epidemiologic studies in the past were hampered by the lack of accurate and easy to use tests for the detection of antibodies against HSV-2 in different populations. Type – specific serologic tests based on glycoprotein G antibodies are the tests of choice to establish HSV infection when no lesions are present, but are still inaccessible in developing countries due to high cost. These tests have been evaluated in developed countries and their sensitivity against Western Blot ranged from 93%-100%, and specificity ranged from 95%-100%. [10] There have been concerns with the performance of some serologic tests in African sera due to observed low specificity,[53, 54] and therefore, these tests require validation in other populations. Universal screening for HSV-1 and

HSV-2 in the general population is not indicated. The challenges to serologic testing is that the tests do not indicate when the infection was acquired, nor does it confirm the diagnosis of a lesion as due to genital herpes; other aetiologies for GUD such as syphilis and chancroid should still be excluded. The other limitations in performing both the HSV PCR and serology tests for routine management of patients with genital herpes infection in resource poor countries is costs and the technical expertise required for these assays. PCR requires sophisticated lab equipment, high level of technical expertise and strict quality control.

The potential benefits for testing is that newly infected HSV-2 persons would be counseled about the increased risk of HIV acquisition. Routine screening for HSV-2 in populations with high HSV-2 and HIV infections such as observed in developing countries is also hindered by the cost and poor evidence supporting the use of anti-herpetic as a strategy for HIV prevention.

1.2.6 Intervention Options

Prevention of HSV-2 infection is of public health importance in the developing countries given that HSV-2 and other STIs including HIV infections share similar risk factors such as age, mobility, early age of sexual debut and high number of sexual lifetime partners and that HSV-2 is a cofactor in HIV transmission and acquisition.

Primary prevention

Ideally an effective vaccine would be the primary preventive method of choice to reduce the acquisition of genital herpes or the severity of HSV disease at an individual level, and potentially the reduction of the burden of both HSV and HIV infections at a public health level. Currently there is no effective vaccine. Three candidate vaccines have been investigated and none were effective. One of the candidates, an HSV-2 glycoprotein D

vaccine based on alum morpholine as adjunct showed efficacy of 40-60% for clinical disease and HSV-2 transmission only in women who were HSV-1 and HSV-2 seronegative at the beginning of the study, but the original study was not powered to show this effect.[55] Microbicides which prevent STIs *in vitro* such as PRO 2000 are another option and would ideally offer women a controlled method of preventing HIV and STIs, including HSV-2 infection. These products are however still under development.

Behavioural counselling is the only available option and focuses on the modifiable risk factors identified in literature review above. Given that HSV-2 infection is a marker of sexual activity, educational interventions should target young persons especially women and promote behavioural changes who bear the brunt of the disease. Risk reduction messages should include delaying sexual initiation, condom use, reducing concurrent partnerships, knowing your partner's HSV-2 status and promotion of health seeking behaviour for sexual advice and STI symptoms. Interventions aimed at behaviour change and ultimately the prevention of HIV have been studied both in Africa and developed countries and demonstrated a reduction in the incidence of HSV-2. A cluster randomised controlled trial which assessed the impact of a behavioural intervention (Stepping Stones) on the incidence of HIV, HSV-2 and sexual practices in a community in the Eastern Cape, South Africa found that the intervention had an impact HSV-2, reducing the incident by 33% and perpetration of intimate partner violence by males but not on HIV incidence or behaviour in women.[31] The explanation offered by the authors is that younger women who tend to have much older sexual partners who have a higher HIV prevalence, lack the ability to change their sexual behaviour in the context of unequal gender power relations. Intense education campaigns on HIV prevention which targeted young homosexual men in Amsterdam observed a strong decline in herpes simplex virus antibodies.[56] This observation was attributed to a change in sexual behaviour. Education campaigns should

also aim to increase awareness among the public and health care providers which will in turn increase symptom recognition and reduce transmission of HSV infection.

Secondary prevention

Anti-viral therapy available for the treatment of genital herpes offers clinical benefit but does not cure the condition. There are two ways in which antiviral therapy can be given to treat HSV infections. Episodic therapy is effective when given at the onset of clinically apparent disease or during the prodromal phase. It may abort the genital herpes outbreak if given during a prodrome or decrease the duration, associated pain and shedding of the virus of a clinical episode of genital herpes. Long-term suppressive therapy reduces clinical recurrences by 70% to 80% in patients with frequent outbreaks (i.e. more than six recurrences per year), and by up to 90% in clinical and subclinical shedding of the HSV. [414] According to mathematical modeling by Blower et al [57], in order prevent transmission of HSV infection; chronic suppressive therapy is likely to be more beneficial than episodic treatment because it reduces both the symptomatic and asymptomatic infectious episodes of HSV infection. The authors suggest that concentrating on identifying and treating persons with high frequency HSV-2 reactivation, the 'virologic core groups', could reduce HSV-2. Core groups are infected individuals with a high frequency of transmission due to high rates of viral reactivation and genital shedding of the virus. Examples of virologic core groups are individuals with newly acquired HSV infection, such as sexually active young adults and women who acquire primary HSV infection in pregnancy; and individuals co-infected with HSV-2 and HIV infection.

Table 1.0 Summarized Studies of Risk Factors for HSV-2 Infection

Author (Year)	Study design, Sample size & Population (Country)	HSV-2 Prevalence (P) /Incidence (I)	Test method	Risk factor (Measure of Effect p-value/OR/RR with 95%CI)	Comments
Ramjee, G (2005)	A cohort of 416 Female Sex Workers (South Africa)	P = 84.0% I = 35.0%/year	Gull ELISA	i. Increasing age (P=0.001) ii. Syphilis (P = 0.004) iii. C. trachomatis (P = 0.059)	1. Primary outcome was HIV incidence 2. Education levels were reasonably high and not associated with HVS-2 3. Condom use was low and also not associated with HVS-2
Auvert, B (2001)	Population-based study of 718 males and 771 females (South Africa)	P ;M = 17.0% P;F = 53.3%	Type-specific Elisa IgG (MRL Diagnostics	i. Increasing age ii. number of lifetime partners	1. Data gathered as part of seroepidemiological study of HIV infection 2. Measure of effect not reported
Tassiopulous, K (2006)	Cross-sectional study of 1050 females (Tanzania)	P =56.3%	Type-specific Elisa IgG (Focus Technologies)	Similar to risk factors reported in the literature	1. Author only reported that RFs were similar to those reported in elsewhere in literature 2. Measure of effect not reported
	A cohort of 360 females	14.2/100 person years	Type-specific Elisa IgG (Focus Technologies)	i. HIV infection [3.36 (1.44-7.87)] ii. Age of sexual debut [6.00 (1.39-25.9)] iii. Alcohol consumption [1.30 (1.01-1.68)] iv. Partner with other sexual partners during follow up [4.91 (1.39-17.31)] v. Ethnic group (Besides Chagga and Pare) [2.34 (1.16-4.70)]	1. Used 1.1 index cut-off value with lower sensitivity equal to 2. overestimation of HSV-2 incidence 3. Condom use not protective 4. Ethnicity reflects sexual behaviour
Ketjland EF et al (2005)	Cross-sectional study of 527 women aged 20-49years (Zimbabwe)	P = 64.5%	Type-specific Elisa IgG Focus Technologies)	i. Non-syphilitic GUD [OR = 9.7 (1.08-22.34)] ii. HIV [OR = 2.92 (1.85-4.65)]	1. H Ducreyi was not isolated in GUDs.

