INTERACTIONS BETWEEN SEXUALLY TRANSMITTED INFECTIONS AND HUMAN IMMUNODEFICIENCY VIRUS IN SOUTHERN AFRICA

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

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DECLARATION

I, Dr Ye Htun declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in Medicine in the branch of Clinical Microbiology and Infectious Diseases in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

28 May, 2005

DEDICATION

This thesis is dedicated to my parents, who taught me to follow Buddha's teaching: "*for the growth of wisdom - associating with a good person; the development of a pure mind or Dhamma; maintaining a right attitude of mind and leading a life in accordance with the Dhamma".*

I am indebted to my mentors Professors RC Ballard and HJ Koornhof, whose guidance and support made these studies and thesis possible.

To my beloved wife, Mon, I thank her for her encouragement, lifelong friendship and companionship.

CONTENTS

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ABSTRACT

Epidemiological information on sexually transmitted infections (STIs) is necessary to assess the magnitude of the burden of infections, to identify vulnerable population groups, to mobilise resources for intervention activities and to monitor the impact of these activities. In addition, specific STI surveillance systems, such as studies on the relative prevalence of aetiological agents of STI syndromes and their antimicrobial susceptibility patterns, are aimed at improving patient care.

The studies included in this thesis were designed and implemented to improve our understanding of the epidemiology of STIs and HIV infection in southern Africa. In all the study populations, we observed that high level STI epidemics preceded the explosive spread of HIV infection among high-risk individuals. The studies reported here also demonstrate the importance of triangulating data collected from different recommended STI surveillance components, using a tiered surveillance approach.

The studies reported here also explored the bidirectional interactions of HIV and STIs. We observed that different STIs have shown different magnitudes of interaction with HIV infection. We found particularly strong interactions between genital herpes and HIV. At the individual level, HIV-seropositive patients with genital herpes were more frequently found to have atypical clinical presentations, delays in spontaneous healing, longer duration of HSV shedding and increased association with HIV shedding from ulcer and genital exudates. Mixed infections involving chancroid and genital herpes were found to be common, particularly in HIV-seropositive patients. The effectiveness of syndromic treatment targeting only bacterial causes of genital ulceration was significantly reduced due to persistent ulcerations as a result of co-infection with genital herpes. The successful treatment of herpes in men and women was found to be associated with a decline or cessation in HIV shedding into ulcer exudates or genital fluid. The studies have also shown that HIV plasma viral load is the main determinant for HIV shedding in both men and women presenting with STIs.

As was the case with HSV infection, there was a strong association between HIV and HPV infection in both men and women. A higher prevalence of HPV infection was found among HIV-seropositive patients in our study population and this may reflect the higher frequency of recurrences and/or longer duration of infection (i.e. persistency).

The studies also found that the biological false positive reactions in syphilis serology (i.e. RPR) are not a common occurrence in our HIV-seropositive study population. On the other hand, syphilis serology could be falsely negative in patients with PCR-confirmed primary syphilis who are co-infected with HIV and other aetiological agents causing GUD.

In conclusion, the findings of our studies have supported the bidirectional nature of interactions between conventional STIs and HIV infection in southern Africa.

FIGURES

TABLES

ANNEXURES

NOMENCLATURE

CIN	Cervical Intraepithelial Neoplasia
CPE	Cytopathic Effect
CT	*Chlamydia trachomatis*
CVL	Cervicovaginal Lavage
DF	Dark Field
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EPRU	Emergent Pathogen Research Unit
FTA-ABS	Fluorescent Treponemal Antibody Absorption Test
GC	*Neisseria gonorrhoeae*
GI	Granuloma Inguinale
GUD	Genital Ulcer Disease
HAART	High Active Antiretroviral Therapy
HC	Hybrid Capture
HCT	Hybrid Capture Tube Test
HD	*Haemophilus ducreyi*
HIV	Human Immunodeficiency Virus
HLF	Human Embryonic Lung Fibroblast
HPV	Human Papillomavirus
HSIL	High-Grade Squamous Intraepithelial Lesion
HSV	Herpes Simplex Virus
IARC	Internationl Agency for Research on Cancer
IgG	Immunoglobulin G
LCR	Ligase Chain Reaction
LCR-GC	Ligase Chain Reaction for Gonococcal Infection
LGV	Lymphogranuloma Venereum
LSIL	Low-Grade Squamous Intraepithelial Lesion
MEM	Minimum Essential Medium
MIF	Microimmunofluorescence
M-PCR	Multiplex PCR
MSM	Men Who Have Sex With Men
NAAT	Nucleic Acid Amplification Test
NAT	Nucleic Acid Testing
NGU	Non-gonococcal Urethritis
NHLS	National Health Laboratory Services
NIV	National Institute for Virology
NRC-STD	National Reference Centre for STD
OR	Odds Ratio

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NRC-STD	National Reference Centre for STD
OR	Odds Ratio
PAF	Population Attributable Fraction
Pap Smear	Papanicolaou Smear
PC	Positive Controls
PCP	*Pneumocystis carinii* Pneumonia
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
PPT	Periodic Preventive Treatment
PVL	Plasma Viral Load
RCT	Randomised Control Trial
RHRU	Reproductive Health Research Unit
RLU	Relative Light Unit
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
RTIs	Reproductive Tract Infections
SAIMR	South African Institute for Medical Research
SD	Standard Deviation
SE	Standard Error
SOPs	Standard Operating Procedures
SRPS	Sexual Relationship Power Scale
SSA	Sub-Saharan Africa
STDs	Sexually Transmitted Diseases
STIs	Sexually Transmitted Infections
TMA	Transcription-mediated Amplification
TMP-SMX	Trimethoprim-Sulfamethoxazole
TP	*Treponema pallidum*
TPHA	*Treponema pallidum* Hemagglutination Test
TV	*Trichomonas vaginalis*
UL	Ulcer Lavage
UNAIDS	United Nations Joint Programme on AIDS
UU	*Ureaplasma urealyticum*
VCT	Voluntary Counselling and Testing

VCT	Voluntary Counselling and Testing
VVC	Vulvovaginal Candidiasis
WHO	World Health Organization
YLL	Years of Life Lost
95% CI	95% Confidence Interval

CHAPTER 1

Historical perspective and recent developments in the field of conventional sexually transmitted infection - human immunodeficiency virus interactions

1.1 Early history of sexually transmitted infections

The history of sexually transmitted diseases (STDs) has been extensively documented in early writings and has been summarized in an excellent chapter in a standard STD manual by Waugh in 1990[1]. Early accounts of STDs include descriptions in papyri from ancient Egypt, dating about 1550 BC of "strictures of the flesh" of men and women[2] and genital inflammation in women[3]. Urethral and vaginal discharges likely to be gonorrhoea were described in biblical times[4] and possibly in the 4th and 5th centuries BC by Hippocrates who used the term "strangury" for what was likely to have been gonorrhoea in men[5]. The clinical manifestations of male gonorrhoea were also described in ancient Chinese and Roman literature[6].

The history of syphilis, especially related to clinical manifestations and epidemiological impact, is remarkable and in many respects (but not in terms of severity of the disease and magnitude of the problem) similar to HIV/AIDS. Both conditions have an insidious course, affect many parts of the body and early in their respective histories carried a poor prognosis with little to offer with respect to effective treatment. Historically, the devastating effect of syphilis in Western Europe was documented during the latter years of the fifteenth century. An epidemic of the disease emerged rapidly, appeared to be spread by sexual contact and was characterized by skin ulcers and eruptions, often causing systemic illness and death[7,8]. The origin and clinical manifestations of syphilis have been debated over many years. Based on the many similar manifestations of yaws, pinta, endemic syphilis and sporadic syphilis and their widespread occurrence in equatorial Africa, Hudson suggested in 1946 that these entities are varieties of the same disease caused by *Treponema pallidum* and that they are evolutionary linked and evolved in sub-Saharan Africa[9]. Evidence provided by paleopathology, based on bone changes likely due to tertiary syphilis, strongly suggests pre-Columbian syphilis in the Americas while such lesions were not found in ancient Egyptian mummies nor

in human remains in Europe prior to 1493[10]. Only in the late seventeenth century did Phillipe Ricord (1799 – 1889) establish the clinical presentation and describe the stages of syphilis[11] and later, Fritz Schaudinn (1871 – 1906) and Erich Hoffmann (1986 – 1959) identified the causative organism of the disease[12].

The history of other 'minor' STDs, including the genital ulcer diseases (GUDs) such as chancroid, lymphogranuloma venereum and granuloma inguinale and causes of genital discharge has also been documented by Waugh[1].

1.2 Recent explosion of knowledge on HIV and STDs

Since the beginning of the 20th century, scientific knowledge on STDs has advanced rapidly and many breakthroughs in diagnosis and management of these infections have been documented. The history of STD epidemiology with evolving scientific knowledge relating to sexually transmitted infections (STIs) in the latter half of the 20th century is highlighted by major advances in STI research. These involve not only innovative approaches to our understanding of the microbiology and pathogenesis of STIs and HIV, but at the practical level led to the development of microbiological and molecular methods that proved to be extremely useful tools for the diagnosis of STIs and investigative epidemiological studies (Figure 1.1)[13].

Despite the achievements listed in Figure 1.1, STIs remain a major public health problem in both industrialised and developing countries[14]. The World Health Organization (WHO) estimated that, globally, 340 million new cases of curable STIs (syphilis, gonorrhoea, chlamydial infections and trichomoniasis) were acquired in 1999 by men and women aged 15 – 49 years[15]. Rapidly changing demographic, economic, and cultural factors in many countries led to an escalation in risky sexual behaviour among vulnerable population groups, which, in turn, facilitated the spread of STIs.

1.2.1 Progress in epidemiological research

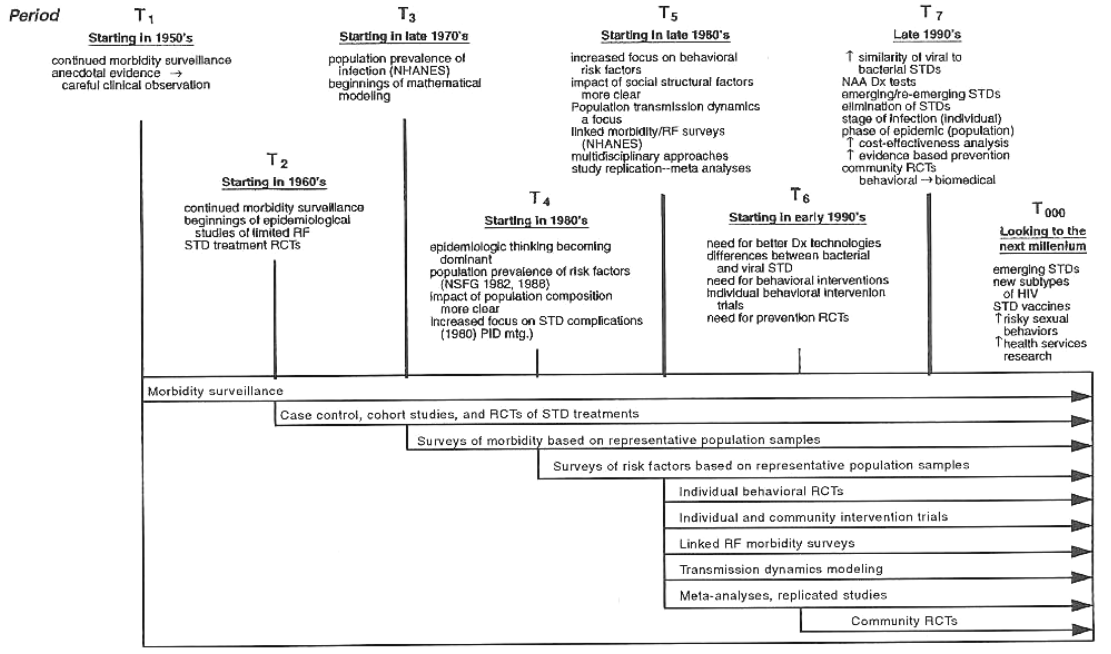

Figure 1.1 History of STD epidemiology and the evolving level of disease-related knowledge (After Aral and Holmes,1999)

1.2.2 The advent of AIDS and discovery of the HIV agent

In the early 1980s, a new infectious disease was recognized mostly among men who have sex with men (MSM), who presented with immunosuppression and associated Kaposi's sarcoma and *Pneumocytis carinii* pneumonia. The causative agent, the human immunodeficiency virus (HIV), was isolated and linked to the disease which was subsequently called acquired immunodeficiency syndrome (AIDS)[16-18]. Later, HIV was detected in genital fluids and it became obvious that the sexual route was the major mode of transmission of HIV globally[19-25]. Since then, HIV infection has spread rapidly throughout the world with devastating effects, particularly in sub-Saharan Africa. An increasing number of babies were born with HIV infection due to the high rate of infection among women in the reproductive age-group. In 2003, WHO/UNAIDS estimated that 40 million adults and children had been infected with HIV globally and that 23.3 million were living in sub-Saharan Africa[26]. Although it accounts for less than 2% of the world's population, southern Africa is currently inhabited by up to 30% of people living with HIV/AIDS worldwide.

1.3 STI-HIV interactions

1.3.1 Risk of HIV acquisition

The bi-directional nature of interactions between conventional STIs and HIV has been documented in many studies[27,28]. It has been recognized that the presence of STIs can increase the risk of acquisition of HIV infection and also promote its transmission[29-31]; likewise the natural history and treatment outcome of STIs might be altered by HIV infection and related immunodeficiency. The magnitude of the impact of this interaction on the HIV epidemic is more prominent in developing countries where the devastating epidemics of HIV infection and STIs exist together and produce a vicious cycle of impoverishment that is less evident in more developed countries (Figure 1.2).

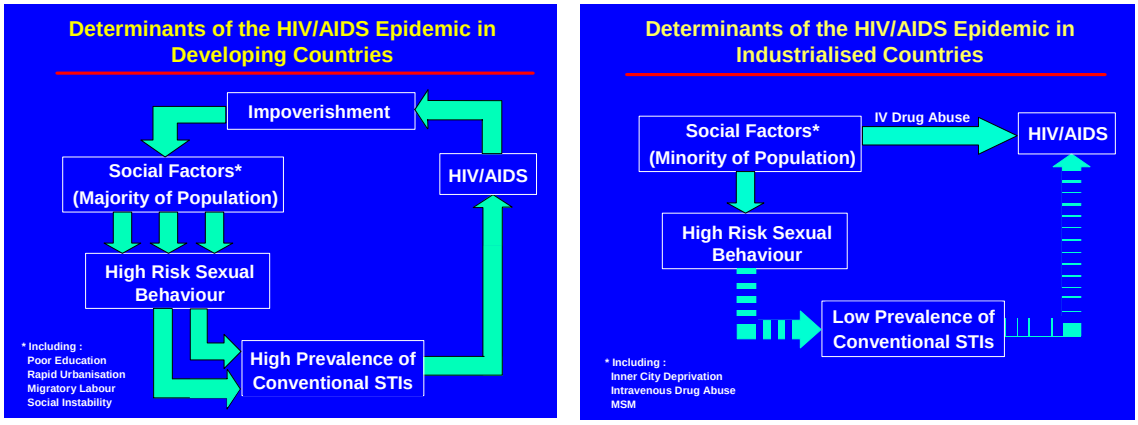

Figure 1.2 Determinants of the HIV/AIDS epidemic

Epidemiological evidence indicates that a significantly higher HIV prevalence occurs among patients with a history of conventional STI episodes when compared with the general population[27]. It is difficult to interpret these epidemiological findings due to the cross-sectional nature of theses early studies, especially as they relate to the excess risk of acquiring HIV as a result of co-infection with an STI. The common high-risk sexual behaviors that predispose individuals to acquisition of both HIV and conventional STIs confound this association. The complexity of factors inherent in the design of these studies, therefore compromises quantification of the attributable risk of active STIs in the acquisition of HIV infection. This situation is more marked in many developing countries where over 90% of conventional STI and HIV transmission occurs through heterosexual transmission.

1.3.2 Risk of HIV transmission related to gender and STI type

During the 1990s, several studies indicated that HIV incidence among patients with repeated STI episodes was significantly higher compared to those without STI. This association was more prominent among those patients presenting with genital ulcer disease (GUD)[32-34]. Statistically significant increases in the risk of HIV seroconversion were found among patients with GUD with an odds ratio (OR) of 3.7 for women and 4.7 for men[35,36]. In the presence of STIs, the risk of HIV infection increases 10 – 50-fold for male to female transmission and a 50 – 300-fold increase for female to male transmission per single sexual act[37]. Although the strength of association was smaller, similar findings were documented for patients presenting with non-ulcerative (discharge-associated) STIs[38]. In recent years, attempts have been made to obtain unbiased information regarding the determinants of HIV transmission by conducting randomised control trials (RCTs)[39]. However, ethical issues related to study design pose problems for, and result in significant delays in the implementation of such studies.

1.3.3 Proposed mechanisms of STI-HIV interactions related to HIV transmission/acquisition

The biological plausibility which explains the mechanisms enhancing the efficiency of HIV acquisition in the presence of active sexually transmitted infections, has been well documented[40-43]. Such enhancement is thought to be due to several mechanisms including the disruption of normal epithelial barriers with either macro- or micro-ulceration and the recruitment of HIV-susceptible cells such as T- lymphocytes or macrophages to the infected area as part of the host's immune response. Studies indicate that HIV target cells such as lymphocytes and macrophages are normally present in male and female genital tract tissues and secretions. Genital inflammation associated with STIs increases the number of target cells in the genital tract and is likely to augment the susceptibility of HIV-seronegative partners by enhancing HIV entry into susceptible host cells[44,47]. Furthermore, epithelial barriers of the genital tract play an important role in host defence against infection. Disruption in the lining of the genital tract, either due to micro- or macro-lacerations is therefore likely to lead to an increase in the susceptibility of individuals to HIV. The scenario outlined above thus

constitutes a compelling argument that the presence of GUD in both men and women could potentially increase the acquisition of HIV infection.

Recent studies have also shown that the presence of an active STI in HIV-seropositive individuals increases the migration of $CD4^+$ cells containing HIV to the genital tract and increases the shedding of HIV in genital secretions[48]. Thus, the presence of STIs could increase the infectiousness of HIV-seropositive partners by increasing the cell-associated viral load in genital secretions. The presence of viable HIV in genital ulcer exudates has also been demonstrated in earlier studies[49,50]. These findings suggest that the transmission efficiency of HIV is increased by co-infection with a symptomatic or asymptomatic conventional STI.

1.3.4 Effect of STIs on the HIV transmission, based on Anderson and May's model

As discussed in Anderson and May's basic reproductive rate of infection model (Figure 1.3)[51] an increase in efficiency of HIV transmission will, theoretically, accelerate the spread of HIV infection. Furthermore, the increase will reduce the effectiveness of behavioural interventions which have been a major focus in strategies to decrease the rate of sexual partner change (D). An overall increase in efficiency of HIV transmission due to conventional STIs therefore becomes an important problem in developing countries where a significant proportion of the population has been infected with HIV.

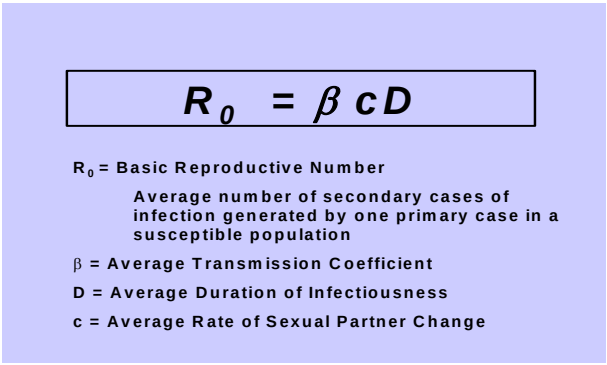

Figure 1.3 Anderson and May's model of the spread of HIV infection

1.3.5 Role of STIs on progression of HIV infection to AIDS

STI episodes are also believed to be associated with the accelerated progression of asymptomatic HIV infection to AIDS. However, confounding variables such as duration of HIV infection at the time of diagnosis, baseline immunological status and other co-factors related to immunosuppression need to be taken into account when designing studies to show this association. The progression from latent HIV infection to AIDS is generally preceded by accelerated viral replication. In most infected individuals, a reduction in $CD4^+$ cell level is paralleled by an increase in viral load, leading to progression to AIDS, characterized by the onset of opportunistic infections. Various infectious agents have been thought to be associated with up-regulating the HIV replication[52]. It has been hypothesized that frequently recurring STI episodes in HIV-seropositive individuals either due to re-infection or reactivation of latent infection, as may occur with genital herpes, might lead to rapid progression to HIV-related immunodeficiency[53]. *In vitro* studies have indicated that viral STIs might enhance HIV infection by various mechanisms, eg. stimulating expression of cell surface receptors that facilitate HIV attachment, coding for gene products that are necessary for HIV growth, or by temporarily or permanently altering HIV expression through phenotypic mixing or genetic recombination[54,55]. Several *in vitro* studies have also suggested that herpesviruses may transactivate HIV. The clinical significance of these findings, including *in vivo* up-regulation of HIV in people co-infected with both viruses, has however not been fully substantiated.

Studies have also indicated reduced efficiency of standard therapies for other common STIs and their complications in HIV-infected patients. Such STIs include chancroid (particularly with single dose therapy), genital herpes (the association of HIV infection with emergence of drug resistant strains) and genital warts[56-61].

As mentioned earlier, the effect of the co-existing STI epidemic on HIV transmission at a community level might include other complex sociocultural determinants. It is therefore necessary to confirm or quantify these co-factor effects of STI on HIV transmission by conducting community-based randomised control trials.

Recently, several community-based RCTs have been conducted to determine the effect of management of STI on the incidence of HIV infection. In order to determine the population attributable fraction (PAF) of improved STI management on HIV transmission, two community randomised control trials (RCT) were conducted in the early 1990s. Firstly, during 1992-94, a RCT was performed in the Mwanza district of Tanzania to assess whether improved treatment services for symptomatic STIs could reduce HIV transmission at the population level[62-64]. The results of the Mwanza RCT showed that improved STI case-management led to a 38% reduction in HIV incidence during the two-year follow-up period. In contrast, no significant difference between the intervention and control communities in the incidence of HIV infection was observed in the Rakai study conducted in Uganda[65,66] where mass treatment for STIs was the intervention. Critical analysis of the possible reasons for the unexpected different outcomes of these two large community-based RCTs produced hypotheses regarding the STI co-factor effect on HIV transmission. The fundamental differences between the two intervention trials that may explain the differences in outcome have been debated in the literature. Differences in study design, type of intervention and the characteristics of the study populations emerged as the most important factors. It became clear that differences in the maturity of the HIV epidemic and the aetiological patterns of STIs in the study populations of the two studies had a major impact on the effectiveness of specific intervention activities[67-69]. In summary, the pattern and magnitude of interactions between STIs and HIV infection appears to vary with different phases of the HIV epidemic and the local patterns and epidemiology of conventional STIs .

Most epidemiological studies conducted to explore the determinants of HIV transmission and STI/HIV interactions were conducted during the early phase of the HIV epidemic. The findings of these earlier epidemiological studies should be interpreted with caution due to the complex nature of the natural history of some STIs even in HIV-seronegative individuals and the lack of reliable gold standard laboratory tests in detecting all stages of infection. With major advances in the knowledge on the natural history of HIV infection together with the availability of newer, more sensitive and specific diagnostic methods, researchers have recently revisited these importance issues.

1.4 Concluding remarks

During the 1990s, great strides were made towards our understanding of the interactions between conventional STI and HIV infection. Although the spread of HIV infection reached pandemic proportions in the 1990s, there is considerable variation in the maturity of the epidemic and the distribution of dominant viral clades in different regions of the world. The diverse nature of the epidemic made generalization of research findings on the HIV-STI interactions problematic. For example, research studies conducted during the late 1990s have shown that the extent of HIV shedding from genital lesions might vary with the aetiology of STIs while co-factors such as immunodeficiency and prevailing HIV sub-types in an area may also play a role. It is therefore important that when research studies are being planned for a specific region, a unique study design be developed for that population group/region.

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CHAPTER 2

Methods used for STI–HIV interaction studies

In order to study the interactions between STIs and HIV infection, several investigational approaches involving appropriately chosen populations at high risk of infection have been followed. In all these studies, core methods have been used for the demonstration of specific aetiological agents of STI syndromes and these are described in this chapter.

2.1. Introduction

Epidemiological information on STIs is necessary to assess the magnitude of the burden of infections, to identify vulnerable population groups, to mobilise resources for intervention activities and to monitor the impact of these activities. In addition, specific STI surveillance systems, such as studies on the relative prevalence of aetiological agents of STI syndromes and their antimicrobial susceptibility patterns, are aimed at improving patient care[1]. For the implementation of such specialised surveillance, comprehensive laboratory support is mandatory.

2.2. Types of tests and their underlying principles

Laboratory tests that are used to determine specific aetiological agent(s) in research studies can be grouped into six categories and are based on the following principles: (i) direct visualization of the causative organism(s) under an ordinary or specialised microscope, (ii) culture of the organism in specific, usually selective, culture media, (iii) detection of antigen by immunological means, (iv) employment of non-amplifying techniques with nucleic acid (DNA/RNA) probes (NAT) for pathogen identification, (v) demonstration of target DNA or RNA of organisms by nucleic acid amplification techniques (NAAT) and (vi) using serological methods for measuring antibody responses to the organism(s)[2,3].