Author (Year)	Study design, Sample size & Population (Country)	HSV-2 Prevalence (P) /Incidence (I)	Test method	Risk factor (Measure of Effect p-value/OR/RR with 95%CI)	Comments
Brown, J et al (2007)	A prospective cohort of 2235 females (Uganda)	P = 51.5%	Type-specific Elisa IgG(Focus Technologies)	i. Being older (P<0.01) ii. Less educated (P<0.01) iii. Married (P<0.01) iv. Past history of genital ulcers (P<0.01)	1. Baseline data
		I = 9.6/100 person years	Type-specific Elisa IgG(Focus Technologies)	i. Less educated (P<0.01) ii. Polygamous marriage (P<0.01) iii. Multiple sexual partners/older partner prior to HSV-2 acquisition (P<0.01) iv. Having circumcised primary partner protective (P=0.04) v. Pregnant (P=0.01) vi. Gonorrhea and Candida infections (P<0.01)	1. Women were more likely to be infected with other STIs at the time of HSV-2 acquisition 2. Male circumcision protection should be explored in more studies
Brown, J et al (2007)	A prospective cohort of 2296 females (Zimbabwe)	P = 53.2%	Type-specific Elisa IgG (Focus Technologies)	i. Being older (P<0.01) ii. Less educated (P<0.01) iii. More lifetime partners (P<0.01) iv. Past history of genital ulcers (P<0.01)	1. The outcome was HIV incidence 2. Partner's HIV and HSV serostatus was not established which could result in confounding.
		8.8/100 person years	Type-specific Elisa IgG (Focus Technologies)	i. Unmarried (P<0.01) ii. Transactional sex (P=0.05) iii. Report GUD (P<0.01) iv. Cervical infections/BV (P<0.01)	1. Neither odds ratios nor risk ratios were reported to assess the direction of effect.

Author (Year)	Study design, Sample size & Population (Country)	HSV-2 Prevalence (P) /Incidence (I)	Test method	Risk factor (Measure of Effect p-value/OR/RR with 95%CI)	Comments
Watson-Jones, D et al (2007)	A cross-sectional study of 1143 women working in bars and hotels Aged 16-24 (Tanzania)	P=66.0%	Type-specific ELISA IgG (Kalon)	i. Older age [OR 4.42(2.6-7.6)] ii. Less educated [OR 2.96(1.5-5.6)] iii. Number of dependents [OR 1.48(1.0-2.1)] iv. Vaginal douching [OR1.48(1.0-2.1)] v. Age of sexual debut[OR1.79(0.9-3.4)] vi. Increasing number of sexual lifetime partners [OR 5.45 (1.9-15.9)] vii. Consistent condom use[OR 0.93(0.6-1.5)] viii. Increasing alcoholic drinks/wk[OR 3.72(1.6-8.4)] ix. Past history of GUD [OR 1.38 (0.9-2.1)]	1. The majority of women were infected by age 20. 2. Alcohol is a marker of risky behaviour
Kaul R,et al (2007)	A prospective cohort of 466 female Sex workers (Kenya)	P=77.4 %	HSV-2 IgG Kalon	i. Nisseria Gonorrhoea [RR 4.3 (1.5-12.2)] ii. Trichoma Vaginalis [RR 2.3 (1.3-4.2)] iii. Syphillis [RR 4.7(1.1-19.9)]	1. Study assessing between prevalent HSV-2 and incident STIs 2. Clinical ulcers were rare
Shivaswamy KN et al (2005)	A case control study of 135 STD cases and 135 controls matched for age and sex (India)	P=89.2%	Type specific IgG for HSV-1 and HSV-2 (UBI-MAGIEEL Enzyme Immuno assay)	None of the socio-demographic and sexual behavioural risk factors were statistically significant	1. The authors concluded that in a setting of high HSV-2 prevalence, HSV-2 cannot be used as a sexual behavioural risk marker. 2. The small sample size could explain the lack of associations.

Author (Year)	Study design, Sample size & Population (Country)	HSV-2 Prevalence (P) /Incidence (I)	Test method	Risk factor (Measure of Effect p-value/OR/RR with 95%CI)	Comments
Msuya SE, at al (2003)	Cross-sectional study of 382 women 15-49yrs (Tanzania)	P=39.0%	HSV-2 IgG Gull (Meridan)	i. Presence of GUD [OR 9.7(2.5-36.9)] ii. HIV [OR 2.3 (1.1-4.7)] iii. Syphillis [OR 4.7 (1.4-4.7)] iv. Genital Ulcers [OR 9.7(2.5-36.9)] v. Age> 30 [OR 3.6 (1.4-9.6)] vi. Early age of sexual initiation [OR 1.6 (1.0-2.5)] vii. >2 sexual lifetime partners [OR1.591.0-2.4)] viii. History of spontaneous abortion [OR 1.9(1.1-3.4)]	1. Prevalence lower than STD clinic attendees 2. Risk factors consistent with other studies 3. Poor recognition of symptoms
Smith J, et al (2001)	A cross sectional study 181 Brazillian and 371 Fillipino women; in hospital setting	P;Brazil= 42% P;Philippines =9.2%	HSV-2 IgG Gull (Meridan)	<u>Brazil</u> i. 2-3 lifetime sexual partners [OR OR 2.7(1.0-11.4)] ii. 3 lifetime sexual partners [OR OR 5.9 (1.8-19.2)] iii. Partners with other sexual partners [OR OR 4.8 (2.0-11.4)] <u>Philippines</u> i. Early age of sexual debut [OR OR 5.2(1.7-16.5)]	1. Age not a risk factor because majority of women were older than 40 years 2. Differences observed in predictors attributed to different sexual behaviour between the populations studied 3. Study supports the use of HSV-2 as a marker past sexual behaviour
Santos FC, et al (2006)	A cross-sectional study of HIV + 67 females and 83 males, PHC clinic attendees (Brazil)	P=52.0%	HSV-2 IgG (Focus)	i. Past history of genital herpes [OR 3.8(1.22-12.6)] ii. Men with reporting >10 lifetime sexual partners [OR 3.5(0.7-19.9)]	1. Weak associations and small sample size limited control for confounding in multivariable analysis

Author (Year)	Study design, Sample size & Population (Country)	HSV-2 Prevalence (P) /Incidence (I)	Test method	Risk factor (Measure of Effect p-value/OR/RR with 95%CI)	Comments
Sucato G, et al (2001)	A cross-sectional study of 381 female and male adolescents ages 14-19 STD clinic attendees (USA)	P=12.0%	HSV-2 Western Blot	i. African–American race [OR 2.3 (1.1-4.8)] ii. Female gender [OR 6.0 (2.3-15.9)]	1. HSV-2 correlated with demographic rather than behavioural factors 2. Most HSV-2 infections unrecognized just as in adults
Wald A, et al (1997)	A cross-sectional study of 610 females and 351 Males ages 15-45 PHC attendees African American comprised of 9% and Whites 80% of the total population (USA)	P=11.0%	HSV-1 &HSV-2 Western Blot	<u>African- American</u> i. Oral sex [OR 2.8(2.4-33.8)] <u>White</u> i. 4-9 lifetime sexual partners [OR 3.4(1.2-10.5)] ii. >10 lifetime sexual partners [10.2 (3.6-32.3)] iii. History of STI [OR 2.4 (1.2-4.8)] iv. Married or cohabiting [OR 3.2 (1.4-7.0) v. Low education level [OR 2.7 (1.4-5.6)] <u>Men (Both races)</u> i. >10 sexual lifetime partners [OR 10.4(1.3-83.4)] History of STI [OR 3.1(1.1-8.6)]	1. Racial differences noted among women but not in men 2. Oral sex a marker of greater number of sexual partners and high risk behaviour 3. Risk of HSV-2 differed by gender and ethnicity

1.3.1 Rationale for the study

The prevalence of HSV-2 has been shown to vary in different population subgroups between and within countries with different demographical and behavioural parameters. Risk factors associated with HSV-2 infection have been established in developed countries and some developing countries, but little is known in South Africa. Because of the synergetic relationship between HIV and HSV-2, understanding and identifying risk factors will improve knowledge of the epidemiology of genital herpes and inform prevention programmes. Because of variable real-world performance of newer type specific serologic tests, a validation of the performance of HerpeSelect® ELISA (Focus Technologies Inc., Cypress Hill, Ca) and Kalon HSV-2 gG ELISA™ (Kalon Biologicals Ltd, Aldershot,UK) against the gold standard HSV-2 Western Blot (WB) test was conducted among women attending family planning services at a clinic in inner city Johannesburg in 2003.[53] The validation study was conducted prior to the implementation of RCTs evaluating the impact of suppressive acyclovir therapy on the acquisition and transmission in South Africa. We performed secondary data analysis to determine the prevalence, risk factors and the extent of symptom recognition in order to inform possible intervention for HSV-2 during and after trials.

1.3.2 Purpose of the Research

The purpose of this study was to examine the influence of certain risk factors on HSV-2 seroprevalence and the extent of symptom recognition in the population of family planning attendees in inner city Johannesburg.

1.3.3 Objectives

- To determine the HSV-2 seroprevalence of the clinic attendees in inner city Johannesburg.
- To describe the extent of symptom recognition among this population
- To assess the risk factors associated with HSV-2 infection.
- To assess the utility of genital ulcers in predicting HSV-2 infection

Chapter Two Methodology

2.1 Study setting

This study involves secondary analysis of the data collected as part of a cross sectional study evaluating the performance of two serological assays against Western Blot (WB) in a South African population. The study was conducted at an urban clinic in inner city Johannesburg which primarily offers family planning and STI services; a few primary health care services such as voluntary testing and counselling (VCT) are available. The clinic is large and with a high patient volume and was therefore ideal as a recruitment site for the two randomised clinical trials. It is situated in Hillbrow which is a densely populated inner city suburb with high rise buildings characterised by HIV/AIDS, poverty, crime and a thriving sex work industry. The population of Hillbrow is comprised of economic migrants from the neighbouring African countries and from rural South Africa. It is currently estimated that the population of Hillbrow is close to 100 000 in an area that covers only 35 hectares,[58] but these estimates could be higher due to the constant influx of economic migrants and asylum seekers driven by economic and political instability experienced by some neighbouring South African countries.

2.2 Study design and sample

A cross-sectional study was conducted between August and November in 2003. This was a convenient sample of two hundred and ten consecutive women attending the family planning and primary healthcare services at Esselen Street clinic in Hillbrow. Participants were included if they were 18 years of age and above (as self reported), sexually active (defined as at least one sexual act in the 3 months prior to the interview), willing and able to undergo an informed consent process and willing to provide a blood

sample for HSV-2 testing. Women were ineligible if they were not willing to undergo the informed process or provide a sample for HSV-2 testing.

2.3 Study methods

2.3.1 Clinical procedures

An informed consent was obtained from all participants prior to any study –related procedures. A detailed history of social, demographic, behavioural, past and current STI symptoms was collected using a standardised questionnaire by trained nurse counsellors in a private clinical room. Enrolled participants were given an individual standardised educational session on genital herpes that included reviewing photographs of herpetic lesions. Photographs of both typical lesions (blisters and ulcers) and atypical lesions (fissures) were shown and the common symptoms (itching, tingling and dysuria) were discussed. In addition, participants were counselled about safer sexual behaviour and offered condoms. Information and education on HSV-2 was given prior to collecting blood for testing for HSV-2. Participants were counselled on the implications of a positive or negative result and invited to return for results at a later date. Participants who reported symptoms including genito-urinary symptoms were referred for treatment.

2.3.2 Lab methods

A 10ml specimen of venous blood was collected by a research nurse and sent to the laboratory for HSV-2 testing. Participants were tested using Focus HerpeSelect® and Kalon tests. Optical density (OD) readings for Kalon and the normalised OD readings for HerpeSelect® were recorded and the results categorised as positive, negative and equivocal.

Subsequent analysis showed that raising the cut off value for HerpeSelect® to 3.4 and testing all equivocal results with Kalon as a resolver test resulted in a testing algorithm which had high levels of sensitivity and specificity than HSV-2 WB [51].

For the purpose of this analysis, true positives were those where the HerpeSelect® OD is greater than >3.4, and true negatives were those where the HerpeSelect® OD reading was < 0.9.The equivocal tests i.e. HerpeSelect® OD readings between 1.1 and 3.4, were resolved by Kalon HSV-2 ELISA.

Following the study, new evidence emerged about the potential role of HIV in reducing the specificity of HSV ELISA tests.[54] Permission was therefore sought to test left over sera for HIV and patients were provided with results. HIV testing was performed using Abbott AxSYM HIV1/2 Go (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany) at the local lab in Johannesburg. There were no equivocal results.

2.4 Data procedures and management

Quality control of questionnaires was performed on a daily basis to detect errors and confirm any discrepant responses to the questionnaires. In addition, standard quality control procedures were implemented prior to data capture to ensure the integrity of the data.

Data from questionnaires was captured manually into an MS Access database. Laboratory results were linked to the behavioural dataset through the participants unique study number. Each variable was coded, labelled and a codebook produced for referencing. Lab results were provided in electronic database and merged with the behavioural data. Data was analysed using STATA (version 9.0, Stat Corp., College Station, Texas).Only the participant's unique study number (PTID) was used in the dataset in order to maintain confidentiality.