In this chapter, standard specimen collection and laboratory methods used in subsequent studies are described. The comparison of performance of different laboratory methods applied in these studies are summarised in Table 2.1.

Table 2.1 Comparisons of performance of different laboratory methods applied in the research studies

Laboratory tests	Sensitivity	Specificity	Comments
Syphilis			Sensitivity and specificity are for detection of primary syphilis. The sensitivity of both nontreponemal and treponemal antibody detection increases for detection of secondary syphilis. The sensitivity of nontreponemal antibody detection decreases for detection of latent and tertiary syphilis. The tests for *T. pallidum* are only relevant when lesions are present in primary and secondary syphilis.
Darkfield microscopy	74% - 86%	97% - 100%	
Multiplex PCR (M-PCR)	91%	99%	
Serology tests for syphilis			
RPR	72% - 100%	93% - 98%	
TPHA	69% - 90%	98% - 100%	
Chancroid			The sensitivity of culture varies depending on the type of medium used and can only be estimated since there is no gold standard on which to base the diagnosis of chancroid. Resolved sensitivity of PCR vs H. ducreyi culture.
Culture	56% - 90%	100%	
PCR	77% - 98%	98% - 100%	
Donovanosis			
Microscopy	60 -80%	100%	
Herpes simplex virus			
Culture	Gold standard	100%	
Multiplex PCR (M-PCR)	more sensitve than culture	98% - 100%	
Gonorrhoea			Sensitivity and specificity are for dectection of N. gonorrhoea in urethral, endocervical and urine samples by culture except for microscopy, which is for detection in urethral samples from symptomatic men.
Microscopy	90% - 95%	98% - 100%	
Culture	81% - 100%	100%	
LCR	95% - 100%	98% - 100%	
Chlamydia			Sensitivity and specificity are for detection of *C. trachomatis* by culture or by DNA amplification test.
Culture	Gold standard	100%	
LCR	90% - 97%	99% - 100%	
Trichomonas			Sensitivity and specificity are for detection of *T. vaginalis* by combnied wet prep and culture results
Microscopy	38% - 82%	100%	
Culture	98%	100%	
Bacterial vaginosis			Sensitivity and specificity are for diagnosis of BV by presence of 3 of 4 criteria and/or positive Gram stain.
3 of 4 Amsel criteria	81%	94%	
Gram stain	89%	93%	
Human papilloma virus			
Hybrid Capture	74% - 93%	64% - 68%	
PCR	80% - 100%[1]	40% - 61%	[1] for detection of biopsy-confirmed high-grade dysplasia

(Adapted from WHO's publication: Laboratory tests for the detection of reproductive tract infections, 1999)[4]

Details of methods employed for specific purposes will be provided in subsequent chapters. In general, the most specific and sensitive diagnostic methods available during the study period were used at the National Reference Centre for STD (NRC-STD) in Johannesburg and/or other local and international collaborating centres. Standard quality assurance practices prescribed by the South African Institute for Medical Research (SAIMR), now the National Health Laboratory Services (NHLS), at the time were in place throughout[5].

2.3. Demonstration of STI aetiologies according to syndromes

2.3.1 Aetiology of genital ulcer diseases

A complete microbiological investigation was conducted for genital ulcer disease (GUD) presenting in patients enrolled in aetiological studies. Standard methods were used to detect the agents of GUD, namely *Treponema pallidum, Haemophilus ducreyi,* herpes simplex virus (HSV), *Klebsiella granulomatis (*formerly *Calymmatobacterium granulomatis*)*,* and the L-serovars of *Chlamydia trachomatis* (lymphogranuloma venereum biovar). Appropriate inclusion and exclusion criteria for patients were established and strictly followed during the studies (See Table 2.1 – Patient eligibility for enrolment in GUD studies).

All specimens were collected from a single target ulcer or the largest ulcer if more than one ulcer was present. When multiple swabs were collected, in order to avoid bias, random allocation of swabs for different laboratory investigations was performed. In addition, serum samples were collected for serological testing for syphilis, and type-specific assays for HSV and chlamydial infections. Where appropriate, urine and endo-urethral swabs were taken for the detection of concomitant urethral infections caused by *Neisseria gonorrhoeae and Chlamydia trachomatis.*

Table 2.2 Patient eligibility for enrolment in GUD studies

The following criteria were used for determining patients' eligibility for enrolment into GUD studies
• Presenting with visible genital ulceration(s) of at least 2 mm in size with or without inguinal and/or femoral lymphadenopathy (bubo)
• No history of treatment with antibiotics or anti-viral therapy during the past 7 days
• Agreement to complete questionnaires and collection of clinical specimens
• Voluntary participation based on informed consent

2.3.1.1 Diagnosis of syphilis (primary and latent infection)

2.3.1.1.1 Darkfield microscopy

After thorough cleansing with sterile saline, material from the ulcer base was collected with a platinum scraper or the lesion was firmly squeezed between the fingers and thumb and serous fluid collected on a microscope slide. The lesion material on the slide was then mixed with saline, and immediately examined by darkfield (DF) microscopy for motile spirochaetes resembling *T. pallidum (TP)* as described elsewhere[6-8].

2.3.1.1.2 Multiplex polymerase chain reaction (M-PCR)

The lack of a laboratory method for the *in vitro* culture of *T. pallidum* has necessitated the use of alternative diagnostic methods. Later, M-PCR assays were used as the gold standard for detecting *T. pallidum* from genital ulcer exudates. M-PCR was also used to detect other aetiological agents of GUD including *H. ducreyi* and HSV simultaneously in the same specimen. Material from the base of the target lesions was collected on a cotton-tipped swab

(Medical Wire and Equipment, Corsham, UK). Each swab was then expressed into 0.2 ml sterile distilled water and stored frozen at –70•C until analysed. Later M-PCR assay (Roche Molecular Systems) was used to detect target DNA from *T. pallidum, H. ducreyi,* and HSV from the swabs. An aliquot of the processed specimen was also used in a *C. trachomatis* PCR test (Amplicor, Roche Molecular Systems). M-PCR assays were performed at the Centers for Disease Control and Prevention, Atlanta, GA, USA using methods described previously[9].

2.3.1.1.3 Serology tests for syphilis (RPR, FTA-ABS & TPHA)

Clearly marked vacuum collection tubes without anticoagulant were used to collect serum specimens from patients. After collection, specimens were allowed to clot at room temperature. Each specimen was then centrifuged at 1200 x g for at least 5 minutes to sediment cellular elements and the serum subsequently stored at 4 °C or frozen at –70° C.

Serum samples were tested using both non-treponemal (quantitative RPR card test, IMMUNOTREP® Omega Diagnostics, Alloa, Scotland, UK) and treponemal tests (FTA-ABS, Marburg, Germany and/or TPHA, Randox Laboratories, Antrim, UK) to determine the serological status of patients for syphilis. All serological tests were performed according to standard operating procedures (SOPs) of the South African Institute for Medical Research (SAIMR)[5].

2.3.1.2 Diagnosis of chancroid

Haemophilus ducreyi was identified from ulcer exudate either by culture using a modified Columbia agar system[5,10,11] or by M-PCR[9].

2.3.1.2.1 Culture

The selective culture medium used to isolate the *H. ducreyi* comprised Columbia agar base (Sigma), activated charcoal, 1% haemoglobin, 1% (vol/vol) IsoVitaleX (BBL Microbiological Systems, Cockeysville, Md.), 5% foetal calf serum (Adcock Ingram Diagnostics, Johannesburg, SA), and 3 μg/ml vancomycin. After thorough cleansing, material from the

base of each target ulcer was collected on calcium alginate swabs (Calgiswab, Medical Wire and Equipment, Corsham, UK) and inoculated directly onto the selective solid medium. The inoculated culture plates were immediately placed in a candle-extinction jar (5-10% CO_2) and kept at room temperature during transport to reach the laboratory within 12 hours after collection. On arrival, the inoculated agar plates were placed in an anaerobic jar without catalyst (BBL Microbiological Systems, Cockeysville Md.) to create a humidified atmosphere containing and incubated at 33-35^0 C for 48-72 hours. Colonies were identified by typical morphology (see below) and the following confirmatory tests:

- Cohesive whitish-grey colonies
- Push test: intact colonies move across agar surface
- Gram stain: Gram -ve short rods in parallel arrangement or "school of fish" appearance
- Production of acid in glucose but not in lactose or sucrose
- Oxidase positive (tetramethyl-p-phylenediamine reagent)
- Produces porphyrin from δ-aminolaevulinic acid (porphyrin test positive)
- Catalase negative (5% H_2O_2 v/v),
- beta-lactamase positive
- alkaline phosphatase positive

Colonies from purified cultures were stored in three polypropylene vials per isolate. A heavy inoculum was placed in DMSO + 20% foetal calf serum (Adcock Ingram Diagnostics, Johannesburg, SA) or 20% skimmed milk + 10% glycerol (Diagnostic Media Products, Johannesburg, SA) and stored at -70^{0}C.

2.3.1.2.2 Multiplex polymerase chain reaction (M-PCR)

M-PCR was used as gold standard for detecting *H. ducreyi* according to the procedure described in Section 2.3.1.1.2[9].

2.3.1.3 Diagnosis of lymphogranuloma venereum (LGV)

Historically, the diagnosis of LGV has been based on a positive Frei skin test, a positive serological test for LGV, isolation of *C. trachomatis (L1, L2, L3)* from infected

tissues/secretions, histological identification and/or PCR[12]. However, the poor sensitivity of the Frei skin test (test usually become positive after appearance of buboes or 2-8 wks after infection) and likewise poor specificity (Frei antigen common to all *Chlamydia* spp. and life-time positivity despite successful treatment) has limited its utility for routine diagnosis[13,14] and is no longer used.

In the present studies, attempts were made to diagnose LGV by applying a combined approach which included *C. trachomatis (CT)* culture, Chlamydial LCR™ (Abbott Laboratories, North Chicago, Ill.) and quantitative measurement of type-specific chlamydial antibodies using the micro-immunofluorescence (MIF) technique. Genotyping of *C. trachomatis* strains was not performed.

2.3.1.3.1 Chlamydial culture

After thorough cleansing, material from the base of each target ulcer was collected on dacron swabs (Hardwood Products Company, Guilford, Maine). Immediately following collection, swabs were agitated vigorously in chlamydial transport medium and discarded after expressing the liquid from the swab to avoid leaving toxic swab material in the transport medium. Sucrose phosphate buffer (2SP) (Highveld Biologicals, Johannesburg, SA) was used as transport medium for clinical specimens. Specimens were kept on ice and transported to the laboratory within 24 hours for immediate processing or stored at -70^0C until batch inoculation. The cell culture method using McCoy cells (mouse fibroblasts) (Highveld Biologicals, Johannesburg, SA) was employed to isolate the *C. trachomatis* from clinical specimens[17]. Cells were grown in Eagle's minimal essential medium (MEM), supplemented with 10% foetal calf serum, 2 mM glutamine, 30μM glucose, 0.75% sodium bicarbonate, 20 μM herpes buffer and 10 μg/ml of gentamicin (all constituents from Highveld Biologicals, Johannesburg, SA)[15,16].

Approximately 0.5-1.0 ml of the clinical specimen in transport medium inoculated into each of two vials of confluent cell monolayers on glass coverslips following standard procedures as described elsewhere[3,5]. After 72 hours' incubation at 35°C in an atmosphere of 5-10% CO_2 in air, the monolayers were transferred onto glass slides and were then fixed in methanol and

stained either with fluorescein-labelled monoclonal antibody (MicroTrak, Syva, Palo Alto, CA). The stained slides were examined under a fluorescence microscope at 40x magnification. In a positive specimen, homogenous, granular cytoplasmic inclusions are found in infected cells exhibiting characteristic apple-green fluorescence.

2.3.1.3.2 Chlamydial serology (microimmunofluorescence test)

Although the microimmunofluorescence (MIF) test has limited value in the diagnosis of active chlamydial infection, it is a useful test for epidemiological studies[18-20].

Elementary bodies of chlamydial organisms grown in the yolk sacs of embryonated eggs are used as antigen. Standard microscope slides with eight clusters containing antigens of immunotypes grouped according to clinical disease entities were used. Pooled antigens groups included were (i) ABC (trachoma serovars), (ii) DEFGHIK (oculo-genital serovars), (iii) L1, L2, L3 (LGV serovars), (iv) *Chlamydia psittaci,* (v) *Chlamydia pneumoniae* and (vi) a normal yolk sack suspension served as a negative control. The testing was done according to standard procedures described elsewhere[3,5]. Antigen clusters were examined by fluorescent microscopy using a X40 objective. Fluorescence was graded according to colour brightness and evenly distributed elementary bodies.

2.3.1.4 Diagnosis of donovanosis (GI)

The characteristics of Donovan bodies in macrophages and epithelial cells of the stratum malpighii were described by Donovan in 1905. Since then, tissue smears stained by the Leishman or Giemsa methods are used as standard diagnostic method[21,22]. There is no established culture system for the routine identification of the aetiologic agent, *Calymmatobacterium klebsiella granulomatis.* Several investigators have claimed the successful isolation of the causative organism since 1913[23-26]. However, more recently Kharsany *et al*[25] in Durban, South Africa and Carter *et al*[26] from Australia reported the propagation of *C. granulomatis* in monocyte and McCoy cell cultures respectively, but these techniques have yet to be used routinely for diagnostic purposes. A molecular amplification method has been developed to characterize DNA sequences of the organism from genital

donovanosis lesions using PCR[27]. Serological tests applying complement-fixation and indirect immunofluorescent technique have been developed and have been used in epidemiological studies[28,29]. In the studies, reported here, only clinically suspicious lesions were investigated for the presence of Donovan bodies using the Giemsa staining method. After thorough cleansing, material from the edges of the target ulcer was collected with a platinum scraper, spread on a microscope slide, fixed with methyl alcohol, stained (with Giemsa's stain), and examined for the presence of Donovan bodies. Donovan bodies are pleomorphic, bean-shaped microorganisms with small intrabacterial inclusions showing a bipolar distribution, and giving the microorganism a closed safety-pin appearance.

2.3.1.5 Diagnosis of herpes simplex virus (HSV) infection

Four categories of test have been used for the diagnosis of genital herpes: rapid detection techniques, tissue culture-based methods, serological methods and molecular amplification methods[30,31]. In these studies, herpes simplex virus culture using human embryonic lung fibroblasts (HLF), type-specific PCR and/or M-PCR techniques were used to demonstrate viable virus or virus-specific DNA sequences in lesions, while type-specific serology was employed in some studies.

2.3.1.5.1 Culture of herpes simplex virus

After thorough cleansing, material from the base of each target ulcer was collected on a dacron swab. Immediately following collection, the swab was agitated in viral transport medium (MEM plus 10% foetal calf serum with antibiotics; Highveld Biologicals, Johannesburg, SA) expressed and then discarded. Specimens were kept on ice for up to 8 hours, transported to the laboratory where they were either cultured immediately or stored at -70^0C until inoculation into cell cultures.

Human embryonic lung fibroblast (HLF) monolayers were cultured in Eagles E-MEM with L-glutamine plus 10% FCS and antibiotics (all constituents from Highveld Biologicals, Johannesburg, SA). The standard procedures, described elsewhere[5], were followed for preparation, storage and trypsinization of cells. Each tissue culture tube (with one containing

a coverslip) was seeded with 2ml of $2x10^7$ cells/ml in growth media plus 10% FCS and antibiotics and incubated at 35ºC in a CO_2 incubator. The medium was changed after 72 hours with growth medium containing 2% FCS prior to inoculation.

Each of two roller tubes was inoculated with 0.2ml of sample in MEM plus 2% FCS and incubated at 35°C in an atmosphere of 5-10% CO2 in air in a roller drum apparatus. The tubes were examined microscopically every day for the presence of cytopathic effect (CPE). Positive tubes with coverslips were fixed in Bouins' fixative (SAIMR-STD Quality Assurance Manual, 1996). The slides were then stained with haematoxylin for one hour and counterstained with eosin for 2 minutes after which they were mounted with DPX and read at 400x magnification. The presence of herpes simplex virus was indicated by a characteristic bulging or "ballooning" of the infected fibroblasts and the presence of a typical cytopathic effect with intracellular eosinophilic inclusions, producing a "stippling effect" with a halo formation. The isolates were typed as either HSV-1 or HSV-2 using fluorescein labelled monoclonal antibodies[3,32].

2.3.1.5.2 Multiplex PCR

M-PCR was used as gold standard for detecting type-specific HSV and the procedure has been described in Section 2.3.1.2 and elsewhere[9].

2.3.1.5.3 HSV PCR

In addition to M-PCR, type-specific HSV PCR testing was performed in some studies, using an in-house PCR method. After thorough cleansing, material from the base of each target ulcer was collected on a calcium aginate-coated dacron swab. Swabs were stored at -70^0C and processed in batches using the method described in the annexure 2.1.

2.3.1.5.4 Herpes serology

Although various type-specific serological tests for HSV infection are available, they are not useful for diagnosing mucosal lesions caused by HSV. In some studies, complementary type-specific serology was used to detect the individuals' previous exposure to herpes simplex

viruses. Standard serological methods such as ELISA and/or Western Blot were applied to detect the type-specific antibodies to HSV-1 and HSV-2. In each case blood samples were collected and allowed to clot at room temperature prior to centrifugation. Serum was then transferred aseptically to a tightly closing sterile container for storage. Samples were frozen at -70°C and processed in batches following thawing. HSV type-specific antibody testing was performed at the CDC, Atlanta, USA, as previously described using Western Blot analysis of recombinant-derived, baculovirus-expressed HSV glycoprotein G1 and G2 from HSV-1 and HSV-2, respectively[33,34]. In some studies the Focus HSV-2 ELISA IgG test (Focus Diagnostics, Cypress, CA) was used for the detection of antibodies to HSV-2. The test procedure was followed according to the manufacturer's instructions[35,36.]

2.3.2 Aetiology of acute urethritis in men

Patients complaining of painful or burning micturition with or without visible signs of urethral discharge, were enrolled into the studies. Asymptomatic men were also enrolled in community-based prevalence assessment studies. Other eligibility criteria for enrolment were the same as in the GUD studies (See Table 2.2).

2.3.2.1 Diagnosis of gonococcal urethritis

The laboratory diagnosis of gonococcal infection[37] includes identification of *N. gonorrhoeae* by;

- microscopic examination of stained smear
- culture
- non-culture diagnostic methods
- non-amplified techniques such as EIA, DNA probe test
- amplified nucleic-acid detection techniques such as LCR, PCR, transcription-mediated amplification (TMA).

In these studies, microscopy of stained smears (Gram stain), culture and LCR tests were employed to diagnose acute gonococcal urethritis in men.

2.3.2.1.1 Microscopy of genital exudates for gonococci

The genital exudate from the urethral meatus was collected using a cotton swab at least two hours following the last micturition. Immediately following collection, the swab was rolled onto two clean microscope slides immediately following collection and allowed to air dry. Smears were fixed with the film uppermost by passing the slide rapidly three times through a flame, avoiding overheating which may distort morphology of the cellular material. The slides were stained using the standard Gram's staining method[5]. The stained smears were examined microscopically for evidence of urethritis (5 or more polymorphs per high power field). The presence of Gram-negative intracellular diplococci supported a presumptive diagnosis of gonococcal urethritis. The absence of diplococci with 5 or more polymorphs per high power field was indicative of non-gonococcal urethritis (NGU).

2.3.2.1.2 Culture for *N. gonorrhoeae*

An endourethral swab, also taken at least two hours after micturition, was inserted at least 2 cm into the urethra and gently rotated. Swabs were then inoculated immediately at the clinic onto New York City (NYC) medium and placed into a candle extinction jar. Inoculated plates were placed at 35°C in a humidified atmosphere in a candle-extinction jar containing an atmosphere of 5-10% CO2 in air and incubated for 24 - 48hrs. Typical translucent colonies of *N. gonorrhoeae* were identified by Gram staining, a positive oxidase test and conventional carbohydrate utilization tests or employing commercially available rapid fermentation tests. All confirmed gonococcal isolates were stored in at least three polypropylene vials per isolate. A heavy inoculum was placed in trypticase soy broth + 20% glycerol and stored in vials at −70°C[3,5].

2.3.2.1.3 Ligase chain reaction for gonococcal infection (LCR-GC)

First catch urine specimens were collected in sterile containers and stored at 4°C for immediate processing or at -70°C for batch processing. The Ligase Chain Reaction (LCx™, Abbott Laboratories, North Chicago, Ill.) was used for the qualitative detection of a specific target nucleic acid sequence in the *opa* gene of *N. gonorrhoeae*. The standard amplification and

detection procedure was carried out according to the manufacturer's instructions provided with the kit and described elsewhere[38].

2.3.2.2 Diagnosis of chlamydial urethritis

First catch urine specimens and/or endourethral swabs from patients presenting with urethritis were tested for the presence of *C. trachomatis (CT)* using either McCoy cell or LCR techniques culture as described in sections 2.3.1.3.1 and 2.3.2.1.3 respectively. A specific target nucleic acid sequence detected by the LCR test is the chlamydial plasmid.

2.3.2.3 Diagnosis of ureaplasma infection

Four species of the order *Mycoplasmatales* are found in the human genital tract: *Mycoplasma hominis, Mycoplasma fermentans, Mycoplasma genitalium* and *Ureaplasma urealyticum.* An association between acute urethritis in men and infection with *Ureaplasma urealyticum* was first documented in 1974 by Shepard[39]. More convincing evidence of an aetiological role for *Ureaplasma urealyticum* in NGU was obtained following human intraurethral inoculation of ureaplasma and the development of signs and symptoms of urethritis[40,41]. Recovery of ureaplasmas from the urethra from a person with NGU does not always indicate that they are the cause of the urethritis. *U. urealyticum* has been isolated from many men who have had no clinical evidence of urethritis[42]. It has been suggested that *Ureaplasma urealyticum* causes 10 to 40% cases of NGU. In theses studies, genital mycoplasmas were detected by using culture methods. Endourethral swabs were collected as described in section 2.3.2.1.2 and *U. urealyticum* and *M. hominis* were isolated using standard culture methods described below. No attempt was made to identify other mycoplasma species during the study period.

2.3.2.3.1 Culture of *U. urealyticum*

Immediately following collection, swabs were agitated in Shepards' U9 broth, expressed against the sides of container, and discarded. Inoculated U9 broth was kept on ice and transported to the laboratory within 24 hours. The broths were placed with loosened lids in an anaerobic atmosphere (anaerobic jar) and incubated at 35°C for 24 - 72hrs. Cultures were examined for a colour change every 12 hours. A change of colour from yellow to light

orange/pink was interpreted provisionally as positive. All positive U9 broths were subcultured on A2 medium for growth of colonies of *U. urealyticum.* The inoculated A2 medium was incubated at 35°C for 24-48hrs anaerobically. The agar plates were examined under light microscope (X10 objective) for colonies which appeared as coccoid particles. *M. hominis* colonies were also detected based on their "fried-egg" appearance.

2.3.2.4 Diagnosis of other causes of urethritis

Studies have shown that 30 to 50 percent of heterosexual men with NGU were negative for both *C. trachomatis* and *U. urealyticum*. Other possible causes of urethritis include *M. genitalium*, *Trichomonas vaginalis,* yeasts, HSV, adenovirus, *Haemophilus* species and other bacteria[43]. Owing to logistical constraints, attempts were only made to identify *T. vaginalis* in selected studies. In most studies, NGU was categorised as either *C. trachomatis* and *U. urealyticum*-positive (CT/UU +ve) group, *C. trachomatis* or *U. urealyticum*-positive group (CT +ve/ UU−ve or CT−ve/UU+ve), and a *C. trachomatis* and *U. urealyticum-negative* (CT/UU –ve) group.

2.3.2.4.1 Diagnosis of *T. vaginalis*

Trichomonas vaginalis can be identified using direct microscopy of urine or stained urethral smears using Gram, Giemsa, periodic acid-Schiff, acridine orange, fluorescein, neutral red or immuoperoxidase staining techniques[44,45]. Culture methods using liquid and semisolid media have remained the “gold standard” tests for the detection of *T. vaginalis*[46-48]. However, better detection rates were reported using PCR when compared to culture. Further evaluations are required to determine both the optimal specimen for PCR testing and the clinical significance of a positive result[49-52].

In these studies only culture methods were used to identify *T. vaginalis* in the urethra of men presenting with urethritis. Endourethral swabs were collected as described in section 2.3.2.1.2 and inoculated directly by agitating the swab in Diamonds' medium. The inoculated bottles of Diamonds' medium were placed at 35°C in an anaerobic atmosphere with loosened lids initially for 24-72 hours. A drop of the incubated Diamonds' medium was examined daily for 7 days on a

clean glass slide covered with a coverslip and scanned microscopically under X400 magnification for motile trichomonads.

2.3.3 Aetiology of reproductive tract infections (RTIs) in women

Non-menstruating women complaining of vaginal discharge with or without lower abdominal pain were enrolled in the studies described in this thesis. Asymptomatic women were also enrolled in community-based prevalence assessment studies.