2.5 Definition of terms

Condom use

As a measure of consistency of condom use, participants who reported condom use as a form of contraception were grouped together with those who reported using condoms as a method of preventing HIV infection. Participants who reported that they perceived themselves to be at risk of STIs including HIV infection due to lack of condom use were categorised as inconsistent condom users.

STI symptoms

STI symptoms were defined by the conventional genital symptoms such as vaginal discharge and genital sores or ulcers. In addition, non-specific genital symptoms such as itch or burning in the genital area, flu-like symptoms like fever (particularly in primary genital herpes pain in thighs or legs and lower abdominal pain which are recognised in literature as symptoms of genital herpes were also included.[14]

2.6 Statistical Analysis

The seroprevalence of HSV-2 was calculated as the proportion of cases in the study population. The frequency distributions of categorical variables and summary of continuous variables were described. The mean, standard deviation (SD) and range of all continuous variables was reported. In the cases where continuous variables were asymmetric or skewed, medians were reported with inter-quartile ranges (IQR) for summary measures. The primary outcome variable, (HSV-2) was dichotomised into a binary response and coded as 0= HSV-2 negative and 1= HSV-2 positive.

Assessing the value of using a history genital ulcer disease (GUD) as a diagnostic test to predict HSV-2 seropositivity

The accuracy of a past history of genital ulcer disease in predicting HSV-2 infection was assessed using test characteristics such as sensitivity, specificity, predictive positive value, negative predictive value and the likelihood ratios. The validity of a past history of GUD was of particular interest since it would be a cost effective, easy to use and accessible diagnostic tool to identify individuals infected with HSV-2 who would benefit from suppressive prophylactic acyclovir therapy should the randomised clinical trials proved efficacious instead of the "gold standard" HSV serology which is expensive and less widely available. [54]

The Likelihood Ratio (LR) is commonly used in clinical epidemiology and expresses the magnitude by which the probability of a diagnosis in a given patient is modified by the test results; it does not prove that the patient has the condition but increases or decreases the odds of that diagnosis by a certain factor. [55]

LR1-10: There is a weak association between a positive test and the disease and the test should be used with caution.

LR>10: There is a strong association between a positive test and the disease of interest.

LR<1: The smaller numbers signify a low risk of having the disease following a negative result.

Analysis of outcome

Cross- tabulation (2x2) of the primary outcome variable and the exposure variable were carried out. Continuous variables were categorised so that roughly an equal number of observations would be in each category. Associations between primary outcome variable and exposure variables were explored in univariate analysis using the Pearson's chi square test (χ^2) or the Fisher's exact test. Both the Pearson's chi square and the Fischer's

exact tests are dependent on the sample size and the Fisher's exact test is useful when the some of the cells have small (>5) observations. The measure of association was expressed as odds ratios (OR) with 95% confidence intervals (CI)

Adjusting for confounders

Confounding occurs when there is an apparent relationship between a risk factor and an outcome. A confounder that was determined *a priori* was age. Since HSV-2 antibodies remain for life in infected people, older participants will be more likely to have antibodies from previously acquired HSV-2 infection. Age was adjusted for in order to avoid overestimation in older participants and underestimation in younger participants thus allowing comparison of the risk of HSV-2 infection. Selected factors that were statistically significant at level above 0.05 in the univariate analysis were stratified by age using stratified (Mantel-Haenszel) analysis. The risk factors were stratified by those that were younger or equal to 25 years of age or older than 25 years of age.

Power Analysis:

A sample size of N=125 would have 99% power to detect an HSV-2 prevalence of 73%, assuming a 55% HSV-2 prevalence based on the results of a population –based survey conducted in Carltonville [30] at a 5% alpha (significance level). A smaller sample size, N=57 was required for a power of 80%; therefore the study was sufficiently powered to detect an HSV-2 prevalence of 73%.

2.7 Ethical Considerations

The informed consent process was conducted in the language of the participant's choice (English, Zulu, Sotho) prior to any study related procedures. Key elements of the informed consent process such as voluntary participation, the option to withdraw without

any consequences, confidentiality, reimbursement for time and travel and risks and benefits of participation were dealt within the informed consent process and counselling. Ethics approval (M 03-003-09) in accordance with the guidelines on human experimentation was obtained from the University of Witwatersrand and the London School of Hygiene and Tropical Medicine (LSHTM) for the validation research study respectively. The information on the validation study is in the process of being published in a peer review journal.

The student sought approval from the Reproductive Health and HIV Research Unit (RHRU) for secondary data analysis. The student also sought clearance from the University of Witwatersrand for the secondary data analysis (see appendix 2). The approval of the original study is also attached (see appendix 3)

Chapter Three Results

3.1 HSV-2 prevalence

A total of 210 participants were enrolled into the study. Of these participants, 153 [73%, 95%CI 67-79] were HSV-2 antibody positive. The prevalence of HSV-2 rose sharply from 56% among those less than 20 years of age to almost 95% among those between 30-35 years of age. Of the participants that had sufficient samples left for HIV testing the prevalence of HIV was 52% (CI 43%-60%). Figure 4.1 shows the prevalence of HSV-2 and HIV by age group. The rate of HSV-2 and HIV co-infection was 45% (CI 37%-54%)

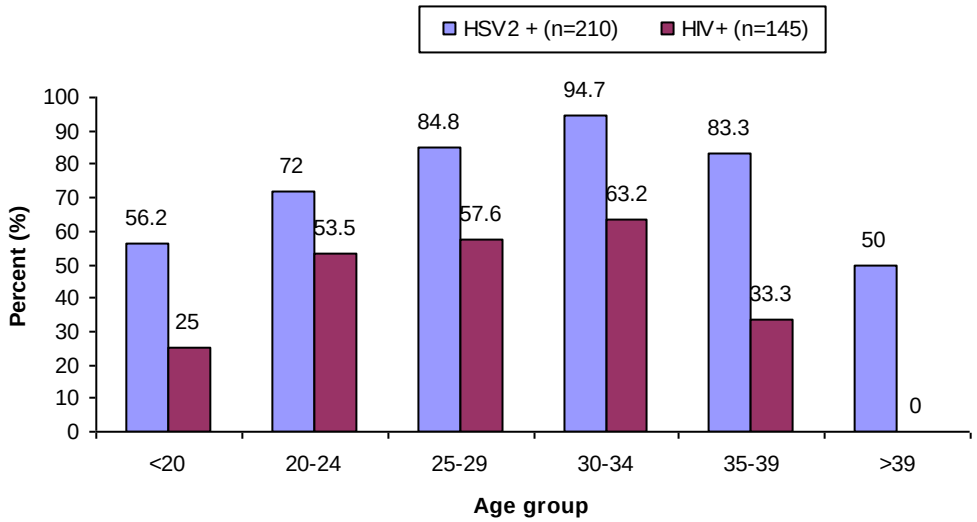

Figure 3.1 Prevalence of HSV-2 and HIV by age group

3.2 Participant characteristics

Table 3.1 Descriptive characteristics of population

Characteristic	No. of women	Percent (%)
Total	***N=210***	
Age (years)		
Mean (range)	25.6 (17-46)	
Home Language		
Zulu	59	28.1
Ndebele	59	28.1
Sepedi	29	13.8
Xhosa	17	8.1
Tswana	14	6.7
Sotho	10	4.8
Other∗	22	10.4
Level of Education		
Primary	12	5.7
Secondary	143	68.1
Post-Matric	55	26.2
Source of income		
Yes	67	31.9
No	143	68.1
Ownership of commodities		
Radio	175	83.3
Television	150	71.4
Car	23	10.9

∗Other South African languages such as Tsonga, Seswati, English and Afrikaans

Table 3.1 shows some of the socio demographic characteristics. All 210 participants were African women. The mean age of the studied population was 25.6 years ([SD] 4years).[a] The majority of the participants were older than 25 years of age (56.2%); mostly spoke Zulu and Ndebele; and were well educated. Despite their high level of education only a third reported having a source income derived from the informal sector (street vendors or domestic or child care workers or labourers) and formal sector (hotel or retail or security officers).

Despite the high impoverished state, levels of ownership of commodities such as a radio and a television set were relatively high, 175 (83%) participants reported owning a radio

[a] Even though one of the inclusion criteria was age 18 years and above ,one participant reported her age as 18 years because she was turning 18 on the year of the interview although her calculated age was 17.

and 150 (71%) owned a television set. The participants owned a median of 2 commodities (IQR 1-2 commodities). Few, 10 (5%) participants reported not owning any commodities. As expected of a population of low socio-economic status, most participants did not have access to ready cash nor could they afford private health care. One hundred and thirty five (65%) said that they would find it very difficult to access R100 for medical expenses and 151(72%) reported using public health care facilities to access healthcare.

Overall, 57% of the participants were born in South Africa. Three quarters of those interviewed reported Hillbrow as their primary residence and had been living in their primary residence for a median of 24 months (IQR 9-36 months).

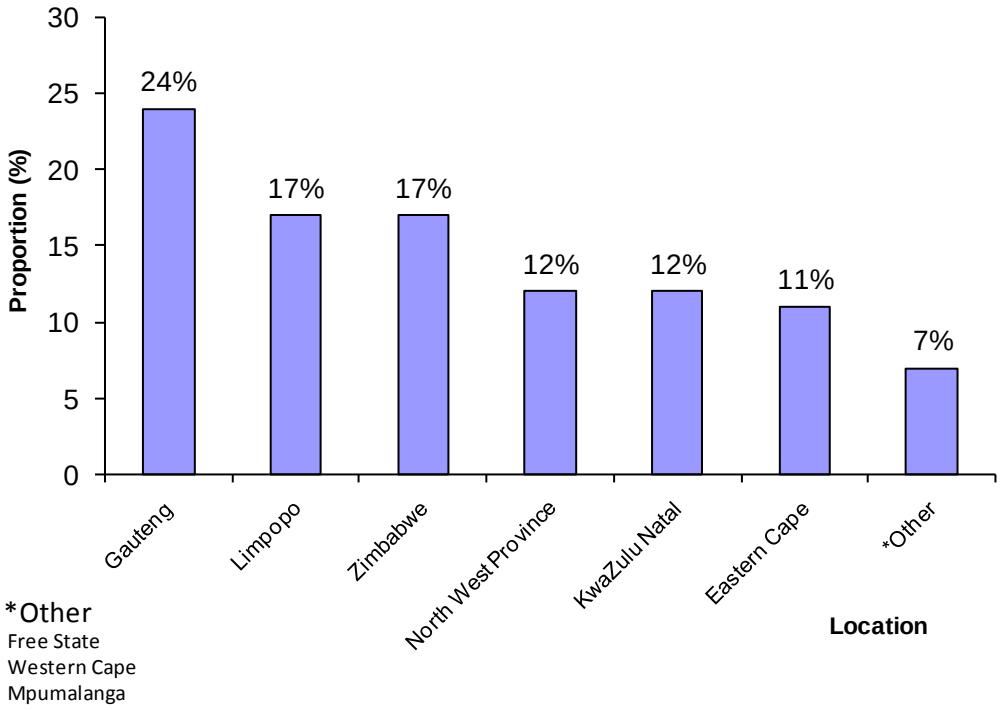

Fig 3.2 Proportion of participants reporting a second residence (N=83)

In addition, 83 (39%) of those interviewed reported having a second household or residence. The proportion of South Africans who had another household was higher,67 (80%) compared to non South Africans 19(20%) The second households were located

either within Gauteng, possibly in the peri- urban areas or spread throughout other provinces in South African as shown in figure 4.2 above. Those participants who reported having another household travelled an average of 12 times per year (IQR 2-18 times per year) visiting the second residence. Overall, 46/210 (22%) participants reported spending an average of 2 nights away from their primary residence (IQR 1 – 4 nights) in the past month irrespective of whether they owned a second residence elsewhere or not.

3.2.1 Sexual Behaviour

The mean age of coital debut reported by this population was 18 years of age (SD 2years). Participants reported first sexual intercourse as young as 12 years, and as old as 26 years. All participants interviewed had initiated sexual intercourse. The number of lifetime sexual partners ranged from 1-35 with a mean of 3.4 partners.

Only 32 (15%) participants were married. The majority of participants, 112 (57%) reported being in a stable relationship but not cohabiting with their partners. Married participants reported the longest median duration of sexual relationship in months (84 months; IQR 42 – 120) compared to those not married (24 months; IQR 12 – 48) and those in casual relationship (5; IQR 3 - 12). A small proportion, 16 (7.7%) reported having more than one sexual partner in the past 3 months with the median number of multiple sexual partners being 1 (IQR 1-2). Of the participants involved in multiple sexual relationships, only 2 (12.5%) admitted to having sex in exchange for money. Consistent condom use was reported by 23 (41,8)%[b] of the studied population.

[b] Condom use not asked of all participants

3.2.2 Self reported history of past and current STI symptoms

A series of both unprompted (open) and prompted (closed) questions were asked of participants about genital symptoms. When participants were asked in an open ended manner if they had "any problems in the genital area", 20 (10%) reported symptoms at the time of the interview. The most common symptom spontaneously reported was genital itch, reported by 8 out of 20 (40%). When the open-ended questions were followed by the enquiry of the presence of specific symptoms amongst all the participants, genital itch remained most common, but now lower abdominal pain was equally common

Only 5 of the 20 participants (28%)[c] with symptoms at the time of the interview had sought treatment for their symptoms and all had visited a local public clinic. Overall, 44 participants (21%) reported having genital symptoms in the past 3 months and of these, the majority 29 (66%) had multiple STI symptoms.