Appropriate laboratory methods were employed to detect the aetiological agents of both vaginal and cervical infections, namely, *T. vaginalis*, *Candida* spp, *Mycoplasma* spp, bacterial vaginosis, *N. gonorrhoeae, C. trachomatis,* Human Papilloma Virus (HPV) and Herpes Simplex Virus (HSV).

2.3.3.1 Diagnosis of bacterial vaginosis (BV)

In 1983 Amsel *et al* [53] established criteria for the diagnosis of bacterial vaginosis based on the presence of at least three of the following four clinical signs;

1. Characteristic homogenous white adherent discharge
2. Vaginal fluid pH >4.5
3. Release of a fishy amine odour from vaginal fluid when mixed with 10% KOH
4. Presence of clue cells (representing at least 20 percent of vaginal epithelial cells).

However, subsequent studies have indicated that the presence of clue cells represents the best single criterion for the diagnosis of BV[54].

Specific guidelines for the reading of vaginal Gram-stained smears were described by Dunkelberg[55] and Spiegel *et al*[56]. Later, in 1991, Nugent developed a standardized scoring system for evaluation of Gram-stained vaginal smears[57]. This system was based on three bacterial morphotypes: large Gram-positive rods (lactobacilli), small Gram-negative or variable rods (*Gardnerella* and anaerobic rods), and *Mobiluncus*[57]. Due to the high number of women without BV (up to 58%) harbouring *G. vaginalis*, culture for *G. vaginalis* has little value

for the diagnosis of BV[58]. However, a specific oligonucleotide probe test adjusted to detect only high concentrations (>10^7/mL of vaginal fluid) of *G. vaginalis* has shown improved specificity in detecting the infection[59]. In the studies reported here the Amsel criteria were applied to diagnose BV. However, the presence of typical clue cells together with absence of lactobacilli is used in some studies where only Gram-stained smears were available to establish the diagnosis.

2.3.3.1.1. Microscopy of vaginal wet mount (Amsel criteria)

The vaginal fluid accumulating in the posterior fornix was collected using sterile cotton-tipped swabs (Medical Wire and Equipment, Corsham, UK). The sample was directly placed onto pH paper immediately following collection. The swab was then agitated in 1ml of saline and a drop of fluid placed on a clean glass slide, a drop of 10% KOH solution was added, and the generation of a distinctive "fishy" odour, due to the releases of amines, was considered indicative of BV. Two drops of fluid were also placed to a clean glass slide and covered with a coverslip. The slide was examined for "clue cells" which are epithelial cells covered with many vaginal bacteria giving the margins of the cells a granular appearance under light microscopy using a X40 objective.

2.3.3.1.2 Gram stain for BV diagnosis

Immediately following collection, the swab was rolled onto a clean glass slide and stained with Gram's stain. The smear was examined under X400 magnification for "clue cells", epithelial cells containing large numbers of Gram-negative or Gram-variable coccobacilli. These bacteria obscure the defined edges of the epithelial cells. This finding together with the absence of lactobacilli was considered indicative of BV.

2.3.3.2 Diagnosis of vaginal trichomoniasis

In all studies, undertaken on women both wet mount microscopy and culture methods were employed to identify *T. vaginalis* in vaginal secretions.

2.3.3.2.1 Wet mount microscopy for *T. vaginalis*

A procedure similar to that used for detecting clue cells (Section 2.3.3.1.1) was used to detect motile flagellate trichomonads. Microscopically, these protozoa have an ovoid globular shape and a characteristic jerky or twitching motility.

2.3.3.2.2 Culture of *T. vaginalis*

Vaginal fluid from the posterior fornix was collected using a sterile cotton-tipped swab (Medical Wire and Equipment, Corsham, UK) and cultured for *T. vaginalis* as described in Section 2.3.2.4.1.

2.3.3.3 Diagnosis of vaginal candidiasis

Over 80 percent of cases of vulvovaginal candidiasis (VVC) have been associated with the species *Candida albicans* while the remainder are caused by non-*albicans* species such as *Candida glabrata*[60]. Yeast cells and pseudohyphae may be readily detected in vaginal secretions using microscopic examination of wet mounts or KOH preparations. Up to 50 percent of patients with culture-positive symptomatic VVC however have been found to have negative microscopy[61]. On the other hand, owing to the carriage of commensal yeasts in the vagina, a positive culture does not necessarily indicate a pathogenic role of the yeasts[62]. It is therefore necessary to consider clinical findings, in conjunction with microscopic examination and vaginal culture for the diagnosis of VVC.

In our studies, women presenting with vaginal discharge who were positive for yeasts and pseudohyphae on wet mount microscopy and/or on culture, were considered having VVC.

2.3.3.3.1 Wet mount microscopy for *Candida* organisms

A procedure similar to that used for detecting clue cells as described in Section 2.3.3.1.1 was employed and examined under microscopy for the presence of yeasts/pseudohyphae.

2.3.3.3.2 Culture for *Candida* spp.

Vaginal fluid from the posterior fornix was collected using a sterile cotton-tipped swab (Medical Wire and Equipment, Corsham, UK). Immediately after collection, the vaginal swab was inoculated onto a dextrose/chloromycetin agar plate and kept at room temperature. Subsequently the inoculated plates were incubated at 33-35^{O}C aerobically for 24-48 hours. The presence of typical colonies on the agar plate and presence of yeast cells on Gram-staining confirmed the presence of yeasts. Differentiation of *Candida* species using the germ tube method was performed in these studies.

2.3.3.4 Diagnosis of vaginal mycoplasmal infections

Although both ureaplasmas and *M. hominis* may colonize the female genital tract without producing disease, several studies have found that *M. hominis* can be recovered more frequently from vaginal and cervical specimens collected from patients presenting with PID[63]. In the present studies, attempts were made to isolate only *U. urealyticum* and *M. hominis* from vaginal secretions.

2.3.3.4.1 Culture of *U. urealyticum*

Vaginal fluid from the posterior fornix of women was collected using sterile cotton-tipped swabs. Similar procedures to those described in Section 2.3.2.3.1 were used.

2.3.3.4.2 Culture of *M. hominis*

Specimens for culture of *M. hominis* were collected as for *U. urealyticum.* Immediately following collection, swabs were agitated in PPLO broth (Diagnostic Media Products, Johannesburg, SA), expressed in the liquid medium and discarded. The specimens were stored on ice and transported to the laboratory within 24 hours. The inoculated PPLO broths were placed with loosened lids in an anaerobic atmosphere (anaerobic jar) and incubated at 35^{O}C for 24-72hrs. The presence of a colour change of the broth from red to dark red was reported as positive. All 'positive' PPLO broths were subculture onto PPLO agar and incubated at 35^{O}C for 3 – 5 days. The agar plates were examined under a light microscope (X10 objective) for colonies with a typical "fried-egg" appearance.

2.3.3.5 Diagnosis of gonococcal cervicitis

Endocervical swabs for culture of *N. gonorrhoeae* were collected by rotating the swab in the endocervical canal after the cervix was wiped clean of accumulated discharge with a sterile ball of cotton wool or gauze. Urine specimens were collected for gonococcal LCR assays. Similar laboratory procedures for culture and LCR for *N.gonorrhoeae* were followed as described in Sections 2.3.2.1.2 and 2.3.2.1.3. Microscopic examination of stained cervical smears has a lower predictive value compare to that of the urethral smear in men with urethritis and therefore direct microscopic examination of Gram-stained cervical smears was not performed.

2.3.3.6 Diagnosis of chlamydial cervicitis

Endocervical swabs for culture of *C. trachomatis* and urine specimen for LCR were collected as described in Section 2.3.3.5. The endocervical swab was tested for the presence of *C. trachomatis (CT)* using the standard culture method as described in Section 2.3.1.3.1. LCR was used to detect *C. trachomatis* in urine specimens as described in Section 2.3.2.1.3.

2.3.3.7 Diagnosis of cervical HSV infection

Cervical HSV infection may be asymptomatic or may present as a mucopurulent cervicitis. Studies suggest that evidence of HSV cervicitis may be found during colposcopy and on Papanicolaou smear microscopy in up to 70 percent of cervical HSV infection[30]. In our studies both culture and type-specific PCR were used to diagnose cervical HSV infection.

After thorough cleansing of accumulated discharge on the cervix, the specimen was collected from the ectocervical area on a sterile dacron swab. HSV infection was detected using culture and PCR methods as described in Sections 2.3.1.5.1 and 2.3.1.5.3 respectively.

2.3.3.8 Diagnosis of human papilloma virus (HPV) infection

Human papilloma viruses cannot be propagated in tissue culture although McLean et al has reported a successful animal model to mimic natural HPV infection using a transplantation technique in mice with a karatinocyte cell line expressing HPV16 E7 or E7[64]. For diagnostics

and epidemiological research purposes, the molecular hybridization and amplification techniques have been used to identify HPV in various specimens[65,66]. Serological tests have been developed mainly for use in seroepidemiological studies of HPV. In our studies, the presence of cervical HPV lesions was detected using PCR and Hybrid Capture Assay (Digene Diagnostics, Silver Spring, MD).

2.3.3.8.1 Hybrid capture assay

Specimens were collected from the endocervical canal and transformation zone using the swab provided in the Hybrid Capture Assay (HCA-Digene Diagnostics, Silver Spring, MD). Immediately after collection, swabs were placed in the transport medium provided by the manufacturer and then stored at -70^{O}C until processed. The presence of low- and high-risk HPV types was tested according to the manufacturer's instructions[67]. HPV types were detected in specimens by using probe A (HPV types 6, 11, 42, 43, and 44) and probe B (HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56).

2.3.3.8.2 HPV PCR (consensus primer; MY9/11)

In addition to the Hybrid Capture System, HPV PCR assays using the consensus primer (MY9/11) were conducted at the Centers for Disease Control and Prevention (CDC), Atlanta, GA. The detailed procedure for HPV PCR has previously been described elsewhere[68].

2.3.4. Diagnosis of HIV infection

HIV infection can be detected by culture of the virus or direct identification of viral-specific antigens or nucleic acid sequences such as P24 antigen, viral DNA or RNA. The demonstration of HIV-specific antibodies is the most widely used diagnostic method owing to its simplicity and low-cost. It cannot, however, detect recently infected individuals because of the presence of the seronegative "window period" which may last approximately 6 – 12 weeks.

In the studies reported here, the HIV status of patients was determined by standard antibody tests. An unlinked, anonymous testing protocol was employed for routine surveillance studies.

Patients who requested voluntary counselling and testing were provided with pre-test and post-test counselling. HIV antibodies were detected using a standard automated ELISA method (AxSYM HIV1/2g0, Abbott Diagnostics, North Chicago,ILL) and/or a rapid test employing a latex agglutination method (Capillus HIV-1/HIV-2, Cambridge Biotech, USA) or chromatographic strip test (Determine HIV, Abbott Laboratries, North Chicago, ILL). In selected studies, all HIV antibody positive participants were further evaluated by performance of a quantitative HIV plasma viral load assay and $CD4^+$ lymphocyte count. Viral isolation, p24 antigen and HIV proviral DNA tests were not performed during these studies.

2.3.4.1 HIV antibody

Blood samples were collected and sera separated as described in Section 2.3.1.1.3. The South African Department of Health and SAIMR HIV antibody testing protocol was employed for all patients who requested voluntary HIV counselling and testing. It comprised screening using a rapid test (i.e., Capillus® HIV-1/HIV-2, Cambridge Biotech, USA) and positive samples were confirmed by an ELISA test (AxSYM HIV1/2g0, Abbott Diagnostics). All indeterminate or low-titre positive specimens were further tested using the Western Blot method (HIV2.2 Blot, Genelab Diagnostics). In studies where unlinked anonymous HIV testing was performed on all participants, only a single rapid test (Capillus® 1&2) was used. The performance of the rapid test used in all studies was evaluated under local conditions[69].

2.3.4.2 HIV plasma viral load measurements

Vacuum blood collection tubes with EDTA were used to collect plasma specimens from patients. After collection, blood specimens were kept at room temperature and subsequently centrifuged at 1200 x g for at least 5 minutes to sediment cellular elements. The plasma was separated and stored at -70°C. Viral load measurements were performed using the Amplicor Monitor Assay (Roche Molecular Systems, Branchburg, NJ) at the National Institute for Virology (NIV), Johannesburg, South Africa. The manufacturer's instructions were followed[70]. A quantitation standard was used to measure RNA with a detection limit of 400 RNA copies/mL. The method detects a range of up to 750,000 RNA copies/mL.

2.3.4.3 $CD4^+$ lymphocyte counts

As the $CD4^+$ lymphocyte is the primary target of HIV, $CD4^+$ cell counts have been routinely used to determine the level of immunosupression in HIV-1 infected individuals. The depletion of $CD4^+$ lymphocytes is strongly associated with the occurrence of specific opportunistic infections[71].

The enumeration of T-cell subsets involves three distinct measurements: the white blood count (WBC), the lymphocyte differential count (percent lymphocytes), and the percentage of T lymphocytes that were $CD4^+$, $CD8^+$ and $CD3^+$ positive. In these studies, the WBC and percent lymphocyte were routinely measured on whole blood samples using an automated haematology instrument (EPICS XL-MCL Flow Cytometer and Coulter T-Q prep, Coulter Corporation, Hlaleah, FL). The $CD4^+$, $CD8^+$, $CD3^+$ percentages were determined on whole blood by flow cytometric immunophenotyping.

Vacuum blood collection tubes containing EDTA were used to collect whole blood specimens from patients. After collection, specimens were kept at room temperature and sent to the laboratory within 24 hours for further processing, as described elsewhere[72]. The following reference ranges for adults were used in the interpretation of results;

White cell count:	4-11 *x* 10^9/l
Total lymphocyte count:	1-4 x 10^9/l
$CD4^+$ cells:	500-2010 x 10^6/ml
$CD8^+$ cells:	250-990 x 10^6/ml
$CD3^+$ cells:	699-3001 x 10^6/ml
CD4:CD8 Ratio:	2:1

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CHAPTER 3

Changes in clinical presentations and aetiology of sexually transmitted infections among South African mineworkers, coinciding with the advent of the HIV/AIDS epidemic

3.1 Introduction

3.1.1 Global perspective

Since the early 1980s, the global epidemiology of sexually transmitted infections (STIs) including HIV and its determinants have been extensively studied by researchers from the sociological, anthropological and medical disciplines[1-5]. Like many other infectious diseases, the epidemics of conventional STIs and HIV/AIDS have been largely driven by social, cultural, economic, behavioural and biological factors. Both epidemics have triggered an intense and ongoing debate over the issues related to wider socioeconomic, racial and gender inequities which have plagued many societies for past decades.

In general, the most common mode of transmission of STIs and HIV is through the practice of unsafe sexual activities. To have effective insights into STIs and HIV epidemics in a defined geographical area, an in-depth understanding of the existing sexual networking patterns in the area is required. This knowledge has assisted many public health authorities to implement effective prevention and control strategies for STIs and HIV/AIDS.

Globally, the existence of diverse social determinants and sexual networking patterns between population groups within countries and regions leads to continuous challenges to our understanding of STI and HIV epidemiology. UNAIDS/WHO has established working definitions of HIV/AIDS epidemics, whereby countries have been classified into three categories based on the magnitude of the problem and the dominant transmission pattern prevailing in a particular country[6-7] (Figure 3.1).

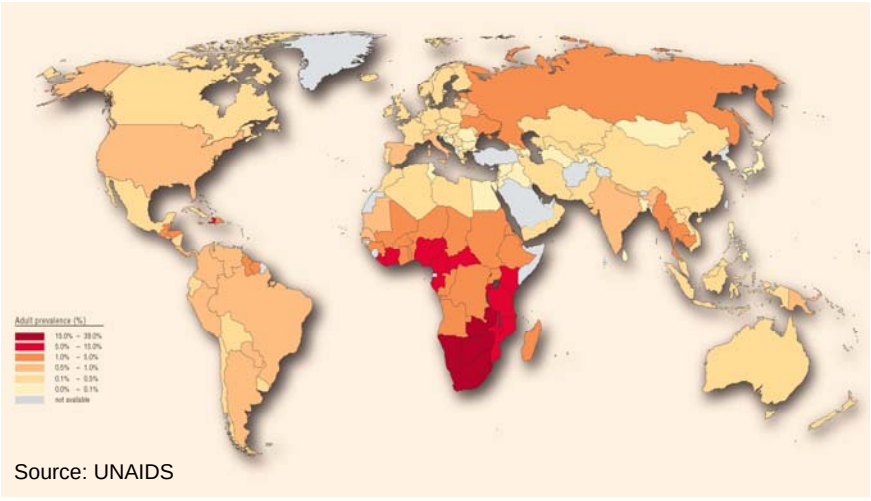


Figure 3.1 Global distribution of HIV infection
(*Numerical proxy for type of HIV epidemics – Generalized epidemic: HIV prevalence >1% in general population & >5% in a high-risk population group; Concentrated epidemic: HIV prevalence <1% in general population but >5% in a high-risk population group; Low-level epidemic: HIV prevalence <5% in high-risk groups)*

3.1.2 STIs and HIV/AIDS in southern Africa

In sub-Saharan Africa (SSA), almost all countries have generalized HIV epidemics where the majority of transmissions occur through unsafe sexual practices with a phase of accelerated spread playing havoc among the sexually active general population (e.g. pregnant women). During the early 1990s, a high burden of STIs and HIV infections had already been reported in many countries in SSA, especially in central Africa.

Rapid and sustained spread of HIV followed and the disease became rampant in many southern African countries during the mid-1990s. This caused major concern, and by 2002, South Africa had become the country with the largest number of HIV-infected people on the continent, despite the fact that the epidemic started much later than in central and west African countries[8]. Similar trends were observed in countries bordering South Africa such as Botswana, Lesotho, Swaziland, Zimbabwe and Zambia as well as in Malawi [8] (Figure 3.2). This southern African epidemic has been characterized by several unique features which could have contributed to its rapid progression in the region[9]. During the 1980s, many countries in southern Africa, particularly South Africa, enjoyed more stable economies when compared to other countries in SSA[10]. The labour-intensive mining and farming industries

played a crucial role in maintaining the country's economy. The creation of a migrant labour system to serve the mines in the early 1900s led to an influx of large numbers of sexually active men who subsequently engaged in internal and cross-border migratory labour activities within the region.

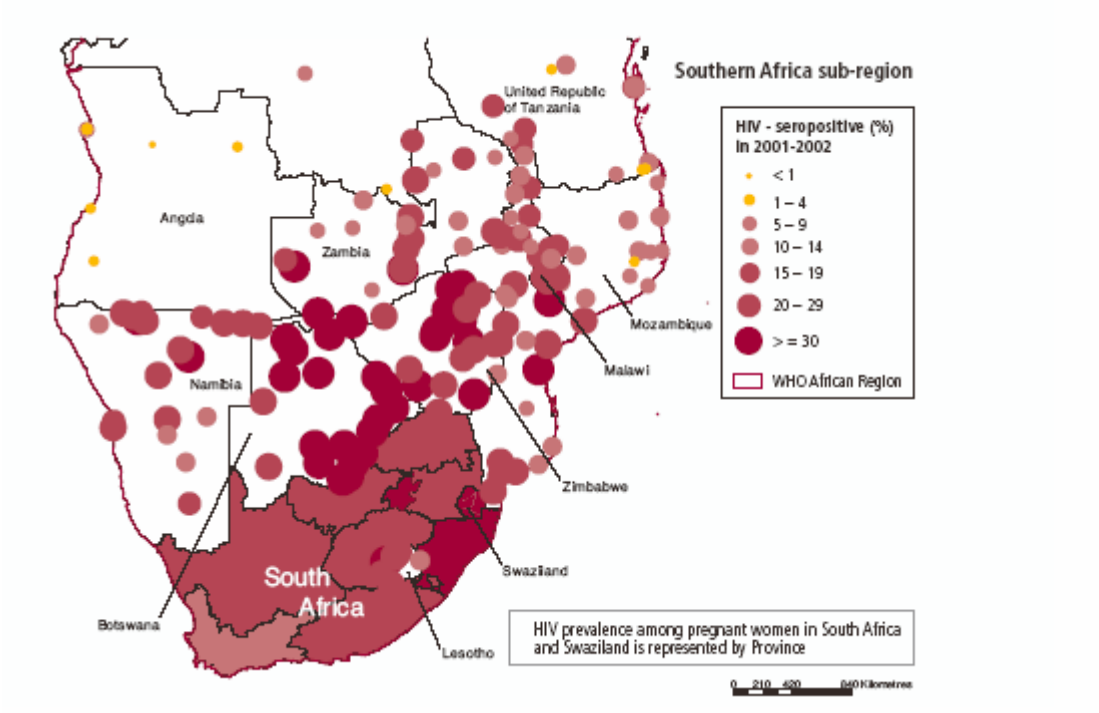


Figure 3.2 HIV prevalence among women attending antenatal care clinics in southern Africa, data reported by clinic site or city, 2001-2002
(Source: World Health Organization 2003)

3.1.3 Genital, including HIV, infections in South African miners

On average, more than 400,000 workers were employed in the mining industry throughout South Africa in 1992, the majority being migrant miners from the former homelands of South Africa (KwaZulu, Transkei, Ciskei, Bophutatswana, Qwa Qwa) as well as neighbouring countries such as Mozambique, Lesotho, Swaziland, Botswana and Malawi[11]. Many of the migrant mineworkers lived in single-sex hostels, having left their sexual partners at their permanent residential place. Anecdotal evidence indicated high levels of STI endemicity in both mining areas as well as rural regions where these miners originated[12]. Extensive mobility of sexually active individuals within regions posed major difficulties in implementing effective STI/HIV control programmes during the early 1990s. The increasing incidence of major conventional STIs cumulated into a hyper-endemic situation in the mines and it was reported

that large numbers of patients attended medical treatment stations with genital ulcer disease (GUD), inguinal lymphadenopathy and urethritis during that period. It is apparent that the STI situation in the early 1990s should have served as an early warning of the forthcoming accelerated spread of HIV infection after its introduction into southern Africa during the early 1980s. Unfortunately, major social and political developments such as the end of the apartheid era in South Africa coincided with the early exponential phase of HIV dissemination in the region. Unlike in other countries, (e.g. Uganda and Thailand), the lack of decisive preventive action against HIV/AIDS and STIs by policy makers and civil societies in the early 1990s allowed both epidemics to rage virtually unabated in southern Africa[13].

3.1.4 Research activities involving sexually transmitted infections in miners, 1981 – 2001

The establishment of the Emergent Pathogen Research Unit (EPRU) of the South African Medical Research Council and the South African Institute for Medical Research (SAIMR), 1981-1993, resulted ongoing STI-related research being conducted among miners in the Carletonville region. These activities were expanded with the establishment, in 1994, of the National Reference Centre for STD (NRC-STD).

Since 1994, the NRC-STD has provided clinical, epidemiological and microbiological assistance to many private and public service providers in southern Africa. In this chapter, the findings of epidemiological and microbiological studies conducted at selected STI clinics situated at the Carletonville gold mines up to 2001 are presented.

3.2 Mine environment and STI-related services during the study period 1992-2000

3.2.1 Geographical location of studies

With the exception of one investigation into the effect of HIV co-infection on the laboratory diagnosis of STIs, notably serological testing for syphilis (see section 4.1), all studies included in this chapter were conducted in a dedicated STI clinic located within the Carletonville gold mining area.

The town Carletonville is situated southwest of Johannesburg approximately 50 km from central Johannesburg. The area measures about 800 sq km and three private mining companies (Goldfields of SA, Harmony and Anglo-Gold) have been operating in the area since the early 1980s. The economy of the district is largely dependent on migrant labour and white collar workers employed by these mining companies. The area is strategically located on the long distance Johannesburg-Cape Town truck route, stretching from Johannesburg via Potchefstroom, Klerksdorp, Bloemfontein or Kimberley, to Cape Town (Figure 3.3).

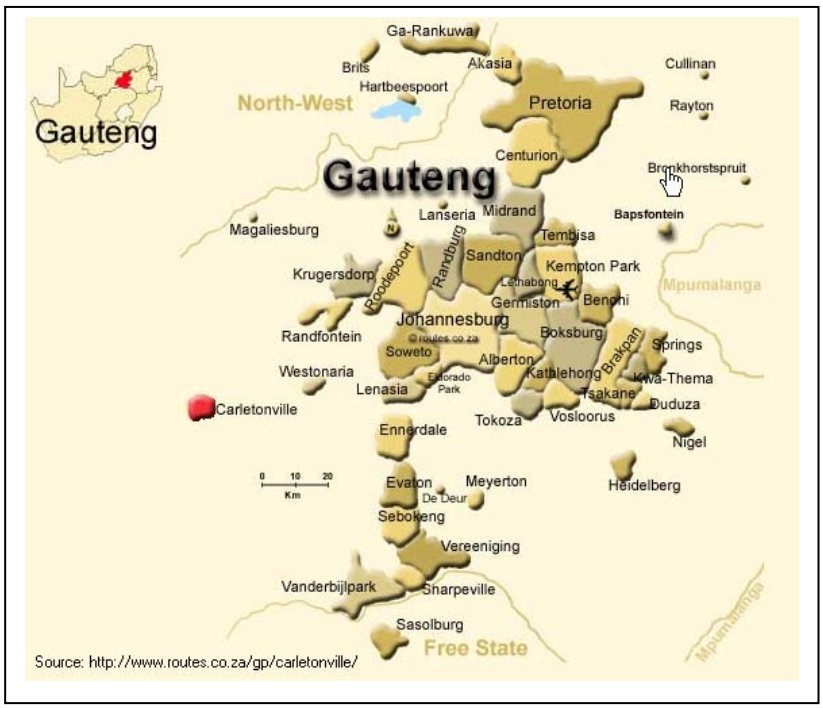


Figure 3.3 Gauteng province and Carletonville study site in relation to other South African provinces and Carletonville District (source: http://www.routes.co.za/gp/carletonville/)

3.2.2 The Carletonville mineworker population

Most residents of the district have either direct or indirect economic and social links with mining companies. In 1995, it was estimated that the district had a population of over 200,000 of which more than 40% were migrant men working in the mines. On average, the mining companies employed over 80,000 miners in the Carletonville area from different regions of southern Africa. There were some fluctuations in the size of the workforce in response to local/international economic situations. However, most employees work for the same company for a significant number of years.

3.2.3 STI services in mines

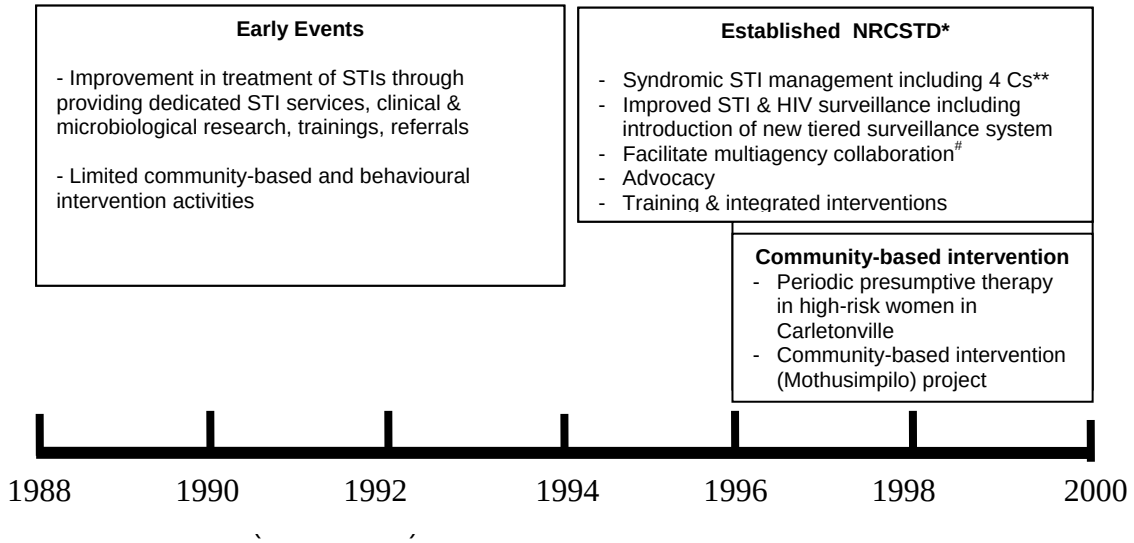
All mine employees are provided with comprehensive health care by the mining companies as part of their employment conditions. Unfortunately, most sexual partners of miners (both locally and at distant location) are required to seek health care at public or private clinics. All symptomatic STI patients from the Goldfields of SA mines were referred to a dedicated STI clinic situated at the referral hospital or a mine medical station. The NRC-STD research team provided comprehensive STI services for these patients on a regular bi-weekly basis. Although systematic health care seeking behaviour studies had not been conducted, key informants (clinic/medical staff, community leaders and miner representatives) reported that over 90% of symptomatic STI patients in mines in the region sought medical care at the STI clinic. This system of service provision remained in place during the period 1992 to 2000. Since 2001, however, the mine medical services and NRC-STD introduced a system of decentralized STI services which operated at primary health care clinics. During 1999 – 2000, surveillance activities introduced by NRC-STD were expanded to determine the burden of selected STIs including asymptomatic infections among miners, as well as local communities surrounding the mines, by conducting serial community-based, cross-sectional, prevalence studies.

3.2.4 Major STI-related events at the study sites (1992 – 2000)

Although major socio-political transformation occurred in South Africa during the mid-1990s, there were no major changes in the mining operations in the Carletonville area. Although there were changes in mine ownership, the composition and broad layout of the workforce was largely maintained throughout the study period. With the establishment of NRC-STD in 1994, the clinical and microbiologically-oriented STI services were augmented by public health-related intervention activities. On-site voluntary counselling and testing (VCT) was introduced for the diagnosis of HIV infection in STI patients by NRC-STD staff, although unlinked anonymous testing for surveillance purposes was still maintained. Syndromic STI management protocols were established and used for patient care. More health education including passive partner notification and management using contact slips were implemented. In 1999, both clinical and public health intervention activities were extended to communities

surrounding the mines. Targeted intervention programmes for women at high risk who had sexual contact with the miners in exchange for kind or cash were also implemented[14]. As mentioned earlier, STI services were decentralized to primary health care clinics in 2001. This major rationalization of health services was preceded by an appropriate training programme which included syndromic management of STIs.

Measures to improve health care delivery during the period 1992 – 2000 will have to be taken into account when interpreting the changing patterns of STIs observed during this period. Some of the changes are presented in Figure 3.4 and include such major events as introduction of syndromic management in the public and mining sectors in 1995 and the periodic presumptive treatment (PPT) programme of women at high risk instituted at that time (Figure 3.4).



* National Reference Centre for STD;
** Condom promotion, Counselling (risk-reduction), Contact tracing, Compliance to treatment.
# Initiate & participate collaborative projects between international NGOs such as Family Health International, Population Council, DFID (UK), the National and Provincial departments of Health, Mining Company such as Goldfields of SA & Harmony Mining Companies.

Figure 3.4 Measures to improve STI prevention and control in Carletonville mining areas during the period 1988 – 2000

3.2.5 Introduction of a routine STI surveillance system

A standard data collection system was established in 1990. Basic personal characteristics such as a unique identifier (i.e. mine employment number), name, date of birth, marital status

and place of origin (permanent residence) were entered onto a simple specially designed form, together with a summary of clinical and routine laboratory findings. All patients presenting with new STI episodes were screened serologically for syphilis, using a quantitative RPR test. VCT for HIV was provided for all new STI patients and the refusal rate was negligible. All HIV-seropositive patients were referred to professional counsellors for further assistance. Only symptomatic HIV-seropositive patients were assessed for reassignment for less labour intensive duties or counseling for medical retirement according to prevailing occupational health regulations at the mine which dictated that such action would be warranted only when miners become sufficiently symptomatic to affect their work performance. No discriminatory measures were taken as a result of a patient's HIV status (for further discussion, see Section 3.3.4).

Patient information was captured in a database at the SAIMR, linked to a unique mine number specifically excluding patients' names. Syndromic management of STIs was fully implemented in 1995 and the data collection form and database were updated to accommodate these changes. The modified surveillance form was used until end of the study period (See Annexure 3.1 - Routine Surveillance Form).

3.3 STI research projects involving HIV-infected patients

Although several STI-related research projects were coordinated by NRC-STD and its predecessor, the EPRU, in the 1980s and 1990s, only those with a relevant HIV component and, specifically, more recent HIV-directed studies involving the author of this thesis, will feature in this chapter.

3.3.1 Cross-sectional aetiology studies

Periodic cross-sectional studies to determine the aetiology of selected STI syndromes were conducted at the dedicated mine clinic at Carletonville. With rapid advances in STI diagnostic methodology, more sensitive, specific and non-invasive laboratory methods became available and were used where applicable and when considered cost-effective or affordable. In these

studies, established laboratory methods were however still included in parallel with new techniques, ensuring comparability of prevalence data.

The cross-sectional studies were augmented by special ad hoc studies such as clinical trials and surveillance of antimicrobial resistance. In general, the basic design and laboratory methods were consistently maintained to provide continuity and afford additional scientific validity to the surveillance programme. Relevant historical data were also used to assess temporal changes in aetiological patterns (Table 3.1).

Table 3.1 Summary description of studies included in Chapter 3

Study Type	Study Population	Sampling Method	Sample Size	Laboratory Method	Year of Study
Record Reviews	Miners presenting with new STI episodes at the Carletonville Mine STI Clinic	All patients treated at the clinic		Clinical diagnosis using pre-defined criteria and serological tests	1992 to 2000
Cross-sectional (Aetiology study)	Miners with GUD	Consecutive	239	Microscopy,Culture, Serology	1986
Cross-sectional (Aetiology study)	Miners with GUD	Consecutive	213	Microscopy,Culture, Serology	1990
Cross-sectional (Aetiology study)	Miners with GUD	Consecutive	232	Microscopy,Culture, Serology, M-PCR	1994
Cross-sectional (Aetiology study)	Miners with GUD	Consecutive	186	Microscopy,Culture Serology, M-PCR	1998
Prospective study (HIV Shedding)	HIV-seropositive miners with GUD	Consecutive	78	Microscopy,Culture, Serology, M-PCR, HIV viral load, $CD4^+$ count	1998 to 1999
Cross-sectional Multi-centre aetiology studies	Patients presenting with GUDs patients at STI clinic in Maseru, Cape Town, Durban, Carletonville	Consecutive	868	Microscopy,Culture, Serology, M-PCR	1993 to 1998
Case-reports	STI patients co-infected with HIV	Observations	-	Microscopy,Culture, Serology, M-PCR	1994 to 2000

3.3.1.1 Study population

All studies were conducted at the STI clinics serving miners from Goldfield of S.A goldmines at Carletonville, near Johannesburg. A retrospective clinic record review was conducted to explore the temporal changes in aetiology of STI syndromes among male migrant miners

from southern Africa. The records of all STI patients presenting with new episodes of STI at the study clinic from 1992 to 2000 were reviewed and analysed by the investigator.
The results of the aetiological studies are reviewed and compared with the studies prospectively conducted between 1994 and 1998 by the investigator. All patients presenting with GUD during each cross-sectional or prospective study during the study period who fulfilled the pre-defined criteria were consecutively enrolled after informed consent was obtained. A sub-group of HIV-seropositive patients with GUD were included in the HIV-shedding study.

3.3.1.2 Sampling method

All patients presenting with genital ulcer disease were offered syphilis serology and voluntary HIV counselling and testing after obtaining written informed consent. The demographic, behavioural and clinical presentations were recorded by interviewers using standard questionnaires (Annexure 3.2). Blood was taken from all consenting patients and tested for syphilis, chlamydial, type-specific herpes (in some studies) and HIV serological tests. Firstly, all ulcer(s) were cleaned with 10ml of normal saline and the washings pooled and collected in a sterile screw-cap container and stored in –70º C until processed to measure HIV viral copies as described in Section 2.3.4. Ulcer scrapings are then taken from the edges of the largest ulcer and tested for *T. pallidum* and Donovan bodies by darkfield microscopy and Giemsa staining respectively. Swabs are taken from the base of active lesion and cultured for *H. ducreyi* and the molecular detection of *H. ducreyi, T. pallidum* and Herpes simplex virus by multiplex PCR (M-PCR) techniques as described in Section 2.3.1. A sub-group of patients who agreed to return after 7 days and who were found to be HIV positive, had $CD4^+$ counts performed, plasma HIV viral load and shedding of HIV in ulcer washings determined. Lastly, all patients were treated using the appropriate management algorithm for genital ulcer syndrome of the NRC-STD (Annexure 3.3). The same procedures were repeated at the return visit on day seven if complete re-epithelialisation has not been achieved. All HIV-seropositive patients were provided with post-test counselling by trained counsellers and appropriate treatment or prophylaxis measures for opportunistic infections was given if indicated.

3.3.1.3 Inclusion and exclusion criteria

All patients presenting with a new episode of STI were included in the record reviews. Patients who returned to the clinic for follow-up visits for any new STI episode were excluded from the analysis.

The standard inclusion criteria for the cross-sectional aetiological studies and the prospective HIV-shedding study included:

- patients presenting with genital ulcerations (> 2 mm in size) during the study period
- no previous history of treatment for current episodes
- availability for follow-up visits for two weeks
- ability to provide informed consent
- agreement for voluntary HIV counselling and testing

3.3.1.4 Questionnaire

Only limited demographic, clinical and serological information were collected for routine STI patients using a standard questionnaire (Annexure 3.1). However, more in-depth interviews were conducted in their own language by the study team member using pre-coded questionnaires. The patients' demographic characteristics, sexual behaviour, presenting symptoms and clinical history of current STI episode were collected during the interview. The detailed clinical description of presenting STI episode was recorded by the investigators using standard clinical criteria. The results of laboratory tests performed on each patient were recorded when available.

3.3.1.5 Laboratory methods

The detailed methods of each laboratory investigation have been described in Section 2.3. All patients were screened for quantitative syphilis serology (by RPR) at the time of presentation with each new episode of STIs. Almost all patients agreed to participate in the voluntary counselling and testing for HIV and the standard test algorithm recommended by the SAIMR serology laboratory was used for those patients. Those patients who refused to provide blood

were excluded from the aetiological studies although a participation rate of over 99% for voluntary counselling and testing was recorded during the study.

Other appropriate microbiological investigations such as microscopic examinations, cultures and nucleic acid amplification tests (NAATs) were only conducted on specimens (e.g. genital ulcer exudates, urethral swabs and urine) collected from the patients enrolled in the aetiological studies. In addition, additional serological tests such as type-specific herpes serology, chlamydial micro-immunofluorescence (micro-IF) assays, and FTA-ABS were also conducted in these cases. Laboratory investigations such as $CD4^+$ counts and HIV plasma viral load (PVL) measurements were conducted only on patients enrolled in the HIV-shedding study.

The detection of aetiological agents and HIV viral load in the plasma and genital ulcer exudates were measured at both enrolment and subsequent follow-up visits when necessary. The detailed methods used in collection of genital ulcer exudates for the measurement of HIV shedding in ulcer exudates is described in Sections 5.1 and 5.2.

3.3.1.6 Treatment protocol

All patients were immediately treated according to the STI management algorithm for the respective STI syndromes at the initial visit (Annexure 3.3). Although “tests of cure” for all possible agetiological agents were not conducted routinely, microbiological, virological and serological investigations were conducted in those participating in follow-up studies or those identified as treatment failures at the follow-up visit. Patients presenting with mixed STI syndromes were treated by using the most logical combination of recommended therapy for the presenting syndromes. Anti-herpes therapy was only provided if there was laboratory evidence of HSV infection or patients presented with a typical clinical presentation of genital herpes during participation in the HIV shedding study, or patients who exhibited clinical failures but had demonstrable bacteriological cure for *H. ducreyi, T. pallidum, C. trachomatis* and donovanosis at the follow-up visit. Valacyclovir 500 mg BD for 5 days was used as standard therapy for suspected or confirmed cases of genital herpes.

3.3.2 Specific STI studies relating to effects of HIV co-infection

During the period 1992 – 2000 several studies were conducted by the NRC-STD where the effects of HIV infection on other infections of the genital tract among mineworkers were assessed.

3.3.2.1 HIV co-infection and clinical presentation of STIs

Patients presenting with defined STI syndromes were enrolled consecutively in the studies. In each case, their HIV status was recorded when, on history-taking, miners who have had a previous STI confirmed at a clinic visit were tested for their serological status through NRC-STD's VCT service. Those who had not been tested previously, were offered VCT at the time of their visit, as were those of unknown serostatus.

Standard questionnaires designed for specific STI syndromes were completed by the investigator. An aetiology-based clinical diagnosis was also made using pre-defined criteria for evaluation purposes. All patients were treated according to syndromic STI management protocols and subjected to syphilis screening by RPR testing (Annexure 3.3) The immunological status of patients whose positive HIV serological status had previously been established was assessed by the use of $CD4^+$ counts and HIV plasma viral load (PVL) determinations.

During the study period, the standard of care for HIV-seropositive individuals did not include provision of anti-retroviral drugs. HIV-seropositive mineworkers were provided with prophylaxis for selective opportunistic infections including tuberculosis and *Pneumocystis jerovicii* (formerly *P. carinii*) pneumonia (PCP) at the referral hospital. Standard laboratory investigations appropriate for STI syndromes as they presented in patients were performed as described in Section 2.3. The nature and severity of clinical presentations were analysed retrospectively according to objective criteria in relation to their HIV status.

3.3.2.2 Effect of HIV co-infection on the laboratory diagnosis of STIs

The performance of routine laboratory tests in studies on the aetiology of STI syndromes in patients from Johannesburg, Carletonville, Durban, Cape Town and Lesotho, particularly syphilis serology in patients presenting with primary syphilis, was evaluated and the effect of co-infection with HIV investigated. Similar enrolment steps as outlined in sections 3.3.1.1 – 3.3.1.3 were followed. Patients who were found to have M-PCR-confirmed primary syphilis at the various study sites were included in the analysis. The same protocol and laboratory methods as described for GUD (see sections 2.3.1.1 and 3.3.1.5) were followed throughout.

3.3.2.3 Effect of HIV co-infection on response to treatment of STIs

This section of the thesis concentrates on patients presenting with GUD. Their HIV status was determined as described in Section 2.3.4. Laboratory investigations to determine the aetiology of STI syndromes, HIV/syphilis serological status and immune function were conducted as described in Section 2.3. All patients were treated using standard treatment protocols for their respective STI syndromes. Follow-up visits were scheduled on Day 7, Day 14 and Day 21 to monitor clinical progress and establish microbiological cure.

3.3.2.4 Lesional shedding of HIV from genital ulcers

Shedding of HIV from genital ulcers, the effect of treatment on viral shedding and the method including a novel specimen collection technique used to determine quantitatively the number of HIV RNA copies in fixed amounts of genital ulcer exduate were performed in male patients at the Carletonville mine clinic and subsequently in female sex workers at the Esselen Street Clinic in Johannesburg. These studies are described in detail in Chapter 5 (male patients) and Chapter 7 (female patients).

3.3.3 Data entry, management and analysis

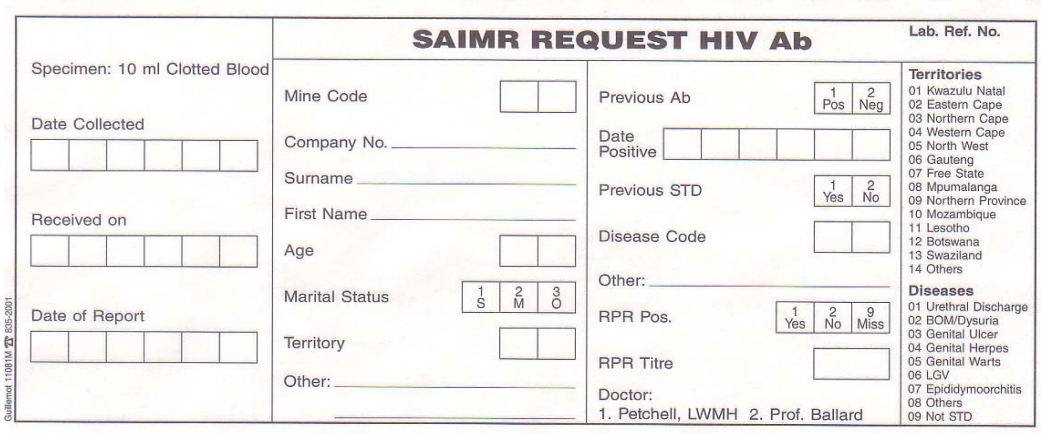
All demographic, clinical and microbiological data were captured in pre-coded databases using EpiInfo Version 6.04 (CDC, Atlanta, GA) and Microsoft Access (Microsoft, SA) programmes. The accuracy of data entry was checked by double entries of randomly selected records into each database. Databases with discrepant information were re-checked, and

double entry of entire databases was performed when necessary. The data were exported into SPSS® Version 8.0 (SPSS Inc., Chicago, IL.) and analysed by the investigator.

3.3.4 Ethical considerations

All study protocols were submitted to the Committee for Research on Human Subjects, of the University of the Witwatersrand for ethical approval. A simple patient information sheet describing study objectives, procedures, risks and benefits was prepared and used to explain to patients in their mother tongue the nature of the study and the ethical issues involved by study coordinators at the enrolment visit. Written informed consent was obtained from all participating subjects. All symptomatic patients were treated immediately using standard syndromic management protocols. Follow-up visits were scheduled where patients were informed about their laboratory results. Additional treatment or referral was provided if necessary. All HIV seropositive patients were provided with post-test counselling and referred to local HIV clinics for follow-up. In unlinked anonymous studies, confidentiality was maintained by de-linking all identifiable personal information before testing for HIV.

Annexure 3.1 Surveillance form and data elements used for routine STI patients at Carletonville mine STI clinic



SAIMR REQUEST HIV Ab

Lab. Ref. No.

Specimen: 10 ml Clotted Blood

Date Collected

Received on

Date of Report

Mine Code

Company No.

Surname

First Name

Age

Marital Status: 1 S | 2 M | 3 O

Territory

Other:

Previous Ab: 1 Pos | 2 Neg

Date Positive

Previous STD: 1 Yes | 2 No

Disease Code

Other:

RPR Pos.: 1 Yes | 2 No | 9 Miss

RPR Titre

Doctor:
1. Petchell, LWMH 2. Prof. Ballard

Territories
01 Kwazulu Natal
02 Eastern Cape
03 Northern Cape
04 Western Cape
05 North West
06 Gauteng
07 Free State
08 Mpumalanga
09 Northern Province
10 Mozambique
11 Lesotho
12 Botswana
13 Swaziland
14 Others

Diseases
01 Urethral Discharge
02 BOM/Dysuria
03 Genital Ulcer
04 Genital Herpes
05 Genital Warts
06 LGV
07 Epididymoorchitis
08 Others
09 Not STD

Guillemot 11081M ☎ 835-2001

Annexure 3.2 Questionnaires used for GUD aetiology and HIV shedding studies

Study Code | | | **Visit** | | |

Patient Code | | | | | | **Date** D D M M Y Y | | | | | | |

Mine Code | | | | ***Mine Num*** | | | | | | | | | | |

1. Name
Surname : _____ Firstname _____ *Initial* | |

2. Age (Years) *Age* | | How long you have been working in the mine? *Year* | | *Months* | |

3. Origin (1=KZN, 2=Lesotho, 3=Ecape, 4=MZB, 5=Botswana, 6= Others) *Origin* | 1 | 2 | 3 | 4 | 5 | 6 |

4. Marital Status (1=Single, 2=Married, 3=Others) *Marital* | 1 | 2 | 3 |

5. Where did you get this disease? (1=Mine, 2=Home, 3=Others) *Place of infect.* | 1 | 2 | 3 |

6. From whom did you get it? (1=Regular partner,2=Casual partner/Prostitute) *Source of infection* | 1 | 2 |

7. Number of sexual partners ; in last month *Partner1* | |
in life-time *Partner 2* | |

8. Have you ever used a condom ; in last month *Condom 1* | 1 | 2 |
in your life *Condom 2* | 1 | 2 |

9. Estimated incubation period (Days - Last sexual exposure to appearance of symptom) *Incubation* | |

When did you have sex last time? *Datesex* : | | | | | | |

When did you first notice this sore? *Datesore* | | | | | | |

Duration of symptoms? (Days) *Duration* | |

10. How did the sore start? (Blister/Rash/Pustule/Abscess etc.) _____

11. Did you have any treatment for this complaint in last four weeks? *RX* | 1 | 2 |

12. History of recurrences of similar sores? *hsores* | 1 | 2 |

How many times in this years? (Also check patient's card) *sorefreq* | 1 | 2 |

13. Did you have sex after you notice the sores? *hsex* | 1 | 2 |

(if yes) Did you use condom? *hcondom* | 1 | 2 |

14. History of any illness in last 30 days (Review Card as well)

	D D M M Y Y
CDis1	
CDis2	
CDis3	
CDis4	

19. Is HIV status known? *Previous HIV* | 1 | 2 | 20. Date diagnosed? *Date HIV* D D M M Y Y | | | | | | |

Other relevant notes

HIV related and other diseases? *Dis* | 1 | 2 | Other Comments ? *Com* | 1 | 2 |

Dis1 :
Dis2 :
Dis3 :
Dis4 :

1. GENITAL ULCER | 1 | 2 |

11.1 Number		
11.2 Tender	1	2
11.3 Deep	1	2
11.4 Irregular/Ragged Margin	1	2
11.5 Purulent Base	1	2
11.6 Size of Target Ulcer (mm)		
11.7 Erythematous Edges	1	2
11.8 Bleed on touch	1	2

2. GLANDS | 1 | 2 |

12.1 Site	Uni	B i
12.2 Matted	1	2
12.3 Ruptured	1	2
12.4 Size (mm)		

3. CLINICAL DX | 1 | 2 | 3 | 4 | 5 | 6 |

(1=Chancroid, 2=Syphilis , 3=G. Herpes, 4=LGV, 5=G.I, 6=Other)