3.2.3 Specific history of genital ulcer disease (GUD)

Table 3.2 The frequency of symptoms* associated with genital ulcer episodes (for the 13 participants who reported symptoms)

Symptoms associated with GUD	Frequency	Percent
Genital tingling	9	27.3
Blisters	7	21.2
Abnormal vaginal discharge	7	21.2
BOM §	4	12.1
Other∞	6	18.2
Total number of symptoms	33	100.0

*Symptoms are not mutually exclusive; they will thus not add up to 13
§ BOM: Burning on micturition
∞Other: fissure, pain in the thigh, lower abdominal pain

[c] Data missing for 2 participants

Overall, only 13 (6%) participants reported a history of GUD. Of these participants, very few,2(20%)[d] reported experiencing recurrent episodes of GUD. None of these participants experienced more than 6 episodes of GUD per year. A range of clinical symptoms associated with presence of GUD were reported by participants (see table 3.2). The most frequently cited symptom was genital tingling. Only 4 of the 13 (2.3%) participants were diagnosed by a healthcare provider with genital herpes. In all cases, the diagnosis was made clinically by a doctor or nurse. No case had serology test or a laboratory test performed to isolate the causative agent of genital ulcers. Even though the participants who had received a diagnosis of genital herpes reported that they were treated with specific anti-herpetic treatment, none knew the name of the treatment received.

Participants who reported a history of genital ulceration were further asked if they engaged in sexual activity during clinical episodes of genital herpes; 9 (69%) reported that they continued having sex during genital herpes episodes. Of these participants, 5(55%) reported that they always or sometimes used condoms, whilst the rest reported that they seldom or never used condoms. Reassuringly, disclosure of symptoms seemed to be common, with ten participants (83%) reporting that they had disclosed their genital ulcer symptoms to their partners.

3.2.4 Genital Herpes Symptom Recognition after Education

General knowledge or awareness of genital herpes was very poor amongst the studied population, only 13(6%) of the participants had ever heard of genital herpes. Only 9[e] (4.4%) participants reported having experienced lesions typical of genital herpes following education and counselling about genital herpes. All these participants were amongst the thirteen participants who reported a history of GUD. Among 194

[d] Data missing for 3 participants
[e] Out of 210 participants ,data was missing for 7 participants

participants who did not report a history of genital herpes, 139_b (72%) participants were HSV-2 seropositive.

3.3 Risk factors for HSV-2: univariate analysis

Table 3.3 Risk factors associated with HSV-2: unadjusted analysis

Characteristic	Positive/Total	% HSV-2 Positive	Unadjusted OR(95%CI)	*P value*
Age groups (years)				0.002
< 25	57/92	62.0	1	
>=25	96/118	81.4	2.6(1.4-5.0)	
Level of Education				0.119
Primary	11/12	91.7	1	
Secondary	106/143	74.1	0.3(0.03-2.1)	
Post-Matric	36/55	65.4	0.2(0.02-1.4)	
Source of income				0.691
No	103/143	72.0	1	
Yes	50/67	74.6	1.1(0.59-2.2)	
Mobility:				
Nights spent away from primary residence				0.024
1-2 nights	15/24	62.5	1	
>2 nights	20/22	90.9	6(1.0-62.7)	
Visit other household				0.834
visit 1-4 times/year	29/39	74.4	1	
visit>4 times/year	26/36	72.2	0.9(0.3-2.8)	
Age (years) of sexual debut				0.071
<15	12/16	75.0	1	
16-20	125/172	72.7	0.9(0.3-2.9)	
>20	15/21	71.4	0.8(0.2-3.6)	
Lifetime number of sexual partners				0.009
1-2	55/87	63.2	1	
>2	98/123	79.6	2.3(1.5-3.4)	
Condom Use				0.039
Yes	9/17	52.9	1	
No	26/32	81.2	3.8(1.0-14.0)	
Past history of STI				0.005
No	114/166	68.7	1	
Yes	39/44	88.6	3.6(1.3-9.5)	
Past history of genital ulcer disease(GUD)				0.289
No	140/195	71.8	1	
Yes	11/13	84.6	2.1(0.4-10.1)	
HIV infection				0.004
HIV negative	48/70	68.6	1	
HIV positive	66/75	88.0	3.3(1.4-7.9)	

Table 3.3 shows the association between HSV-2, socio demographic, sexual behaviour and clinical characteristics. Older age (> 25) was significantly associated with HSV-2 seropositivity [OR 2.6, 95%CI 1.4-5.0]. Mobility appeared to be associated with an increased risk of HSV-2 infection. Participants who spent more than 2 nights away from home in the past month had greater odds of infection (OR=6; 95%CI 1.0-67.2) .Further analysis revealed that participants who were HSV-2 positive spent a mean of 3.75 (range 2.5-4.5) nights away from home, versus HSV-2 negative participants who spent a mean of 1.72 nights (range 1.1-2.2) away from home. Participants who travelled frequently to a second residence did not have an increased risk of HSV-2 [OR 0.9,95%CI 0.4-1.6 p=0.834]. Non-South Africans had a lesser risk of being HSV-2 positive although this was not statistically significant, [OR 0.7, 95% CI 0.3-1.4, P=0.321].Other demographic factors such as the level of education, marital and socio-economic status were not associated with HSV-2 infection.

On examination of sexual behavioural characteristics, the participants who reported more than two lifetime sexual partners had a two fold increased risk of being HSV-2 positive [OR 2.3, 95%CI 1.5-3.4, P=0.009] compared to those who reported one to two lifetime sexual partners. Inconsistent condom use and the age of sexual debut were not associated with HSV-2 infection in the studied population. A past history of an STI as well as HIV infection were associated with HSV-2 infection, [OR 3.6, 95% CI 1.3-9.5, p=0.005] and [OR 3.3, 95% CI 1.4-7.9, p=0.004] respectively. A past history of genital ulcer disease was not associated with HSV-2 infection.

Table 3.4 Age-adjusted Odds Ratios of HSV-2 seropositivty with factors significant at p<0.05

Independent variable	*cOR	**aOR	95% CI	P-value
Mobility; >2 nights	6.0	6.6	1.0-41.2	0.031
>2 lifetime sexual partners	2.2	2.0	1.1-3.9	0.027
Past history of STIs	3.6	3.6	1.2-9.5	0.010
HIV	3.4	3.2	1.3-7.7	0.008

*cOR= crude Odds Ratio **aOR= adjusted Odds Ratio

Table 3.4 shows the age-adjusted odds ratios of HSV-2 seropositivity with selected factors. The difference between adjusted ORs and the crude ORs was less than 10% indicating that there was no significant confounding.

3.4 The utility of GUD history in predicting HSV-2 seropositivity

Table 3.5 Cross tabulation for calculating the accuracy of a GUD history in predicting HSV-2 infection

History of GUD	HSV-2 Pos	HSV-2 Neg	Total
Yes	11	2	13
No	140	55	195
Total	151	57	208

Sensitivity= 7% Specificity= 96%
Positive predictive value (PPV) = 84% Negative predictive value (NPV) = 28%
Positive likelihood ratio test (LR) = 2.07 Negative likelihood ratio test (LR) = 0.96

A history of GUD would have correctly identified 7% of cases with HSV-2 infection. By contrast, specificity was 96%. The PPV was 84% with a negative predictive value of 28%. The positive likelihood ratio test is 2.07 and the negative likelihood ratio test is 0.96.