4. URETHRAL DISCHARGE | 1 | 2 |

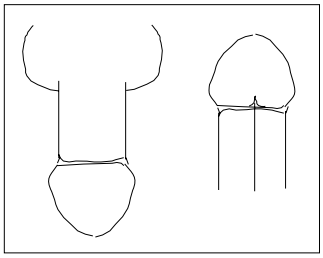
BOM/Dysuria | 1 | 2 |

5. OTHER STD | 1 | 2 |

15.1 HERPES SIMPLEX	1	
15.2 G. WARTS	2	
15.3 EPIDIDYMOORCHITIS	3	
15.4 SCABIES	4	
15.5 Molluscum	5	

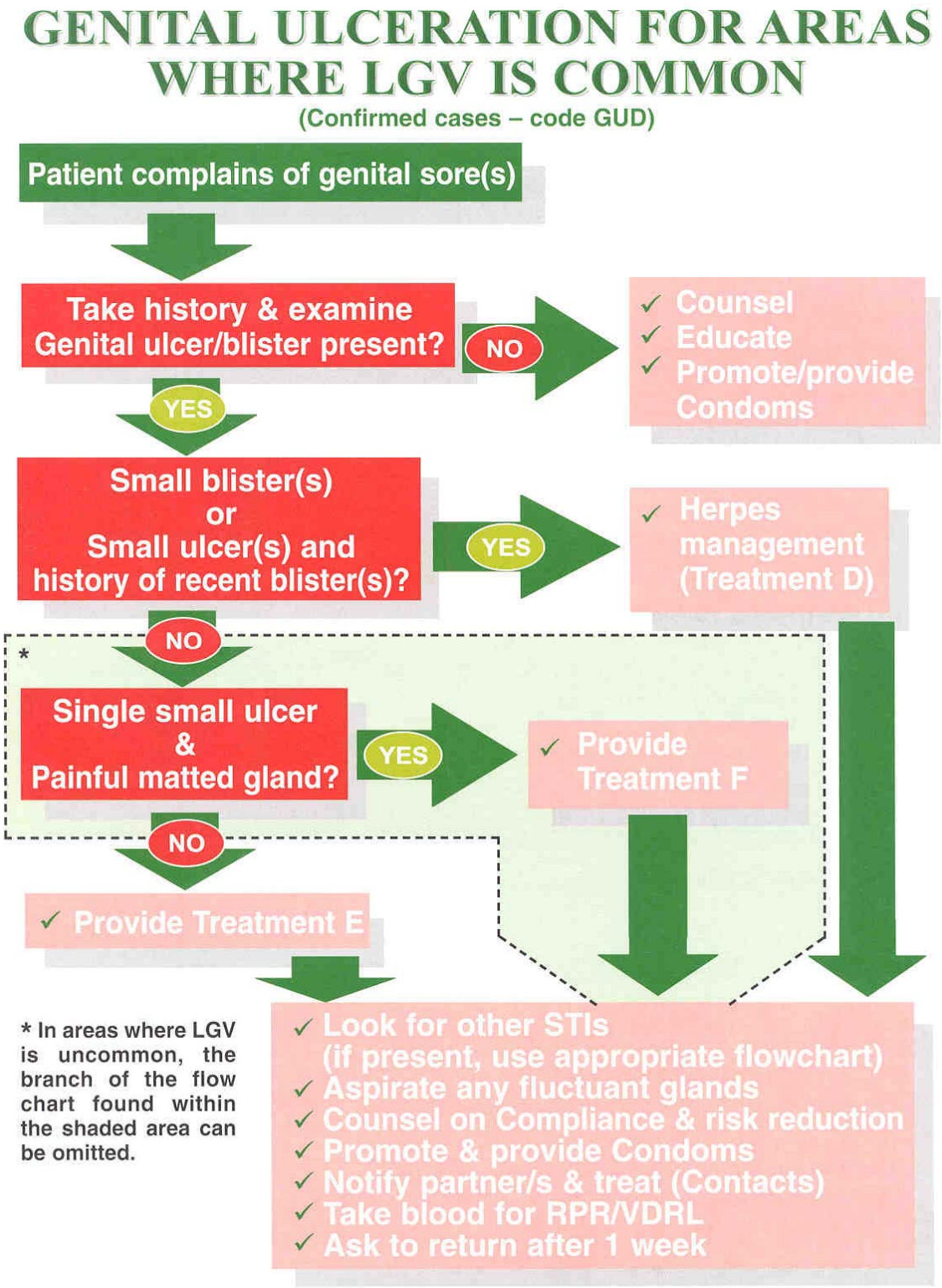
6. Photo taken | 1 | 2 |

7. Photo Num. | | | | | | |



Other Relevant notes

Annexure 3.3 Syndromic management of GUD (1994 – 2000)



TREATMENT OF GENITAL ULCERS (GUD)

TREATMENT OPTIONS – D

Management of Genital Herpes

- Counselling on nature of disease with emphasis on possible recurrences.
- Lesions should be kept clean and dry, using talcum powder.
- Secondarily infected herpes lesions should be treated.
  Cotrimoxazole 1 double strength tablet (160/800 mg) p.o. bd for 7 days OR
  Erythromycin 500 mg p.o. qid for 7 days
- Pain relief if necessary
- If available, specific anti-herpes therapy may be provided
  The dosages indicated are those recommended for primary herpetic episodes:
  Acyclovir 200 mg 5 times daily p.o. for 7-10 days OR
  Famciclovir 250 mg p.o. tds for 7-10 days OR
  Valacyclovir 1 g p.o. bd for 7-10 days
  (Consult page for dosages recommended for recurrent episodes)

TREATMENT OPTIONS – E

Treatment for Early Syphilis

*Benzathine penicillin L.A. 2.4 Mu i.m. stat OR
*Procaine penicillin G 600 000 u.i.m. daily for 10 days OR
Tetracycline 500 mg p.o. qid for 15 days OR
Doxycycline 100 mg p.o. bd for 15 days OR
*Erythromycin 500 mg p.o. qid for 15 days

PLUS

Treatment for Chancroid

*Erythromycin 500 mg p.o. qid for 7 days OR
Ciprofloxacin 500 mg p.o. stat OR
Ofloxacin 400 mg p.o. stat OR
*Ceftriaxone 250 mg i.m. stat OR
*Azithromycin 1g p.o. stat

TREATMENT OPTIONS – F

Treatment for Early Syphilis

*Benzathine penicillin L.A. 2.4 Mu i.m. stat OR
*Procaine penicillin G 600 000 u.i.m. daily for 10 days

PLUS

Treatment for Lymphogranuloma venereum and Chancroid

*Erythromycin 500 mg p.o. qid for 14 days

Treatments marked * are safe for use during pregnancy/breastfeeding

3.4 Main findings of studies in miners

3.4.1 STI and HIV trends in Goldfields miners at Carletonville (1992 – 2000)

During the study period, the average daily workforce at the Goldfields of SA mines where the study was conducted (i.e. East Driefontein, West Driefontein, Deelkraal and Doornfontein) ranged from 38468 in 1992 to 18300 in 2000. A sharp decline in the number of miners employed occurred in 1997. This was due to the restructuring of the Goldfields Mining company while the Deelkraal mine was sold to another mining company. The average daily workforce in the two major mines, East Driefontein and West Driefontein, was, however, relatively stable with a moderate decline documented between 1997 and 2000. Overall trends in the size of different mine populations are shown in Figure 3.5.

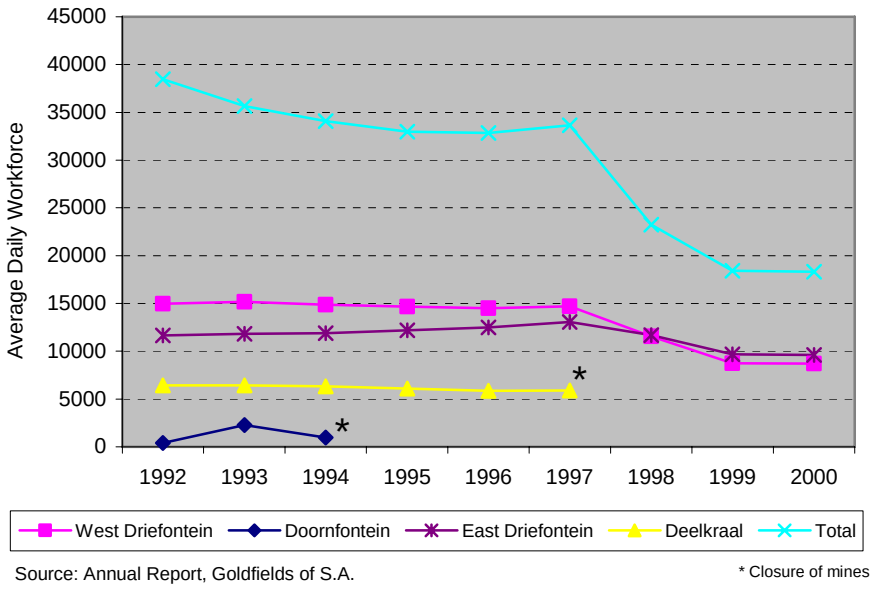


Figure 3.5 Average daily workforce at the Goldfields of SA mines in Carletonville (1992 – 2000)

During the study period, 36,686 new STI episodes were treated at the clinic and the mean annual STI incidence rate was 137.4 per 1000 miners (range – 99.6 to 162 per 1000). The highest annual incidence occurred in 1994 when 5521 new STI episodes were treated. The incidence gradually declined to its lowest, 1822 episodes in 2000 (Figure 3.6). A significant increase in the annual STI incidence rate was found in 1994 when compared to that of 1993 (114 vs. 162 per 1000, $\chi^2 = 338.94$, $p<0.0001$). The STI incidence remained at a high level throughout 1994–1999 although a slight decline was seen in 1997. The annual STI incidence

rate has significantly declined from 157/1000 in 1999 to 100/1000 in 2000 (χ^2 = 266.3, p <0.0001).

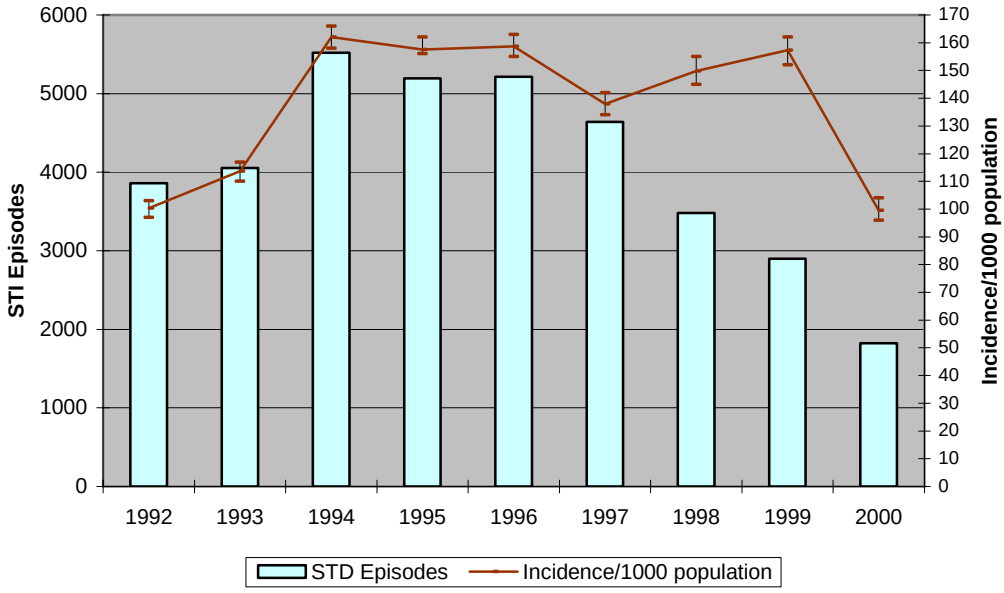


Figure 3.6 Annual incidence of STI episodes seen at the Carletonville mine STI clinic (1992 – 2000)

Over 90% of STI episodes occurred among miners from South Africa, particularly from KwaZulu-Natal and the Eastern Cape province, as well as from other neighbouring countries such as Lesotho, Mozambique, Swaziland and Botswana (Table 3.2). The ethnic distribution of miners was not available and therefore the ethnic-specific incidence of STI was not calculated. Table 3.2, however, records the regions/countries from where miners were recruited.

Table 3.2 Residential origin (area of permanent residence) for miners presenting with STIs, Carletonville (1992 – 2000)

ORIGIN OF MINERS	NUMBER	%
KWAZULU NATAL	9055	24.7%
LESOTHO	8186	22.3%
EASTERN CAPE	7191	19.6%
MOZABIQUE	4569	12.5%
SWAZILAND	2230	6.1%
BOTSWANA	2130	5.8%
NORTHERN PROV.*	843	2.3%
GAUTENG	313	0.9%
NORTHWEST	222	0.6%
NORTHERN CAPE	212	0.6%
FREE STATE	106	0.3%
MPUMALANGA	75	0.2%
WESTERN CAPE	48	0.1%
MALAWI	5	0.0%
OTHERS	788	2.1%
MISSING	713	1.9%
Total	36686	100.0%

* Now called Limpopo Province

Overall, the mean age of STI patients was 30.9 years (range 18 to 65 years), the majority (64.5%) being in the 25 – 39 year age group. The proportion of miners in older age groups (i.e. 25 – 39 and above 39 years) increased in recent years. Of 5194 episodes treated in 1995, 861 (16.6%) occurred in the 18 – 24 year age group compared to 11.2% in 1999 and 7.6% in 2000 (χ^2 = 43.04, p<0.0001) (Figure 3.7). The majority of miners (65.3%) were married but 32.8% denied ever being married.

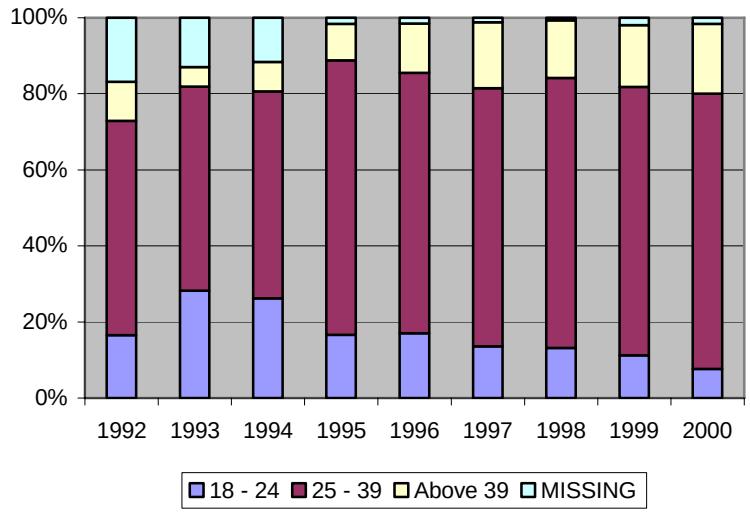


Figure 3.7 Age distribution of miners with STIs (1992 – 2000)

All miners with STIs were offered voluntary counselling and testing for HIV antibody and the majority (over 99%) accepted. A total of 35,789 HIV tests were performed during 1992 – 2000 and the overall HIV prevalence was 35.3% (95% CI – 34.8% - 35.8%). During 1992 – 1994, the HIV prevalence was high but stable within the range of 18% to 20.8%. It, however, increased significantly in 1995 (20.7% in 1994 vs. 42.4% in 1995, $\chi^2 = 579.6$, $p<0.0001$), and this increasing trend continued until 1998. The HIV prevalence peaked at 51.6% in 1998 (95% CI – 49.9% - 53.3%) followed by a gradual decline to 47.3% (95% CI – 36.7% - 41.3%) in 2000 (Figure 3.8).

A variation in HIV prevalence was detected among groups from different regions presenting at the STI clinic. Over the years, the HIV prevalence of STI patients originating in Mozambique and Eastern Cape was consistently lower than that among patients from Swaziland, KwaZulu-Natal and Botswana. Except for those from Mozambique, the HIV prevalence rate peaked in 1998 in miners from all regions. The HIV prevalence was consistently higher among STI patients from Swaziland with a peak prevalence of 63.5% (95% CI – 57.1% - 69.4%) in 1998.

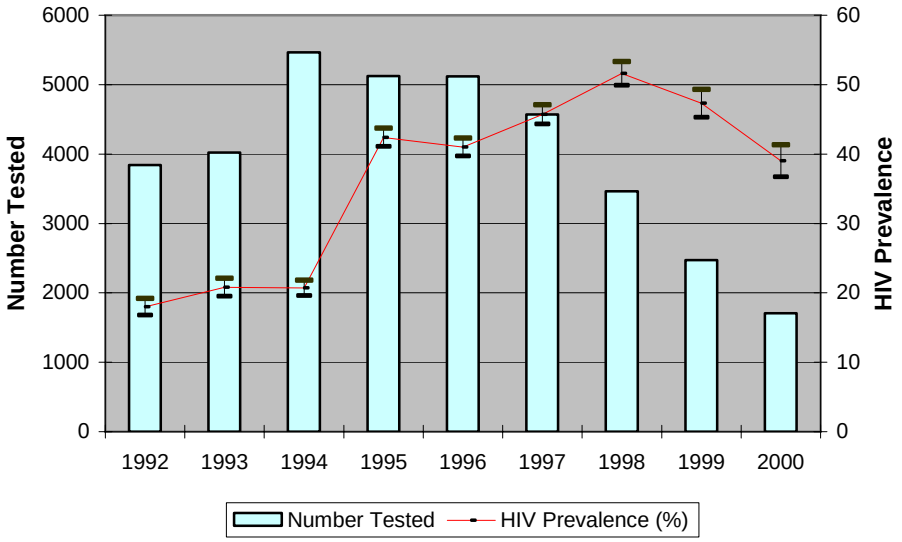


Figure 3.8 Number of miners tested for HIV at STI clinic and HIV prevalence, Carletonville (1992 – 2000)

The lowest HIV prevalence was found in STI patients from Eastern Cape which peaked in 1998 with 43.8% (95% CI – 40.5% - 47.2%). As of 2000, the lowest HIV prevalence of 31%

(95% CI – 26.8% - 35.5%) was found in miners from Eastern Cape attending the STI clinic. The highest HIV prevalence of 50% (95% CI – 39.9% - 60.1%) was found in those from Swaziland (Figure 3.9).

In summary, there was a rapid surge in the prevalence of STIs among miners up to 1994, followed by a step-wise increase during the period 1994 – 1998, and a gradual fall in prevalence during the succeeding three years. This rapid increase in HIV prevalence among miners with STIs peaked in 1998 at a rate marginally exceeding 50%. HIV prevalence rates among STI patients varied substantially in miners with different residential origins.

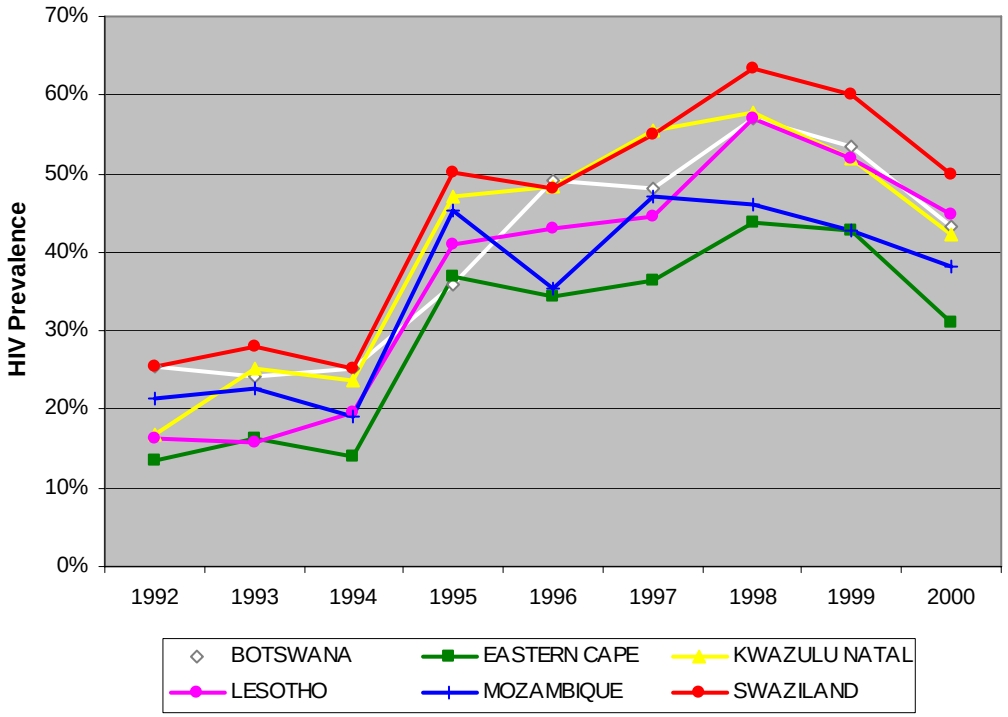


Figure 3.9 HIV prevalence among migrant miners by region of origin attending an STI clinic at Carletonville (1992 – 2000)

3.4.2 Changing Patterns of STI Syndromes (1992 – 2000)

STI syndromes such as genital ulcer diseases (GUD), urethritis, genital warts and epididymo-orchitis were the most frequent presentations recorded during the period 1992 – 2000, representing 91% of total episodes. The relative prevalence of the major STI syndromes is shown in Figure 3.10.

The relative prevalence of GUD, including clinically diagnosed herpes and lymphogranuloma venereum (LGV), was significantly higher than urethritis during the period 1992 – 1998. The increasing trend in relative prevalence of GUD was found during the period 1992 – 1994 rising from 50.6% (95% CI – 49% - 52.1%) in 1992 to 57.1% (95% CI – 55.8% - 58.4%) in 1994 (χ^2 = 38.6, p<0.0001). Thereafter, a decline in the proportion of cases presenting as GUD occurred. A sharp decline was found in 1998 with only 48% of 3482 STI episodes were GUDs compared to 54.4% in 1997 (χ^2 = 32.7, p<0.0001).

Meanwhile, there was a steady increase in the relative and absolute prevalence of urethritis. The definition of urethritis includes those complaining of burning on micturition and dysuria. Urethritis was the most common presentation among STI patients in 1999 and 2000, representing 44.3% and 43.5% of cases respectively. Similar increases were also found when comparing the relative prevalence of genital warts and epididymo-orchitis. The relative prevalence of genital warts and epididymo-orchitis increased from 0.8% in 1992 to 4% (χ^2 = 70.6, p<0.0001) and 0.8% to 1.7% (χ^2 = 9.2, p=0.002) respectively, in 2000.

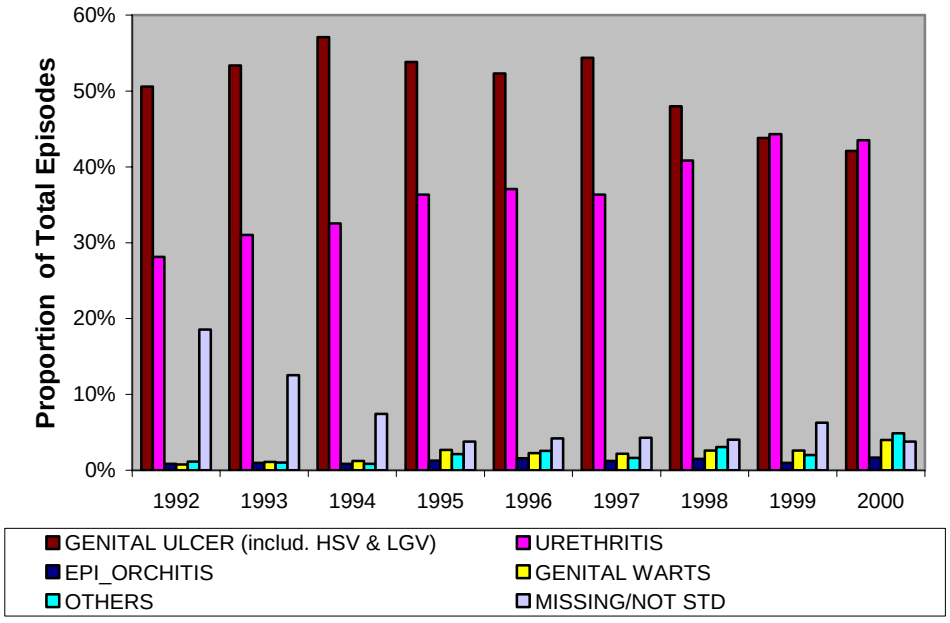


Figure 3.10 Proportion of specific STI syndromes diagnosed in Carletonville mine clinics (1992 – 2000)

As shown in Figure 3.11, the proportion of GUD cases clinically diagnosed as herpes significantly increased from 9.1% (of 1951 GUD episodes) in 1992 to 36% (of 767 GUD episodes) in 2000 (χ^2 = 285.5, p<0.0001). The relative prevalence of cases clinically diagnosed as LGV ranged between 8.8% in 1993 and 15.3% in 2000. A sharp decline in non-herpetic, non-LGV GUD episodes occurred between 1992 and 2000 (80.5% in 1992 vs. 48.8% in 2000, χ^2 = 272.9, p<0.0001). The majority of these ulcerations were clinically diagnosed as chancroid (RC Ballard and Ye Htun, personal observations).

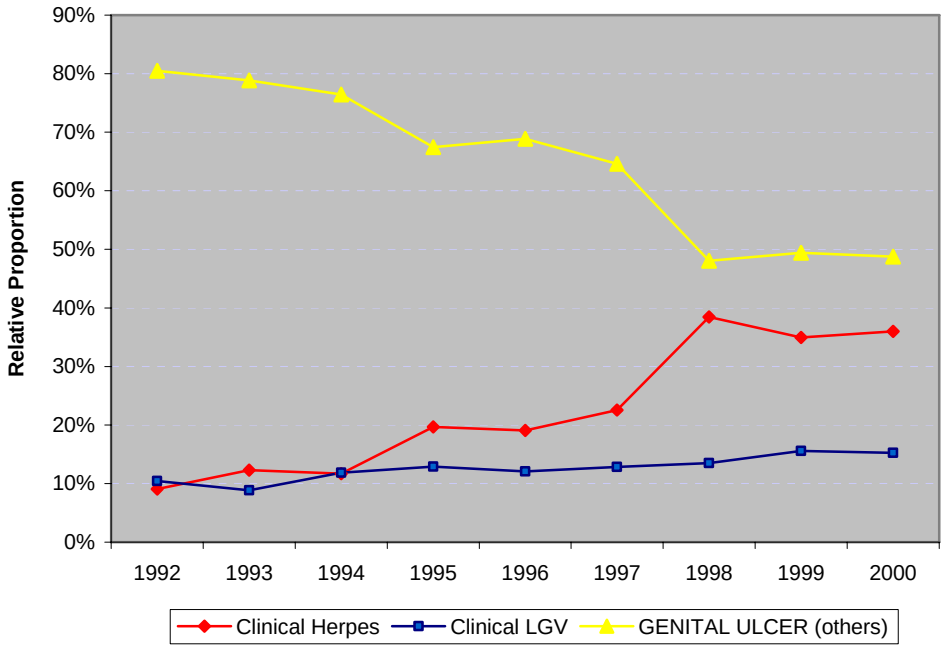


Figure 3.11 Relative prevalence of clinical presentations of GUDs, Carletonville (1992 – 2000)

The incidence of GUD, which was not clinically compatible with genital herpes or LGV, increased sharply in 1992 – 1993, peaked at 70.8/1000 population in 1994 and then declined steadily to 20.4/1000 in 2000 (Figure 3.12). The incidence of clinically diagnosed herpes increased significantly from 4.6/1000 population in 1992 to 27.6/1000 in 1998 (χ^2 = 62.8, p<0.0001). In contrast, the incidence of urethritis continued to increase during the period 1992 – 1999 and then stabilized in 2000.

In summary, GUD was the most common STI presentation among miners during the period 1992 – 1998. The decline in incidence of GUD was mainly attributable to a reduction in the number of GUD which were clinically incompatible with genital herpes or LGV. Although, there was a sharp decline in GUD cases, in more recent years this syndrome contributed to 42% of STI episodes seen at the clinic. With a substantial reduction in clinically diagnosed non-herpetic/non-LGV GUD, other causes of GUD, particularly genital herpes together with urethritis, have contributed significantly to the STI burden on the mines.

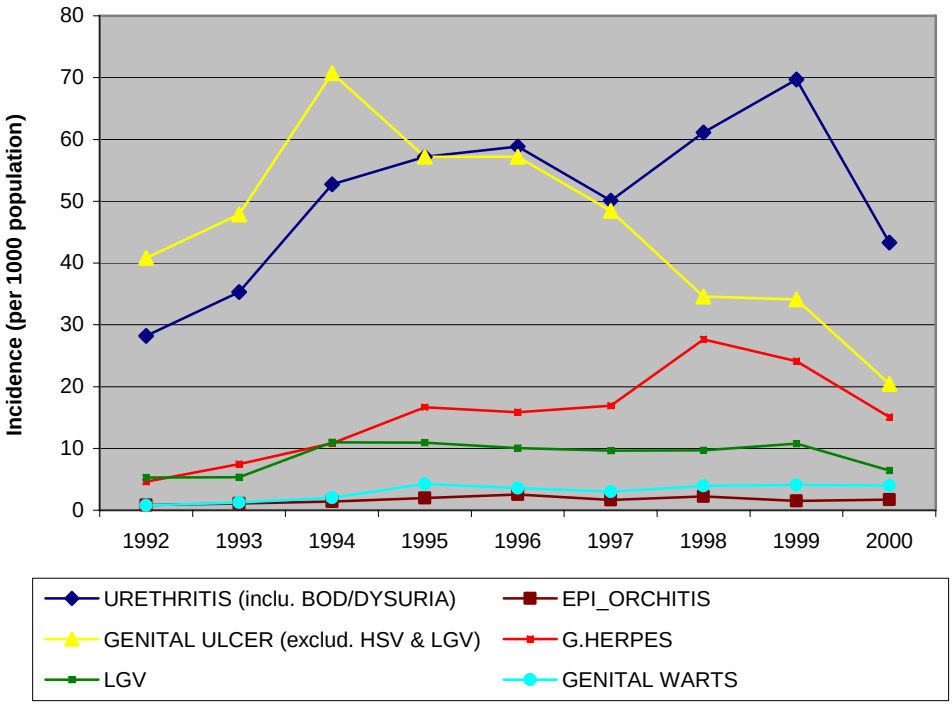


Figure 3.12 Disease-specific incidence of STI among miners, Carletonville (1992 – 2000)

As mentioned in Chapter 3.3.1, the HIV prevalence among STI patients significantly increased from 18% in 1992 to reach a prevalence of 51.6% in 1998. (χ^2 = 918.0, p<0.0001) Similar trends were found among patients presenting with other STI syndromes. The HIV prevalence was significantly higher among patients with genital warts (59.8%, 95% CI 56% - 63.4%), followed by GUD (40.1%, 95% CI 39.4% – 40.8%) and epididymo-orchitis (36.3%, 95% CI 41.2% - 31.8%). The HIV prevalence among urethritis patients was the lowest (29.6%, 95% CI 31.8% – 41.2%), and was significantly lower than that of among epididymoorchitis patients (29.6% vs. 36.3%, χ^2 = 8.89, p 0.003) (Figure 3.13).

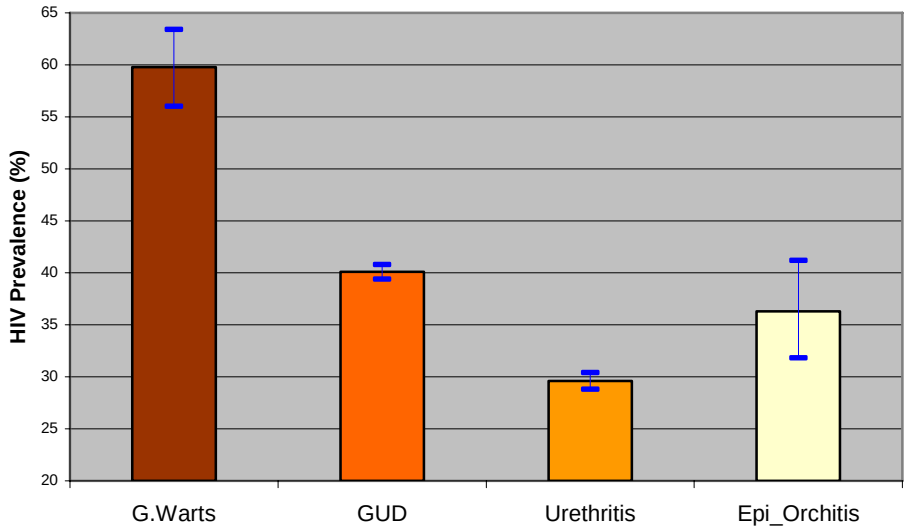


Figure 3.13 HIV prevalence and its 95% confidence interval among miners presenting with STI syndromes, Carletonville (1992 – 2000)

The trend in HIV prevalence among miners presenting with different STI syndromes is shown in Figure 3.14. A consistent increase in HIV prevalence among all patients with the different STI syndromes during the period 1992 – 1998 was recorded. The prevalence peaked in 1998 and a steady decline followed during the period 1998 – 2000. When GUD was compared based on clinical diagnosis, patients with non-herpetic genital ulcerations had a higher HIV prevalence rate than those with clinical herpes. However, the overall difference in HIV prevalence rates between the two groups was not statistically significant (41.4% vs. 40.5, χ^2 = 0.98, p 0.32). The association between HIV and different GUD pathogens was further explored using microbiological surveillance.

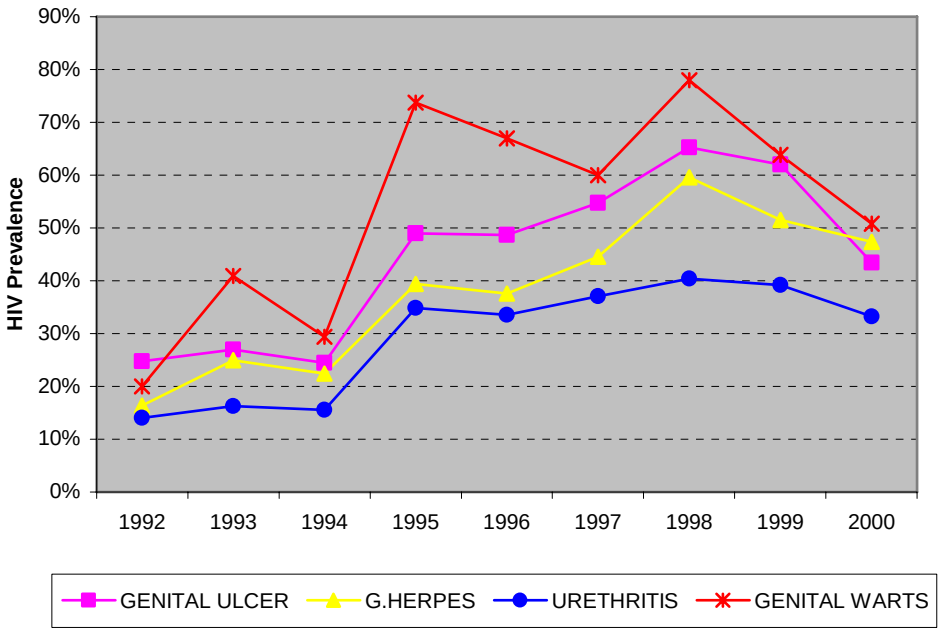


Figure 3.14 Trend in HIV prevalence among miners presenting with different STI syndromes, Carletonville (1992 – 2000)

During 1995 – 2000, 17,659 patients with new STI episodes were screened serologically for syphilis, using quantitative RPR tests and 1688 (9.6%, 95% CI – 9.1% - 10%) were found to be positive. The annual syphilis seropositivity rate ranged from 11.7% (95% CI – 10.3% - 13.1%) in 1995 to 5.2% (95% CI - 4.1% - 6.1%) in 2000. The decline was most evident in 1999 and was consistent for patients presenting with all major STI syndromes (Figure 3.15). There was a slight increase in the syphilis seropositivity rate among genital ulcer patients in 2000, although this increase was not statistically significant (χ^2 = 1.47, p 0.22).

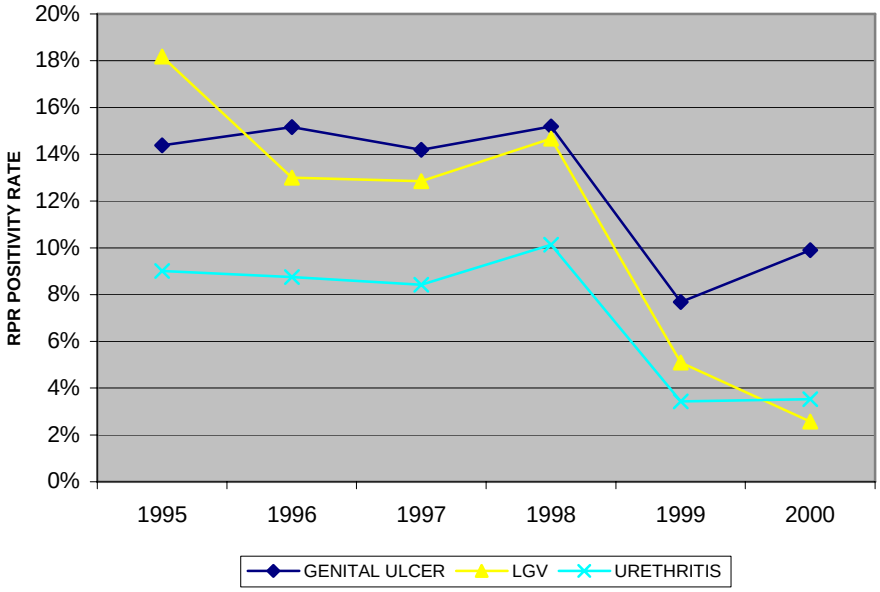


Figure 3.15 Trend of syphilis seroprevalence among STI patients, Carletonville (1995 – 2000)

3.4.3 Changing aetiology of STI syndromes

Cross-sectional studies to detect the aetiologies of STI syndromes were conducted at four-yearly intervals using the most sensitive and specific laboratory tests available at the time. During the study period (1992 – 2000), two cross-sectional studies were conducted using standard study protocols as described in Chapter 3.2. The newly developed multiplex PCR (M-PCR) method was used to detect *T. pallidum, H. ducreyi* and HSV in ulcer exudates. To maintain comparability with previous studies conducted in 1986 and 1990, the traditional methods of culture for *H. ducreyi* and HSV were also employed in the 1994 and 1998 studies. The results of previous studies conducted in 1986 and 1990 were included for comparison. The comparative aetiology of GUD using non-NAAT technology is shown in Table 3.3. No significant change in the proportion of patients with a single aetiological agent was detected during the study period 1986 – 1998.

Table 3.3 Aetiology of genital ulcer diseases, Carletonville (1986 – 1998)

	1986		1990		1994		1998	
	N = 239		N = 213		N = 232		N = 186	
Single Aetiology	**167**	**69.8%**	**129**	**60.6%**	**142**	**61.2%**	**120**	**64.5%**
Chancroid	127	53.1%	107	50.2%	93	40.1%	71	38.2%
LGV	8	3.3%	2	0.9%	6	2.6%	9	4.5%
G. Herpes	3	1.3%	3	1.4%	23	9.9%	31	16.7%
RPR +ve	29	12.1%	17	8.0%	20	8.6%	9	4.8%
Mixed Infection	**41**	**17.2%**	**27**	**12.7%**	**28**	**12.1%**	**15**	**8.1%**
Chancroid + RPR	28	11.7%	18	8.5%	19	8.2%	10	5.4%
Chancroid + LGV	6	2.5%	3	1.4%	1	0.4%	1	0.5%
Chancroid + LGV + RPR	0		1	0.5%	0		0	
LGV + RPR	2	0.8%	0		1	0.4%	2	1.1%
Herpes + LGV + RPR	0		1	0.5%	0		0	
Herpes + LGV	0		0		0		0	
Herpes + RPR	2	0.8%	0		2	0.9%	1	0.5%
Herpes + Chancroid + RPR	1		0		1	0.4%	0	
Herpes + Chancroid	2	0.8%	4	1.9%	4	1.7%	1	0.5%
Indeterminate	**31**	**13.0%**	**57**	**26.7%**	**62**	**26.7%**	**51**	**27.4%**

* *H.ducreyi culture positive*
*** C.trachomatis culture positive from ulcer exudates*
**** HSV culture positive*

However, the proportion of patients with positive *H. ducreyi* (HD) culture alone declined significantly from 53.1% in 1986 to 38.2% in 1998 (χ^2 = 9.42, p 0.002). The overall relative prevalence rate of HD (single and mixed) had also declined from 68.6% in 1986 to 44.6% in 1998 (χ^2 = 24.7, p<0.001) (Figure 3.16).

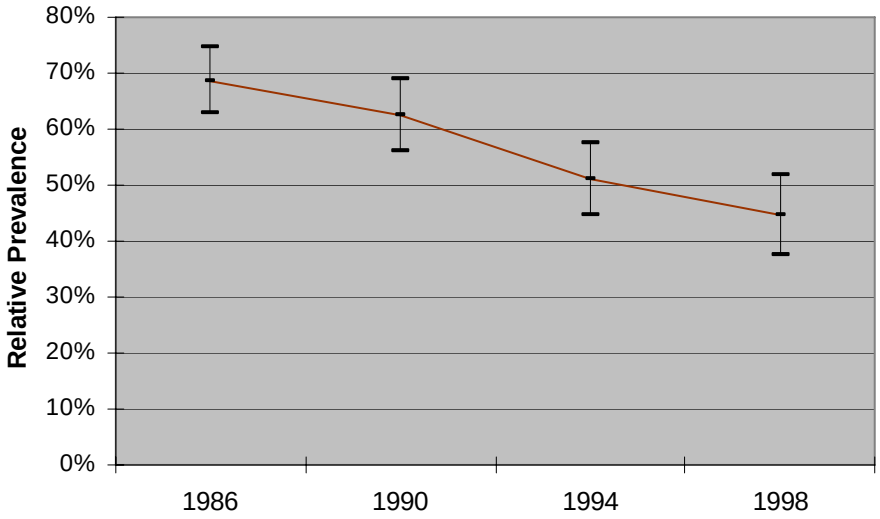


Figure 3.16 Trend of culture-confirmed chancroid among GUD patients, Carletonville (1986 – 1998)

Similarly, rates of syphilis seropositivity among GUD patients also declined steadily from 25.9% in 1986 to 11.8% in 1998 (χ^2 = 13.1, p<0.001) (Figure 3.17).

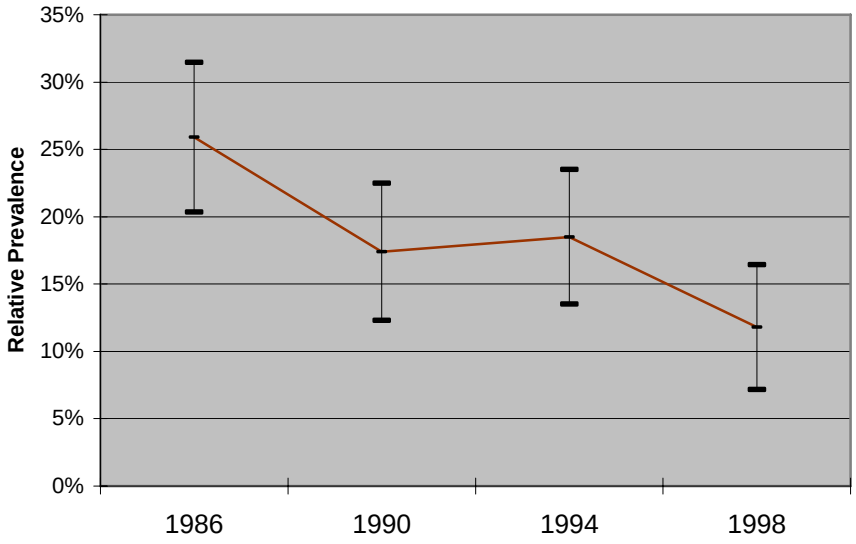


Figure 3.17 Trend of syphilis seropositivity rate (RPR-positive) among GUD patients, Carletonville (1986 – 1998)

In contrast, the relative prevalence of HSV increased significantly from 3.3% in 1986 to 17.7% in 1998 (χ^2 = 24.9, p<0.001) (Figure 3.18).

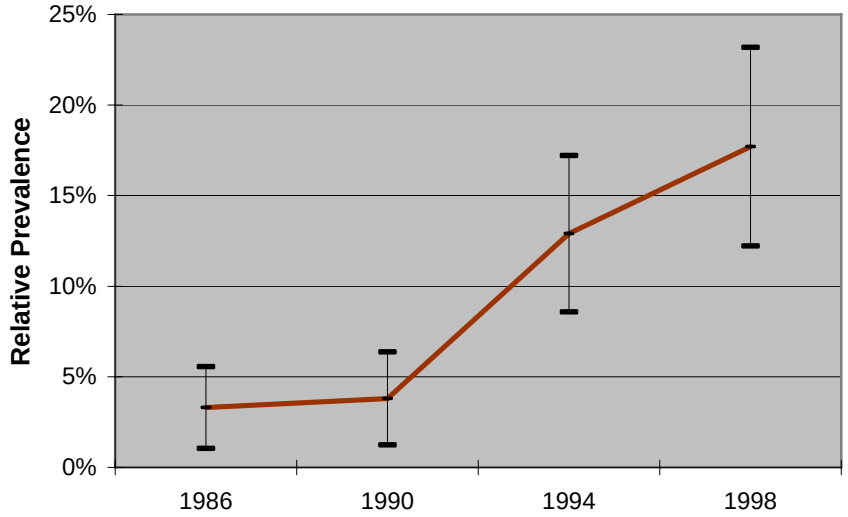


Figure 3.18 Trend of relative prevalence of culture-confirmed HSV in GUD patients (1986 – 1998)

There was an increasing trend in the prevalence of HIV among patients presenting with GUD during the study period (Figure 3.19). The HIV prevalence rapidly increased from 1.5% in 1986 to 22.1% in 1990 and 56.0% in 1994 stabilizing at 58.8% in 1998.

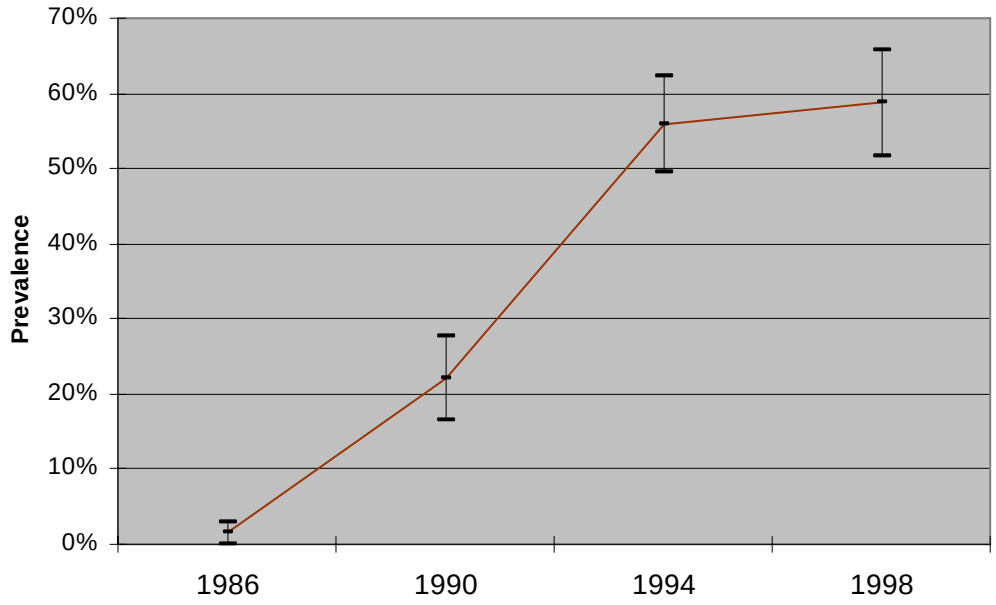


Figure 3.19 HIV prevalence among GUD patients, Carletonville (1986 – 1998)

During the period 1986 – 1994, the relative prevalence of genital herpes has rapidly increased among GUD patients with HIV co-infection: 0% in 1986, 4.7% in 1990 and 20.8% in 1994 (χ^2= 56.6, p<0.001) (Figure 3.20). However, a stable trend of the relative prevalence of HSV was found among GUD patients without HIV co-infection: 3.3% in 1986, 3.9% in 1990 and 2.8% in 1994, respectively (χ^2 = 0.09, p=0.96).

By 1998, the proportion of those with genital herpes among GUD patients co-infected with HIV stabilized at 19.6% while a significant increase was found among those without HIV co-infection (2.8% in 1994 to 14.7% in 1998, χ^2 = 8.1 6, p 0.004). Although the relative prevalence of genital herpes was still higher among GUD patients with HIV infection in 1998, the difference was smaller and not significant (42.1% in 1994 vs 29.6% in 1998, p=0.09) as a result of a similar increase in the relative prevalence of genital herpes among HIV-seronegative patients with GUD. The attributable risk of HIV in the increase of HSV as cause of GUD was 17% in 1990, 86.5% in 1994 and 25% in 1998.

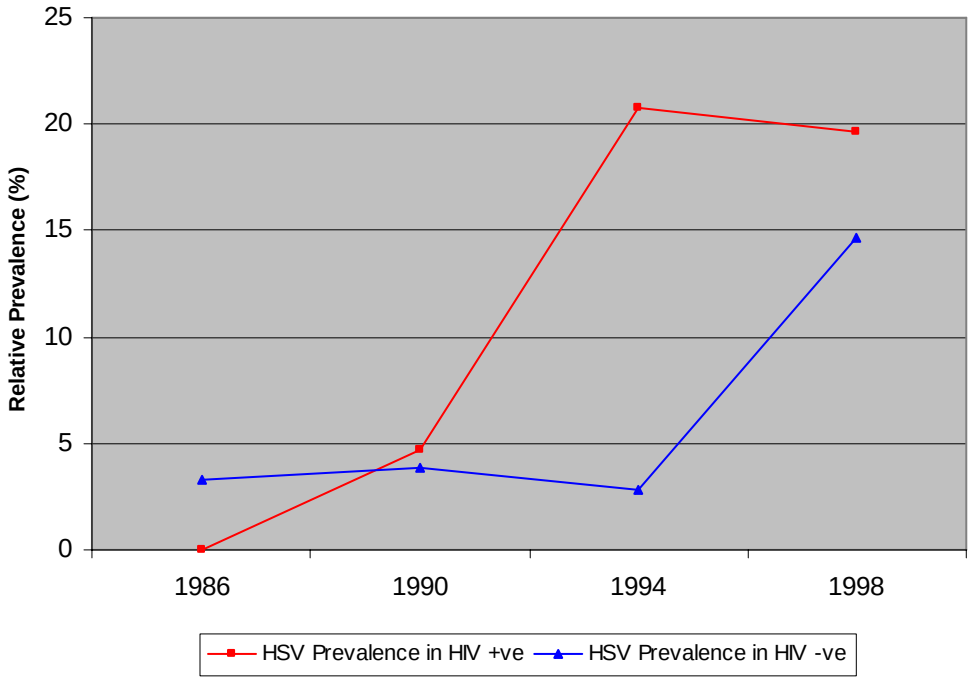


Figure 3.20 Association between HIV and HSV infection* among GUD patients (1986 – 1998) (* Based on HSV culture)

Only in the studies conducted in 1994 and 1998 was multiplex PCR (M-PCR) used for HSV, HD and TP and the results are shown in Table 3.4.