Chapter four Discussion

The aim of the study was to determine the prevalence of HSV-2, its correlates, the utility of GUD history in predicting HSV-2 infection and the extent of recognised symptomatic herpes in the studied population.

HSV-2 Seroprevalence

The prevalence of HSV-2 was very high in this population at 72% (95% CI 67% - 79%). Other epidemiologic studies in South Africa have also demonstrated high HSV-2 prevalence in young female adults in the Eastern Cape (32%)[31], and Carltonville situated west of Johannesburg (53%)[30], HIV positive individuals (63%)[59] , and female sex workers in Durban (84%)[29]. The HSV-2 prevalence in this population however approaches that of female sex workers. One of the possible explanations is that demographic characteristics suggest that these are young women of reproductive age who are economic migrants but are unemployed despite having a relatively high level of education. These participants would therefore be vulnerable to high risk behaviour such as exchanging sex for money or gifts to for livelihood, which would increase their risk of acquiring STIs including HSV-2. However, reported transactional sex was not high possibly due to social desirability bias. On the other hand, at high prevalent HSV-2 levels in the general population, low rates of partner change are sufficient to sustain the prevalence because HSV-2 infection persists for life whereas STIs of brief duration such as gonorrhoea and Chlamydia are limited to subsets of the population with high rates of partner change.

The risk of being infected with HSV-2 was associated with older age, mobility, having more than 2 lifetime sexual partners, a past history of STIs and HIV infection.

Socio-demographic risk factors

Significant confounding is present if (aOR-cOR) /cOR = > 20-30%. The difference between adjusted ORs and the crude ORs was less than 10% indicating that significant confounding was not present. Therefore age was not a confounder but an independent risk factor for HSV-2 infection.

Older age was significantly associated with HSV-2 seropositivity. As has been observed in other studies, HSV-2 prevalence increased with age peaking at the 30-34 age group and thereafter levelled off after age 40. More than half (56.2%) of the participants less than 20 years of age had HSV-2 antibodies suggesting that HSV-2 infection occurs during the first few years following sexual debut. Therefore, young women should be targeted for HSV-2 prevention strategies. The effect of travelling was significantly associated with HSV-2. Migration and travel has been observed to be associated with STIs including HSV-2 and HIV among migrant women and men in South Africa and other developing countries.[8,60] Travelling may be a proxy of risky sexual behaviour such as having multiple concurrent sexual partners which in turn increases the risk of STIs. There was no data on recent concurrency collected in the study which would support this observation.

Education and the socio-economic status were not associated with HSV-2 infection and this effect has been observed in other seroepidemiological studies.[8,19, 61] It is possible that the overall high level of education and a low economic status in the studied population made it difficult to observe associations between HSV-2 and these explanatory variables due to lack of power for comparisons.

Behavioural risk factors

Participants who reported having three or more lifetime sexual partners had double the risk of HSV-2 infection compared to participants reporting fewer lifetime sexual partners. The association between lifetime number of sexual partners and HSV-2 is consistent with data from the African, US and European countries (see table1) and is a measure of increased sexual activity.

Condom use was not associated with HSV-2 infection. It is possible that condom use was not consistent with every sex act but per perceived risky sexual encounter and, thus the benefit derived from condom use was lost.

In other seroprevalent studies, other sexual habits markers such as cohabiting with ones sexual partner and early sexual debut were associated with an increased risk of HSV-2 infection. In this population however, none of these factors were associated with HSV-2 infection. Being married and cohabiting with a sexual partner is thought to be associated with high HSV-2 prevalence due to frequent sexual contact in a HSV-2 serodiscordant relationship. The majority of participants (57%) in the studied population were not cohabiting with their sexual partners and very few (15%) were married. In addition, their partners were not tested for the presence HSV-2 antibodies. Early initiation of sexual intercourse has been found to be associated with HSV-2 infection[27, 45, 61], but not in this population. The likely explanation is that, not many reported sexual debut less than 15 but on the other hand ,older age of sexual initiation was more likely to be protective (OR<1) although this difference did not reach statistical significance.

Sexually transmitted infections

As in other studies, the likelihood of HSV-2 infection was found to increase among study participants reporting a past history of an STI. If an individual presents with an STI chances of having more than one STI are high due to riskier sexual behaviour and having

a high risk sexual partner. Participants also reported multiple STI symptoms which might translate to infection with more than one STI pathogen. Genital examination and laboratory tests to ascertain the aetiology of STI symptoms were however not conducted in this study. In addition, HSV-2 confers genital tract immunological changes that increase the risk of acquiring other sexually transmitted infections. The biological basis for the immunological changes due to HSV-2 is thought to be due to the intense proliferation of proinflammatory cytokines and chemokines which are a final pathway for many inflammatory stimuli observed in the female genital tract. These immunological changes have been demonstrated to enhance infection by N gonorrhoea amongst others. It is possible that control of HSV-2 would not only control HIV infection but other STIs as well.

Treatment for STI related symptoms was only sought by 28% of the participants. These numbers are disappointing as access to STI and primary health care services is free in South Africa. It is difficult to elucidate from the data collected the reasons for this poor health-seeking behaviour. A study done in a clinic cohort in Uganda, found that the participants' decision to seek STI treatment in health facilities might be hampered by perceived poor quality of service, overcrowding and inefficacy of treatment prescribed especially in the case of recurrent genital herpes symptoms.[62] At the time of the study, anti-herpes therapy was not part of the syndromic case management of GUD in the public sector in South Africa and the treatment was therefore likely to be ineffective if HSV-2 was the causative organism.

A substantial proportion of individuals continued to engage in sexual activity despite the manifestation of symptoms and almost half of the women had unprotected sex. This risky

behaviour has also been observed in men in a STI clinic in Uganda and in Durban, South Africa.[62,63] This risky behaviour could be an indication of lack of knowledge regarding sexual transmission of STIs especially genital herpes as observed in this population or the inability to negotiate condom use by women. Another possible explanation is that women were not willing to admit to partners that they had a problem, although this is unlikely given that most of the participants with genital herpes reportedly disclosed their ailment to sexual partners. Whatever the reasons for continuing to engage in sexual activity whilst symptomatic are, they can facilitate the spread of both HIV and HSV-2 and are a public health concern.

The likelihood of HSV-2 infection was three times higher amongst those with HIV infection, and this is consistent with data worldwide. The HIV and HSV-2 co-infection rates of 54% could be an underestimate in this population since not all subjects had suffient sera for HIV testing. It is not clear which infection comes first but HIV and HSV-2 co infected persons experience severe and frequent genital herpes symptoms and also have higher HSV-2 and HIV shedding from the genital tract. If antiherpes treatment is not included in management of GUD, delayed ulcer healing results in prolonged HIV shedding and increased risk of HIV transmission.