Table 3.4 Results based on M-PCR for TP, HD and HSV (1994 & 1998).

M-PCR	1994		1998	
	232		186	
Single Aetiology	**190**	**81.9%**	**116**	**62.4%**
H.ducreyi	146	62.9%	67	36.0%
HSV	28	12.1%	39	21.0%
T.pallidum	16	6.9%	10	5.4%
Mixed Infection	**17**	**7.3%**	**31**	**16.7%**
H.ducreyi + *T.pallidum*	5	2.2%	3	1.6%
H.ducreyi + HSV	9	3.9%	18	9.7%
H.ducreyi + *T.pallidum* + HSV	1	0.4%	6	3.2%
T.pallidum + HSV	2	0.9%	4	2.2%
Indeterminate	**25**	**10.8%**	**39**	**16.8%**

As the sensitivity of M-PCR for HD and HSV is greater than that of culture, the relative prevalence of HSV and HD among GUD patients is higher than if cultures were performed (Table 3.5). In addition, M-PCR was able to detect the presence of TP in genital ulcer

exudates; therefore, for the determination of the relative prevalence of primary syphilis, M-PCR provided improved sensitivity and specificity when compared to the results based on darkfield microscopy (DF) and/or serology.

Table 3.5 Sensitivity and specificity of HSV & HD cultures compared to M-PCR

Performance of *H.ducreyi* culture compared to MPCR (1994)

		MPCR - HD		
		Pos	Neg	Total
HD Culture	Pos	119	7	118
	Neg	42	64	106
	Total	161	71	232

		95% CI	
SS	73.9%	*66.3*	*80.4*
SP	90.1%	*80.2*	*95.6*
PPV	94.4%	*88.5*	*97.5*
NPV	60.4%	*50.4*	*69.6*

Performance of *H.ducreyi* culture compared to MPCR (1998)

		PCR		
		Pos	Neg	Total
HD Culture	Pos	81	2	83
	Neg	13	90	103
	Total	94	92	186

		95% CI	
SS	86.2%	*77.2*	*92.1*
SP	97.8%	*91.6*	*99.6*
PPV	97.6%	*90.8*	*99.6*
NPV	87.4%	*79*	*92.8*

Performance of HSV culture compared to MPCR (1994)

		MPCR - HSV		
		Pos	Neg	Total
HSV Culture	Pos	28	2	30
	Neg	12	190	202
	Total	40	192	232

		95% CI	
SS	70.0%	*53.3*	*82.9*
SP	99.0%	*95.9*	*99.8*
PPV	93.3%	*76.5*	*98.8*
NPV	94.1%	*89.6*	*96.8*

Performance of HSV culture compared to MPCR (1998)

		MPCR - HSV		
		Pos	Neg	Total
HSV Culture	Pos	30	3	33
	Neg	37	116	153
	Total	67	119	186

		95% CI	
SS	44.8%	*32.8*	*57.4*
SP	97.5%	*92.3*	*99.3*
PPV	90.9%	*74.5*	*97.6*
NPV	75.8%	*68.1*	*82.2*

SS = Sensitivity, SP = Specificity; PPV = Predictive Value Positive, NPV = Predictive Value Negative

The relative prevalence of HSV detected among GUD patients with HIV co-infection was 26.2% in 1994 and 42.1% in 1998 (χ^2 = 6.68, p 0.009). Similarly, there was a significant increase in the relative prevalence of HSV among GUD patients without HIV co-infection; 5.9% in 1994 to 28% in 1998 (χ^2 = 11.4, p<0.001) (Figure 3.21). Similar trends, but with lower relative prevalence rates, were observed based on HSV culture findings.

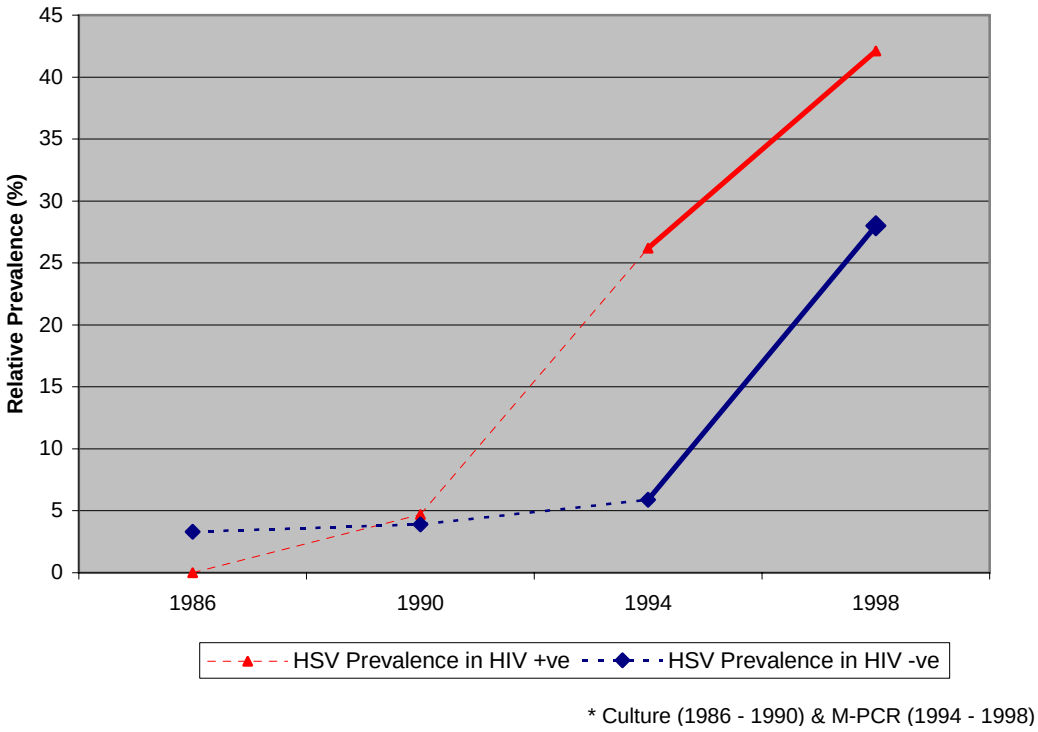


Figure 3.21 Relative prevalence of HSV among GUD patients by HIV status (1986 – 1998)

A significant decline in the relative prevalence of HD among patients with GUD was recorded between 1994 and 1998 [69.4% in 1994 to 50.5% in 1998 (χ^2 =15.4, p<0.001)]. Unlike HSV, the decline was observed in HIV-seropositive and HIV-seronegative patients (64.3% to 46.7% in HIV-seropositive patients; χ^2 = 7.25, p 0.007, and 75.5% to 57.3% in HIV-seronegative patients; χ^2 = 6.64, p 0.009).

The relative prevalence of TP (primary syphilis) increased from 10.3% in 1994 to 12.4% in 1998 although the increase was not statistically significant (χ^2 = 0.42, p 0.5). No significant difference in the relative prevalence of TP between HIV-seropositive and HIV-seronegative patients was observed in the 1994 and 1998 studies (8.7% vs 12.3% in 1994, p 0.4; 11.2% vs 14.7% in 1998, p 0.5).

3.5 Discussion

3.5.1 STI and HIV trends among Carletonville miners: a southern African perspective

Although AIDS was first recognized among homosexual men in the United States in 1981[15-17], HIV infection is thought to have spread, largely unnoticed, throughout most of the world by the mid-1980s. Starting in the late 1980s, HIV infection spread extensively across sub-Saharan Africa. The epidemic extended from West Africa across the continent and reached the Indian Ocean in the early 1980s. In the meantime, countries north of the Sahara and in southern Africa remained apparently untouched. It was only by 1987 that the epidemic began to move south, culminating in explosive waves of spread involving all the countries in southern Africa by the mid-1990s[18].

In Uganda in 1990, the HIV prevalence among pregnant women in urban areas was 30% and 45.3% among STI patients during the same period[19]. Meanwhile, the HIV prevalence among pregnant women in South Africa was found to be 0.7% in 1990 and increased to 2.2% in 1992[20]. In 1990, the HIV prevalence among STI patients in South Africa was less than 6% based on sentinel surveillance[21]. A similar trend was observed in neighbouring countries during the same period, for example rates of less than 6% in pregnant women and less than 10% in STI patients were recorded in Lesotho[22-23].

In most countries in southern Africa, the mining industry contributes significantly to the economy. For example, in South Africa, the mining industry contributes 6.6% of the gross domestic product and more than 9% of gross investment[24]. About 691 mines and quarries employ 410,000 people, many from neighbouring countries, representing about 3% of the economically active population in South Africa. A significant proportion of adult males in their productive years are employed in various mines as migrant labourers. In South Africa, the mining companies hired a large number of migrant male workers from neighbouring countries. Among these countries, Botswana, Lesotho, Swaziland, Mozambique and Namibia shared unique socioeconomic determinants for rapid spread of HIV/AIDS during the early 1990s.

Most migrant workers continue to live in single-sex hostels on the mines while their spouses or regular sexual partners reside at their permanent homes. A thriving informal sex trade was maintained at localities surrounding the mines as well as in many urban areas frequented by migrant workers during their periods of leave. The sexual networks among migrant miners, their spouses or regular sexual partners, and casual sexual partners in so-called 'hot-spots' have contributed towards a sustained high prevalence of STIs during the 1980s and early 1990s. These sexual networks have also fuelled the rapid spread of STIs not only in the mining areas, but also at the miners' places of origin. Among the risk determinants, the following are the most important factors creating a favourable environment for engaging high-risk sexual practices undertaken by miners: long-term separation from their regular sexual partners and family units; easy access to affordable commercial sex establishments; rapid sexual partner change; excessive alcohol consumption and the influence of high-risk peers. Studies have also suggested that parallel sexual networks at the towns or villages where migrant miners are recruited, involving their spouses or regular sexual partners and local sexually active male residents play a role in STI spread[25].

Despite the rapid introduction of HIV into the population, most notably among high-risk local groups as had been reported in the early 1990s, the general complacency of communities and their respective health authorities in many countries in the region delayed a scaled-up implementation of intervention activities. Although comprehensive statistics on STI incidence were not readily available in these countries, anecdotal evidence clearly showed that STIs were one of the top ten disease groups causing morbidity in early-1990s. Most countries had either underestimated or ignored early warning indicators derived from STI incidence data and information of existing complex sexual networks associated with cross-border migratory labour systems. As a consequence, most countries failed to take advantage of the window of opportunity to contain the HIV epidemic before it could reach its exponential growth phase.

The events leading to the rapid spread of HIV, as documented in our studies among miners, clearly support the existence of epidemiological synergies operating between STIs and HIV in southern Africa. Thus the high STI incidence among miners, coupled with existing complex

social and sexual networks in the region might have led to the rapid and explosive spread of HIV in the mid-1990s. By 2003, HIV prevalence in these countries reached extremely high levels - often exceeding 30% among pregnant women. Such levels have been recorded in South Africa, Botswana, Lesotho, Namibia and Swaziland[26].

Similar trends were observed in our study population. Although systematic HIV surveillance among miners was not implemented at the beginning of the HIV epidemic, cross-sectional studies conducted in the early 1990s indicated that the prevalence of HIV among miners with STIs was very low (<1%)[27]. Meanwhile, the STI incidence among miners was extremely high in the late 1980s, a situation which likely served as fertile soil for the subsequent rapid spread of HIV infection in this population.

Unfortunately, strategies for the control of the STI epidemic in the mining industry during the 1980s - mid-1990s, focused mainly on providing appropriate treatment for symptomatic STIs. This traditional approach in STI control seems to have had a limited impact on the STI epidemic in sexually active male migrant workers in the mines. As a result, the STI incidence among migrant miners in Carletonville remained high during the mid-1990s, despite significant improvements in the management of STIs on the mines. Likewise, the HIV prevalence among STI patients also reached its highest level in the mid-1990s. The overall situation in the mines triggered the rapid spread of HIV from relatively small high-risk “core” groups into the wider general population in the late-1990s. It was only in the mid-1990s that several targeted intervention and community-based STI/HIV prevention activities were implemented in the Carletonville region. These measures, together with epidemiological and social research projects on migration and HIV/AIDS conducted in mining areas contributed significantly in improving HIV/AIDS prevention and control activities in mining communities. Similarly, recommendations and policy changes were made by international bodies, governments and private mining companies to control further spread of HIV[28].

Our study findings have highlighted that STI control activities targeting a high-risk migrant population group must be accompanied by appropriate outreach intervention components

including prevention and treatment of STIs among the miners' regular and casual sexual partners in the community. Overall, STI control activities targeting only the mine workforce did not prove to be cost-effective and did not achieve long-term sustainable control of STIs. It is believed that a significant decline in STI incidence could, however, be achieved by intervention activities aimed at comprehensive coverage, conducted with a high degree of intensity and aimed at sustainability in all vulnerable population groups.

The present studies have demonstrated the ability of STI surveillance to provide indications of the level of vulnerability to the HIV epidemic. This approach has been discussed widely at the international level. UNAIDS and WHO recommended in their guidelines for second generation HIV surveillance that STI surveillance in individuals with high levels of risky sexual behaviour be used as a physical marker of unprotected sex with multiple partners[29]. The incidence of acute STIs is closely monitored in many developed countries and has provoked rapid response when resurgence of acute STIs has occurred, eg, prompt action was taken when infectious syphilis was recognised as an emerging problem in Europe[30-31]. With a better understanding of the interactions between the epidemiology of HIV and STI, many countries have used STI prevalence as a barometer of HIV vulnerability and recognised STIs as contributing factors to the spread of HIV. In a recent WHO meeting in Treviso, Italy, participants reaffirmed that selected STI incidence data as provided by comprehensive STI surveillance systems, could be utilized as early warning risk indicators for HIV in vulnerable population groups[32]. The epidemiological interactions between STIs and HIV are, however, strongly dependent on different phases of the HIV and STI epidemics. Therefore, caution should be exercised in utilising STI surveillance data as proxy early warning indicators of HIV vulnerability in various population groups, especially in situations when the HIV epidemic has reached a stage of relative maturity and STIs such as genital herpes may be missed[33].

In summary, our studies contribute towards better understanding of important epidemiological determinants which have fuelled the rapid spread of HIV in southern Africa. In addition, the findings highlight the utility and usefulness of STI surveillance data in predicting the magnitude of vulnerability to HIV transmission among high-risk population groups. The

importance of linking these two surveillance systems should be promoted and applied in tracking the HIV epidemic globally. It is also crucial for individual countries to implement an adequate STI surveillance system as recommended by WHO[34].

3.5.2 Changing aetiological patterns of STIs

Studies have shown that the clinical diagnostic accuracy of STI in developing countries with high level of mixed infections is extremely poor[27]. The clinical diagnostic accuracy is likely to be worse in geographical areas where all aetiological agents causing a STI syndrome are endemic. In southern Africa, all pathogens causing major STI syndromes were highly prevalent during the 1980s and 1990s. For example, the bacterial agents which cause genital ulcerations (*T. pallidum, H. ducreyi, C. trachomatis* (LGV strains), and *Klebsiella granulomatis*) were highly endemic. Similarly, the aetiologic agents causing sexually transmitted urethritis were also highly prevalent. Due to the lack of easy availability and accessibility of sensitive and specific STI laboratory tests in many developing countries, WHO recommended the use of syndromic management of STIs[35]. In our study, all STI episodes seen at the clinic were recorded syndromically except for selected diseases such as genital herpes and LGV.

Syndromic surveillance alone, however, was not sufficient to adequately monitor the changing pattern of STIs. It is possible that the incidence of an STI syndrome may remain unchanged while significant changes may occur in the relative proportion of specific aetiologies causing the syndrome. Therefore, a new surveillance approach was established and implemented in our STI clinic at Carletonville for a better understanding of changing profile of STI aetiologies (Figure 3.22). Epidemiological data obtained from two systems were analyzed and interpreted in an integrated manner. This approach was implemented in the Gauteng Province and a national STI surveillance programme for South Africa based on the model described here was constituted in 2003.

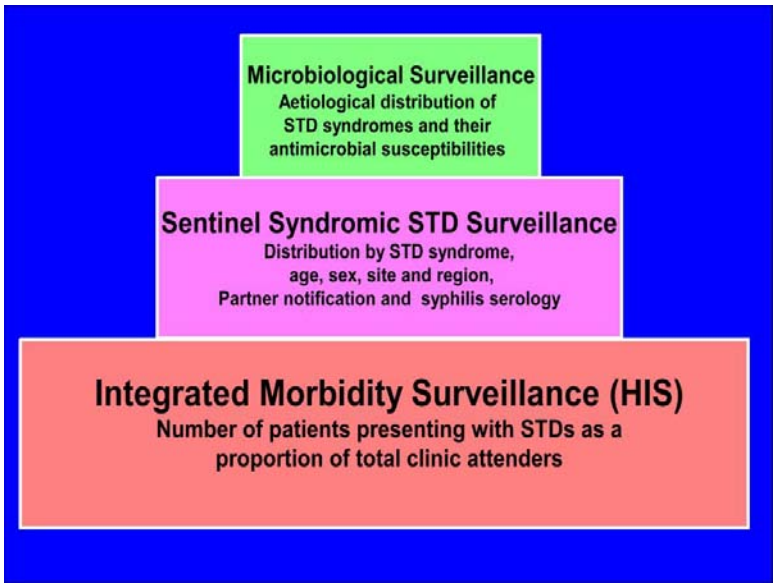


Figure 3.22 Tiered syndromic STI surveillance systems

Syndromic STI surveillance data indicated that there was a high incidence of genital ulcerations among miners during the early phase of HIV epidemic. This sustained high incidence of GUDs in the early 1990s was followed by a rapid increase in HIV prevalence in 1995. Comprehensive information on the aetiological patterns of GUD syndromes was obtained from the periodic cross-sectional aetiological studies conducted at 4-yearly intervals using the same study design.

During 1992 – 1998, the relative prevalence of genital ulcer disease (GUD) was significantly higher than other STI syndromes with the highest proportion of GUD amongst STI syndrome was reported in 1994. Overall, more than 50% of STI episodes presented at the clinic were genital ulcerations in the early-1990s, and majority of cases were non-vesicular GUD. Their clinical presentation was mostly compatible with bacterial causes of GUD particularly that of chancroid. This observation was confirmed by the aetiological studies conducted in 1986 and 1990 when over 60% of GUD were bacteriologically proven to be caused by *H. ducreyi*. Similarly, the highest RPR seropositivity rate was found among GUD patients during the early 1990s. In the GUD aetiology study conducted in 1994, the relative prevalence of primary syphilis and chancroid was in excess of 10% and 69% respectively when more sensitive M-PCR assays were used. Meanwhile, the relative proportion of vesicular ulcerations (clinically

suggestive of genital herpes) was less than 10% of total STI burden in 1992 – a finding confirmed in aetiological studies that laboratory-confirmed genital herpes in 1990 was less than 5% of GUDs.

This period (1992 – 1994) could be described as **'**the early phase of HIV epidemic' in southern Africa when HIV prevalence increased steadily in both high-risk and low-risk population groups[23,36]. During this early phase, our surveillance systems indicated a high incidence of GUDs due to chancroid among the miners, and showed that the prevalence of HIV among patients with GUD was almost two-fold higher than among urethritis patients. The syphilis seroprevalence as identified by a positive RPR test confirmed with a treponemal test was also high among STI patients with a higher prevalence of positive tests being found in patients with GUD.

Subsequently, 'the accelerated HIV transmission phase' was documented during the mid-1990s, and continued until the epidemic peaked in the late-1990s. During this period, there was exponential growth in HIV prevalence in many countries in the region, including miners with STIs in Carletonville. The HIV prevalence among STI patients in the mine doubled from 20.8% in 1994 to 42.4% in 1995. This doubling in HIV prevalence was found across all STI syndromes. The highest HIV prevalence was found among patients presenting with genital warts. Our observation does not explain the nature of the associations between genital warts and HIV. However, this phenomenon might have been due to an increase in persistence and severity of genital warts related to HIV-induced immunodeficiency as reported elsewhere[37-39]. Although there was a slight increase in patients presenting with new or recurrent episodes of genital warts, these lesions represented less than 5% of the total STI burden throughout the study period.

A similar stable trend was found in the case of LGV and epididymo-orchitis. The HIV prevalence among patients presenting with epididymo-orchitis was significantly higher than those presenting with urethritis (i.e. 29.6% vs. 36.3, χ^2 = 8.89, p 0.003). The relative prevalence of epididymo-orchitis also increased from 0.8% in 1992 to 1.7% in 2002. Although

no epidemiological and clinical studies elsewhere have reported a similar association, it is plausible that HIV positive patients may have higher risks of developing ascending infection due to the STI agents causing urethritis. In our study, we did not conduct the radiological or sonographic investigations required to exclude other causes such as tuberculous epididymo-orchitis. However, we observed that most patients presenting with epididymo-orchitis in the mines have either a past history of or presented with concurrent urethritis, and a high titre of type-specific chlamydial antibody based on chlamydial microimmunofluorescent serology tests (Ye H and RC Ballard personal observation). Prospective studies are still required to confirm this association.

The relative proportion, as well as absolute incidence of non-vesicular GUD declined while vesicular ulcerations (clinically suggestive of genital herpes) increased rapidly. This trend was supported by aetiological studies conducted during the period, where the relative prevalence of laboratory-confirmed chancroid declined to its lowest level in 1998, and genital herpes increased significantly. The relative prevalence of genital herpes among HIV-seropositive GUD patients increased sharply and genital herpes was highly associated with HIV-seropositivity. Due to the nature of our cross-sectional study design, we were unable to verify whether the association was due to an increase in the frequency of recurrent herpes induced by HIV-related immunodeficiency or an increase in the acquisition of HIV in patients with genital herpes due to recurrent micro/macro ulcerations caused by genital herpes. Our study confirmed, however, that the attributable risk of HIV associated with the increase of genital herpes among miners with GUDs could have been over 80% in 1994. The rapid increase in the relative prevalence of genital herpes among HIV-seronegative patients occurred in 1998. This could have been due to a rapid decline in bacterial GUDs responding to effective intervention activities in and around the areas, and/or an increase in transmission of genital herpes attributable to an increasing pool of infectious individuals associated with HIV infection. An increase in the absolute incidence rate of clinically suspected herpes ulcerations among miners was also observed during the study period and it is likely that both a decline in bacterial STIs and an absolute increase in genital herpes contributed to the overall changes in aetiology of GUDs.

In 1994, improved STI management was implemented in mine clinics and all GUD patients were treated with effective antibiotic therapy covering both primary syphilis and chancroid. All patients presenting with new STI episodes were routinely screened for syphilis using a quantitative RPR and treatment for syphilis was provided if they were found positive. Since then, the incidence of non-vesicular GUD among miners has decreased significantly and a similar decline in the relative prevalence of chancroid was found in the aetiological study conducted in 1994.

Following enhanced syphilis screening and treatment activities initiated in the mine, RPR seropositivity rates among GUD patients steadily declined to their lowest level in 1998-99. The RPR seropositivity rate was even lower in STI patients presenting with urethritis in 1999. Surprisingly, the RPR positive rate among patients with non-ulcerative STIs in 1999 was lower than that of pregnant women residing in the Carletonville mining town. This interesting phenomenon can possibly be attributed to the successful implementation of enhanced syphilis screening and treatment activities in the mine STI clinic. The high-risk miners frequently attending the clinic with new STI episodes had been repeatedly screened and treated for syphilis since 1994. Therefore, the probability of latent syphilis being detected and treated was much higher for a high-risk miner with repeated STI episodes than for a pregnant women who had the opportunity to be screened only when she became pregnant.

Many studies have reported that the probability of HIV acquisition and transmission is higher in patients with ulcerative STIs[40-44]. During 1992 – 1994, STI episodes due to non-vesicular GUDs and urethritis have proportionately increased in miners. This trend was associated with accelerated spread of HIV among miners with STIs. It is also epidemiologically plausible that the high incidence of genital ulcerations in the mine during early 1990s may have contributed to the rapid and explosive increase in HIV prevalence among miners with STIs. Most GUD-causing pathogens exhibit a higher transmission efficiency than HIV and the GUD epidemic in southern African countries during 1990s provided a fertile ground for the subsequent rapid spread of HIV. In addition, a high incidence of GUD has been documented among regular partners of miners at their places of origin as well as the sexually active population at various

major urban centres (Lesotho personal observation). Although the most effective antibiotics for treatment of bacterial STIs were available at the mine clinics, this was often not the case at public clinics outside the mines. Lack of effective treatment in these areas presumably led to continued transmission of major STIs within the sexual networks.