The performance of GUD history in predicting HSV-2 infection

In the studied population, a history of GUD was not useful in predicting HSV-2 infection and would actually lead to under diagnosis of genital herpes. The poor sensitivity of a clinical diagnosis of genital herpes is similar to observations made in other studies and can be explained by the low recognition or asymptomatic genital herpes. The high specificity and PPV indicate that a history of GUD would yield fewer false positive, thus

increasing the probability of a person being infected HSV-2 and in turn reduce the psychological trauma caused by inappropriate labelling. In addition, overtreatment is less likely if a past history of GUD is used to identify participants that would benefit from acyclovir therapy which is reassuring in the face of high drug costs. Both the positive and the negative LRs confirm that there is no value in the history of GUD in separating those who are HSV-2 positive from those that are HSV-2 negative. However, characteristics such as high sensitivity and high LR would have been desirable in order to correctly detect and treat all participants with HSV-2 infection to prevent transmission of HSV-2 and possibly HIV infections. This difficulty in using the presence or the history of GUD in predicting HSV-2 infection is further complicated by mixed infections with other STI pathogens and time to presentation for treatment.

With HSV-2 currently the leading cause of GUD and the epidemiologic evidence that HSV-2 is a co-factor in HIV transmission and acquisition, developing countries are currently reviewing STI syndromic management guidelines. However, decision about who would benefit from anti herpes treatment and what screening tools, if any could be used to identify those most at risk still pose a dilemma for policy makers. RCTs of herpes episodic treatment of symptomatic genital herpes were conducted in South Africa, Malawi, Ghana and Central African Republic to evaluate the clinical and virologic benefit of adding acyclovir in the GUD management guidelines in Africa. According to Nagot et al, these studies have shown little impact on ulcer healing among HIV patients and no impact on immunocompetent individuals.[64] The South African trial demonstrated an accelerated time to healing of ulcers by one to two days among HIV positive patients who presented early, and based on these on the findings, GUD syndromic management guidelines have been reviewed to include acyclovir.[66]

In addition, proof of concept clinical trials assessing the impact suppressive antiviral therapy among individuals co infected with HSV-2 and HIV all but one demonstrated a reduction in both plasma and genital HIV viral load.[64] These trials used genital shedding of HIV as an endpoint measure and were of relatively short duration. It is hoped that transmission of sexually acquired HIV infection can be reduced by anti-herpetic treatment judging by the evidence generated by these proof of concept trials if one assumes that a reduction in the genital and plasma HIV-1 RNA levels is a proxy for decreased transmissibility. The results of two RCTs that measured the impact of suppressive anti herpes therapy on HIV acquisition have been published and showed no impact. The researchers postulated that poor adherence [66] and different acyclovir pharmacokinetics [67] were reasons behind the failure to show effect. Feature health policy will be guided by the results of the impact of suppressive HSV-2 therapy among couples infected HSV-2 with discordant HIV status, where the index partner is both HSV-2 and HIV-1 infected. The high levels of HIV and HSV-2 co-infection in the studied South African population suggest that episodic and possibly long-term suppressive therapy would be of benefit in the reduction time to healing of ulcers and HSV-2 and HIV genital shedding for those participants who have clinical HSV-2 symptoms if started early.

Recognition of genital herpes symptoms

In this population, there was low recognition of genital herpes with few participants reporting a diagnosis of genital herpes amongst those with HSV-2 infection following education and counselling which included pictures of genital herpes.

This observation could be explained by the high HSV-1 prevalence common in South Africa, which masks the clinical expression of HSV-2. However, HSV-1 testing was not

conducted in the studied population and therefore this explanation is ade with caution. It has been documented that the recognition of genital herpes symptoms following detailed education, counselling about genital herpes coupled with close follow up increased by up to 42%-60%.[34] It is possible that follow up visits might have resulted in increased recognition of symptoms were they conducted in this study.

Of note is the fact that GUD prevalence was low despite the high rate of HIV and HSV-2 co-infection. Among HSV-2 and HIV-1 dually infected people, HIV-1 alters the natural history of genital herpes resulting in extensive and slow healing ulcers especially in WHO stage 3 and 4 HIV disease.[46] Unfortunately, neither WHO staging nor CD4 count testing was done in this study. In addition, very few participants reported extensive and frequent genital herpes recurrences. It is possible that most of the dually infected women had higher CD4 levels and therefore the frequency of symptomatic genital herpes infection was not common. Even though recognition of symptoms is essential in the prevention of transmission of genital herpes, a herpes transmission study involving highly motivated heterosexual couples found that sub-clinical HSV infection was responsible for nearly all cases of genital herpes transmission.[68] Advice should thus be given to patients about HSV shedding as part of primary prevention.

Limitations

One of the limitations of this study is that which is common to other cross-sectional studies; temporality cannot be ascertained. The study also relied on self –reported sexual behaviour, history of sexually transmitted diseases including genital ulcer disease which are subject to recall bias and social desirability bias in the case of sexual behaviour.

The sample size had enough power to detect the prevalence of HSV-2 but not enough to detect small differences between the groups that were HSV-2 positive from the group that

was HSV-2 negative. According Peduzzi P et al, one needs at least ten outcomes for every independent variable to be able to convene a multivariate model for multivariate analysis.[69] Because the conditions of having at least ten outcomes for each variable stated above was not met in our dataset, a model for multivariate analysis was difficult to convene. Finally, this data is based on women attending primary health care facilities and thus limiting generalizability of HSV-2 infection rates to the general population.

Chapter Five Conclusion and Recommendations

Conclusion

In conclusion; the prevalence of HSV-2 seropositivity in this studied population is high and knowledge about this disease is low. High risk behaviour such as a higher number of partners, a self reported past history of STIs and HIV infection should be used as markers to identify those at risk of HSV-2 infection.

A paradigm shift is required in the management of HSV-2 in South Africa, to include anti-herpes treatment, education, and improved clinical skills at primary care. The public health challenges are abound however and include, but are not limited to lack of an affordable and acceptable serologic test, which population to target for screening and increasing accessibility of antiviral therapy. A survey examining barriers to the access of acyclovir in the public and private sector in sub-Saharan Africa including South Africa was recently completed. Findings were that demand for acyclovir was low due to cost and lack of evidence for efficacy. The cost was USD $1 in the public sector and USD $ 2-7 in the private sector.[70] Acyclovir patent has expired and generic acyclovir should be more affordable and the South African trial has provided evidence for efficacy among HIV and HSV-2 positive males. In summary, a package of services for people with STIs and high risk behaviour should include HIV testing and counselling and for those who test HIV positive, provosion of antiherpes treatment because of high HIV and HSV-2 co-infection rates. Counselling to improve recognition of herpes symptoms, early initiation of episodic anti herpetic treatment and counselling on condom use and abstaining from sexual intercourse during herpes outbreaks is also essential.

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Appendices

Appendix 1 The validation questionnaire

Appendix 2 Copy of Ethics Clearance certificate (student's)

Appendix 3 Copy of Ethics Clearance certificate (Original study)