Since 1995, several intervention activities have been implemented which targeted both high and low-risk population groups in the community residing in the areas surrounding mines. These activities included (1) improvement in quality of STI care (by training and implementation of syndromic management)[45]; (2) behavioural interventions including condom promotion[46-47]; and (3) targeted interventions for high-risk women using periodic presumptive therapy for STIs[48] . A more effective contact tracing system was also implemented through collaboration with a network of private and public STI services situated in areas surrounding mines[49]. Within the two most common STI syndromes in the mines (GUD and urethritis), a rapid and sustained decline of GUD of bacterial origin was observed among miners and their casual sexual partners residing in informal settlements surrounding mines. The provision of azithromycin monthly to high-risk casual partners of miners could have contributed to the rapid decline in chancroid cases seen among miners[50-52]. As documented in other studies, targeted intervention activities focusing on high-risk women were shown to have had a positive impact in controlling curable STIs among their partners or clients[50-57]. However, the impact of these activities on the wider community is dependent on the size of bridging populations between core group at high-risk and other groups. The sexual networks between these groups have also contributed to the impact of targeted intervention activities on STI and HIV transmission in the general population. In addition, in the case of some STIs, the majority of infected individuals are either asymptomatic or have inapparent infections despite the fact that they are infectious (e.g. gonococcal/chlamydial infection in women and genital herpes). To contain the spread of these diseases, behavioural modification activities targeting all vulnerable population groups are needed. Other community-based prevalence studies conducted in Carletonville have found that the prevalence of gonococcal and chlamydial infections in various population groups in this area has not changed significantly despite a significant decline in symptomatic STIs among miners.

In summary, the syndromic STI surveillance data are useful but there should be caution when interpreting trends suggested by cumulative data. Dynamic changes in the relative prevalence of specific aetiological agents causing a syndrome in response to the effective prevention as well as treatment and outreach interventions, together with the phase of the HIV epidemic must also be taken into consideration. The high incidence of GUD in the gold miners and their sexual partners at the early phase of HIV epidemic fueled the rapid spread of HIV among high-risk miners and probably their sexual partners in the region. Improvement in the quality of STI management in the mines had a limited impact in controlling GUD epidemic in the mines, but this measure, together with the implementation of successful intervention activities targeted at casual or paid female sexual partners of miners, resulted in containment of the GUD epidemic to its lowest level in the late-1990s. Unfortunately, the relative prevalence of genital herpes has increased significantly, particularly among those patients with HIV co-infection and management of genital herpes with antiviral drugs was not considered in the GUD management algorithm until the mid-1990s. Based on our findings on the changing aetiological patterns of GUDs, an urgent review of GUD management protocols is required in order to improve the performance of the GUD management algorithm. In addition, further studies to explore the potential role of genital herpes in transmission and acquisition of HIV in southern Africa have either been undertaken, or are planned[58-59].

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CHAPTER 4

Influence of HIV/AIDS on the clinical presentation and diagnosis of genital ulcer disease in southern Africa

4.1 Aetiology of genital ulcer disease in southern Africa

4.1.1 Cross-sectional observational studies

Eight hundred and sixty-eight patients with genital ulcerations were enrolled in aetiological studies conducted in Maseru (Lesotho), Johannesburg, Durban and Carletonviile STD clinics during 1994 - 1999. All patients were of black African ethnicities and the majority (97%) were male. The overall mean age was 29 years (range 14 – 65 years) and the HIV prevalence rate was 46.7%.

Of 868 patients included in the analysis, *H. ducreyi* (HD) was detected by M-PCR in 436 (50.2%), *T. pallidum* (TP) in 163 (18.8%)**,** herpes simplex virus (HSV) in 268 (30.9%) and *C. trachomatis* (CT) in 29 (3.3%) patients. No organism could be demonstrated in 88 (10.1%) patients. A single aetiology was detected in 672 (77.4%) and multiple aetiologies in 108 (12.4%) patients. Among those with a single aetiology, HD was detected in 346 (51%), HSV in 203 (30.2%), TP in 106 (15.8%) and CT in 17 (2.5%) patients. The most common mixed infections involved syphilis, chancroid and genital herpes (Table 4.1). Of 163 TP-positive patients, 57 (34.9%) were co-infected with other aetiological agents, namely: 33 (57.9%) with HD, 13 (22.8%) with HSV and 7 (12.3%) with both. Co-infection with HD and HSV was also common (Table 4.1).

Overall, 211 (24.3%) of sera obtained from these patients were positive by both RPR and FTA-ABS tests, a further 236 (27.2%) were positive by the FTA-ABS test alone, and 421 (48.5%) were negative for both. No false positive RPR reactions were detected. Among RPR sero-positive patients, 137 (64.9%) had a titre of 1:8 or higher. The overall geometric mean titre was 11.5 (Figure 4.1).

Table 4.1 Aetiology of GUD among patients in South Africa and Lesotho (1994 – 1999)

Aetiology	No of Patients	%
Single Aetiology (N=672)		
Primary Syphilis	106	12.2
Chancroid	346	39.9
Genital Herpes	203	23.4
LGV	17	2.0
Multiple Aetiology (N=108)		
Primary Syphilis & Chancroid	33	3.8
Primary Syphilis & G.Herpes	13	1.5
Primary Syphilis, Chancroid & G.Herpes	7	0.8
Primary Syphilis & LGV	3	0.3
Primary Syphilis, Chancroid & LGV	1	0.1
Chancroid & G.Herpes	43	5.0
Chancroid & LGV	6	0.7
G.Herpes & LGV	2	0.2
Indeterminate	88	10.1
Total	868	100.0

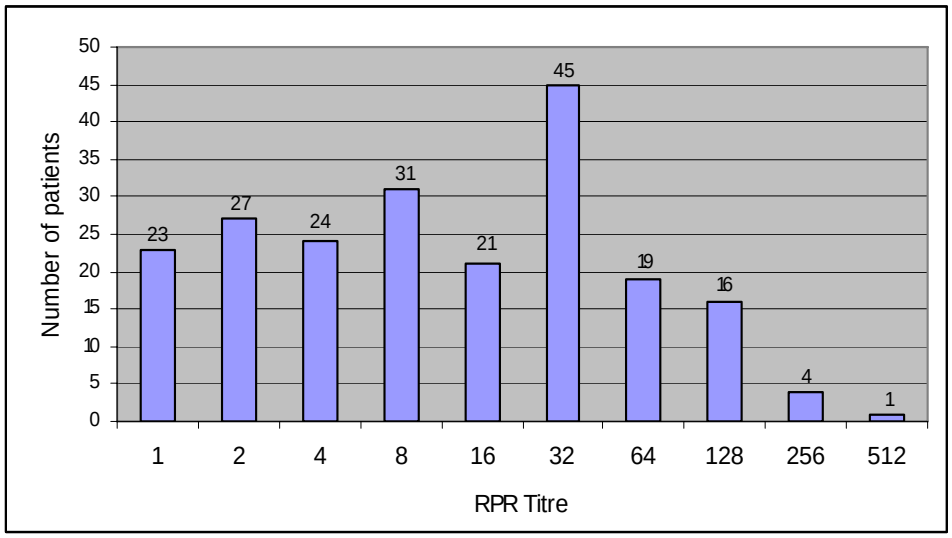


Figure 4.1 Distribution of RPR titres in 211 GUD patients with a positive RPR test

4.2 Primary Syphilis

4.2.1. Presentation of primary syphilis among HIV-seropositive and -seronegative patients

Of 106 patients who were M-PCR (TP) positive alone, complete information on clinical presentation of their disease was available in 58 patients. These data are presented in this section. The median duration of ulceration at the time of presentation was 9.5 days (range 1 – 44 days) with the majority (56.9%) seeking medical attention within 10 days of the appearance of a nodule/ulcer. Only two of 17 (11.8%) HIV co-infected patients presented with a single ulcer while 14 out of 41 (34.1%) HIV-seronegative patients with primary syphilis presented with a single ulcer. However, this difference was not statistically significant (Fisher's Exact test, p 0.11). The majority of patients in both groups complained of pain and/or tenderness around the ulcers (88% of HIV-seropositive and 73% of patients HIV-seronegative patients, respectively). Only three of 17 patients (17.6%) in the HIV-seropositive group, compared to 19 of the 41 HIV-seronegative patients (46.3%) had deep ulcerations (χ^2 = 4.2, p 0.04). Inguinal lymphadenopathy was associated with primary syphilis in both HIV-seropositive (41.2%) and seronegative patients (68.3%, χ^2 = 3.69, p 0.054) and the majority presented with bilateral discrete lymph nodes. Three of 17 (18.8%) HIV-seropositive patients and 13 of 41 (31.7%) seronegative patients with primary syphilis alone were correctly diagnosed clinically as primary syphilis. This difference was not statistically significant (χ^2 = 0.59, p 0.4).

4.2.2 Impact of HIV on diagnosis of primary syphilis

The rates of positive syphilis and HIV serology among patients with different GUD aetiologies is shown in Table 4.2. The highest RPR positivity rate of 69.3% was found among patients with positive M-PCR (TP). Of 163 M-PCR (TP)-positive patients, the RPR positivity rate was higher in patients with single TP infections (79.2%) than in those with mixed aetiology involving TP and other agents (50.9%) (χ^2 = 14.03, p 0.0001) (Table 4.2). The RPR seropositivity rate among patients with a negative M-PCR (TP) was significantly lower than that recorded among patients with positive M-PCR (TP) (13.9% vs 69.3%, χ^2 = 216.9,

p<0.001). The difference was more obvious when the rates between patients with TP only (79.2%) and those with aetiologies other than TP (11.8%) were compared (χ^2 = 241, p<0.001).

Table 4.2 RPR positivity by GUD aetiology

Aetiology of GUD	RPR				Total
	Positive		Negative		
	No	%	No	%	
Only M-PCR (TP) +ve	84	79.2	22	20.8	106
Mixed TP & Others	29	50.9	28	49.1	57
Other Aetiologies	73	11.8	544	88.2	617
Indeterminate	25	28.4	63	71.6	88
Total	211	24.3	657	75.7	868

The geometric mean titre of RPR tests in those solely with treponemal GUD was similar to that of patients with mixed treponemal GUD (1:17.1 vs 1:16.8). The geometric mean titre was, however, significantly lower in RPR-seropositive patients with non-treponemal GUDs (1:5.9) (ANOVA, F statistics=2.9, p<0.05).

All RPR-seropositive patients were confirmed to be truly positive by FTA-ABS testing. No biological false positive reactions were detected. The overall FTA-ABS seropositivity rate was 35.9%. Of 657 RPR-seronegative patients, 236 (35.9%) were positive by the FTA-ABS test. As was the case with the RPR test, GUD patients who were only M-PCR (TP) positive had a higher FTA-ABS seropositivity rate than those with other aetiologies recorded (81.8% vs. 33.6%, χ^2 = 21.4, p <0.001) (Table 4.3).

Table 4.3 FTA-ABS seropositivity by GUD aetiology

Aetiology of GUD	RPR -ve & FTA-ABS +ve				Total
	FTA-ABS +ve		Both Negative		
	No	%	No	%	
Only M-PCR (TP) +ve	18	81.8	4	18.2	22
Mixed TP & Others	15	53.6	13	46.4	28
Other Aetiologies	183	33.6	361	66.4	544
Indeterminate	20	31.7	43	68.3	63
Total	236	35.9	421	64.1	657

The overall HIV seroprevalence among GUD patients was 46.7%. Of 163 patients who were M-PCR (TP)-positive, 47 (28.8%) were co-infected with HIV. Among patients with a single aetiology, the lowest HIV prevalence was found among the patients infected with TP (20.8%). A higher HIV prevalence rate (43.9%) was however found in patients with mixed TP and other aetiological agents (43.9% vs 20.8%, $\chi^2 = 9.64$, p 0.001). The highest HIV prevalence (53.3%) was found in patients infected with non-treponemal agents of GUD (Table 4.4).

Table 4.4 Prevalence of HIV by GUD aetiology

Aetiology	HIV				Total
	Positive		Negative		
	No	%	No	%	
Only M-PCR (TP) +ve	22	20.8	84	79.2	106
Mixed TP & Others	25	43.9	32	56.1	57
Other Aetiologies	329	53.3	288	46.7	617
Indeterminate	29	33.0	59	67.0	88
Total	405	46.7	463	53.3	868

The RPR test was, however, positive in 27 patients (57.4%) in the HIV-seropositive group and 86 patients (74.1%) in the HIV-seronegative group (OR 0.47, 95% CI 0.23 – 0.96, p<0.05). Irrespective of their RPR status, no significant difference in FTA-ABS seropositivity rates was found among HIV-seropositive and seronegative patients with TP infection (83% vs. 92.2%, NS), Eight HIV-seropositive patients (17%) and 9 HIV-seronegative patients (7.8%) with TP

infection were negative by both serological tests (Table 4.5). There was no significant difference in the geometric mean titre of the RPR test between HIV-seropositive and seronegative patients with a positive RPR test (1:13.7 vs 1:18.2, respectively).

Table 4.5 Syphilis serostatus of primary syphilis patients by HIV status

Syphilis Serostatus of MPCR-TP Positive Cases	HIV +ve (N = 47)		HIV -ve (N = 116)		OR (95% CI)	p
	No	%	No	%		
Positive RPR	27	57.4	86	74.1	0.47 (0.23 - 0.96)	<0.05
Positive FTA-ABS*	39	83.0	107	92.2	0.41 (0.15 - 1.14)	NS
Both RPR & FTA-ABS Negative	8	17.0	9	7.8	2.4 (0.88 - 6.8)	NS

*with or with RPR +ve

Among 163 M-PCR (TP) positive patients, 106 (65%) were solely infected by TP and 57 (34.9%) had mixed infections with other aetiological agents. Of 106 patients with a single aetiology, 22 (20.8%) were co-infected with HIV (Table 4.6). There was no significant difference in RPR positivity rates between HIV-seropositive and seronegative groups with a single TP infection (81.8% vs. 78.6%, Fisher's exact test NS). There was, however, a significant difference in the RPR seropositivity rate among HIV-positive and negative groups with mixed GUD aetiologies (36.0% vs 62.5%, $\chi^2 = 3.9$, $p<0.05$).

Of 705 patients with a negative M-PCR (TP) test, 358 (50.8%) were co-infected with HIV and the overall RPR seropositivity rate was 13.9%. The RPR seropositivity rate in HIV-seropositive patients was 12.3% and in HIV-seronegative patients 15.6% ($\chi^2 = 1.6$, p 0.2). The RPR seropositivity rate was significantly higher in patients with indeterminate laboratory findings when compared with that seen in patients with known aetiologies other than TP (28.4% vs. 11.8%, χ^2 17.7, $p <0.001$). This significance was independent of HIV serostatus (HIV-seropositives: 24.1% vs. 11.2%, $\chi^2 = 4.1$, p 0.04; HIV-seronegative group: 30.5% vs. 12.5%, $\chi^2 = 12.1$, p 0.001).

Table 4.6 Syphilis and HIV seroprevalence by GUD aetiology

Aetiology	Number of Patients	Both RPR & FTA-ABS +ve		FTA-ABS +ve only		Both RPR & FTA-ABS -ve		HIV Positive	
		No	%	No	%	No	%	No	%
Single Aetiology									
Primary Syphilis	106	84	79.2	18	17.0	4	3.8	22	20.8
Chancroid	346	45	13.0	115	33.2	186	53.8	163	47.1
Genital Herpes	203	14	6.9	49	24.1	140	69.0	126	62.1
LGV	17	3	17.6	5	29.4	9	52.9	10	58.8
Multiple Aetiology									
Primary Syphilis & Chancroid	33	17	51.5	6	18.2	10	30.3	14	42.4
Primary Syphilis & G. Herpes	13	6	46.2	6	46.2	1	7.7	7	53.8
Primary Syphilis, Chancroid & G. Herpes	7	4	57.1	2	28.6	1	14.3	3	42.9
Primary Syphilis & LGV	3	2	66.7	-		1	33.3	1	33.3
Primary Syphilis, Chancroid & LGV	1	-		1	100.0	-		-	
Chancroid & G. Herpes	43	9	20.9	10	23.3	24	55.8	27	62.8
Chancroid & LGV	6	2	33.3	3	50.0	1	16.7	2	33.3
G. Herpes & LGV	2	-		1	50.0	1	50.0	1	50.0
Indeterminate	88	25	28.4	20	22.7	43	48.9	29	33.0
Total	868	211	24.3	236	27.2	421	48.5	405	46.7

The association of the median duration of ulcers and HIV seroprevalence rates in three groups of patients with different syphilis serological status is shown in Table 4.7. No significant differences were found between the groups.

Table 4.7 Median duration of ulcer and syphilis serostatus among GUD patients

Median duration of ulcer	RRP & FTA-ABS Positive	RPR Negative & FTA-ABS Positive	Both RPR & FTA-ABS Negative
Treponemal GUD	10 days (2 - 90 days)	10 days (1 – 120 days)	10 days (2 – 35 days)
Non-treponemal GUD	7 days (1 – 132 days)	7 days (1 – 90 days)	7 days (1 – 150 days)

A comparison of the performance of syphilis serology in HIV-seropositive and seronegative patients with single and mixed infections is shown in Table 4.8. Eighty-eight patients with unknown or indeterminate aetiology were excluded from this analysis. The overall sensitivity of RPR to detect primary syphilis in our setting was 69.3% and its specificity was 86.1%. The sensitivity of syphilis serology improved to 89.6%, when using the FTA-ABS test. The sensitivity of the RPR test was found to be significantly higher in HIV-seronegative than HIV-seropositive patients (74% vs 57.4%, $\chi^2 = 4.94$, p 0.026). The sensitivity of the RPR test decreased further to 36% in HIV-seropositive patients with mixed infections. Among HIV-seropositive patients, there was a significant difference in the sensitivity of the RPR test in patients infected solely by TP as a single infection when compared to those who had mixed TP infections (81.8% vs 36%, p 0.001). This difference was, however, not statistically significant among those patients who were HIV-seronegative.

Table 4.8 Performance of syphilis serology in the diagnosis of primary syphilis

	RPR		p	FTA-ABS		p
	Single infection	Mixed infection		Single infection	Mixed infection	
HIV-seropositive						
Sensitivity	18/22 (81.8%)	9/25 (36%)	**0.001**	21/22 (95.5%)	18/25 (72%)	0.08
Specificity	271/299 (90.6%)	21/30 (70%)	**0.002**	175/299 (58.5%)	14/30 (54.5%)	0.58
PPV	18/46 (39.1%)	9/18 (50%)	0.4	21/145 (14.5%)	18/34 (52.9%)	**0.001**
NPV	271/275 (98.5%)	21/37 (56.8%)	**0.001**	175/176 (99.4%)	14/21 (66.7%)	0.27
HIV-seronegative						
Sensitivity	66/84 (78.6%)	20/32 (62.5%)	0.08	81/84 (96.4%)	26/32 (81.3%)	**0.02**
Specificity	233/267 (87.3%)	19/21 (90.5%)	0.9	160/267 (59.9%)	12/21 (57.1%)	0.8
PPV	66/100 (66%)	20/22 (90.9%)	**0.02**	81/188 (43.1%)	26/35 (74.3%)	**0.001**
NPV	233/251 (92.8%)	19/31 (61.3%)	**0.001**	160/163 (98.2%)	6/18 (66.7%)	**0.02**

4.3 Genital herpes

As described in the previous chapter (Sections 3.4.2 and 3.4.3), the true incidence as well as the relative prevalence of genital herpes among GUD patients increased significantly in southern Africa during the period 1994-2000. In this section, the clinical presentation and natural history of genital herpes diagnosed either by culture and/or M-PCR (HSV) among the patients recruited into the studies described in Chapter 3.3.1 will be presented. The impact of the increasing incidence and relative prevalence of atypical herpes (either as a single or mixed infections) among GUD patients and the relevance of these findings to treatment outcomes based on the WHO recommended syndromic management protocol (1995) is also outlined in this chapter.

4.3.1 Atypical presentations of genital herpes

During 1998 - 2000, 396 GUD patients who fulfilled the inclusion criteria (i.e. non-vesicular ulcers of > 2 mm in largest diameters and no history of treatment received) were enrolled in a clinical trial designed to explore the efficacy of different treatment options for non-vesicular

ulceration in men. Of those, a single HSV infection was detected by M-PCR in 49 cases (15.4%) and of these, 36 (73.5%) were HIV-seropositive. Among these patients, the median $CD4^+$ count was 335 cells/mm^3 (range – 63 –787 cells/mm^3) at the enrolment visit and 60% had $CD4^+$ counts of less than 350 cells/mm^3. Five patients (14.3%) had $CD4^+$ counts of less than 200 cells/mm^3 although no AIDS-defining illnesses were detected.

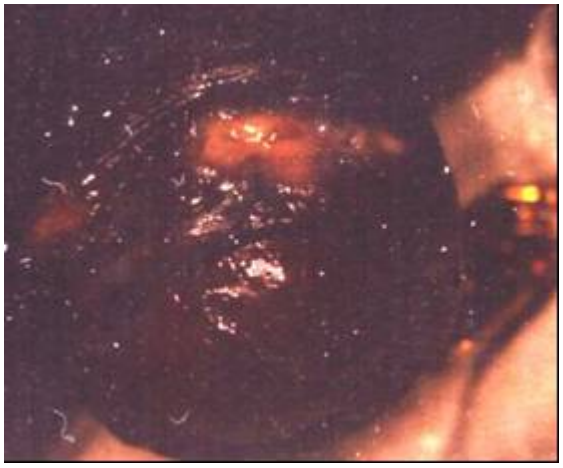
All patients presented with large, deep and purulent ulcerations with or without inguinal and/or femoral lymphadenopathy and thus fulfilled the clinical criteria for a diagnosis of chancroid. The patients did not recall any vesicular lesions during the early stages of their disease and no vesicles were detected on examination. A summary of cases that presented with chronic atypical ulcerations due to HSV infection follows:

Case 1

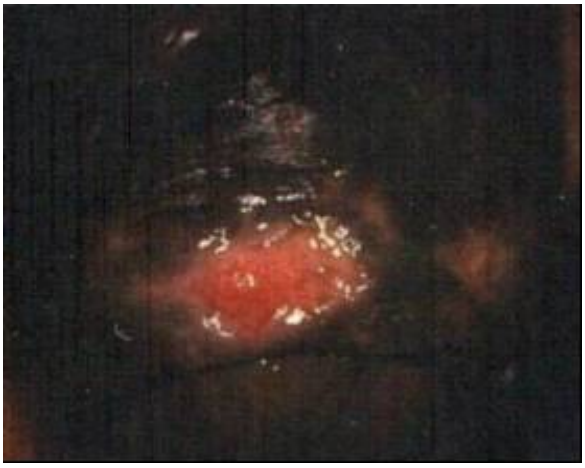
An HIV-seropositive male patient presented with three extensive purulent penile ulcerations of 7 days duration. The largest ulceration measured approximately 20 mm in diameter. The patient was treated syndromically using antibiotics for primary syphilis and chancroid on Day 1. His plasma viral load (PVL) at Day 1 was $8.7x10^5$ copies/ml which increased to $1.1x10^6$ copies/ml by Day 21. Anti-viral treatment was initiated only on Day 14 (due to unavailability of acyclovir at the clinic). The ulcer diminished in size after initiation of anti-viral therapy.

Case 2

An HIV-seropositive male patient presented with two deep, purulent and painful ulcerations of 3 days' duration. The size of the largest ulcer on Day 1 was approximately 9 mm in diameter. His HIV PVL on Day1 was 10,000 copies/ml and $CD4^+$ count was 470 cells/mm^3. He received syndromic treatment with antibacterial antibiotics on Day 1. At follow-up on Day 7, his PVL had increased to 180,000 copies/ml and then declined to 13,000 copies/ml by Day 21. No clinical improvement was noted on the Day 7 or 14. Clinical improvement was observed only after anti-viral therapy with valaciclovir was initiated on Day 14.



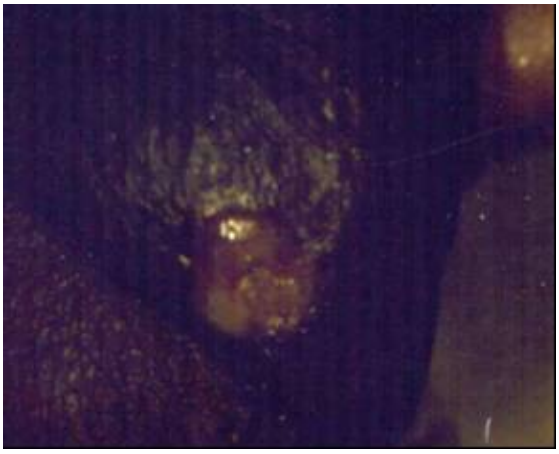
Case 2: Clinical presentation of Genital Ulcer on Day 1



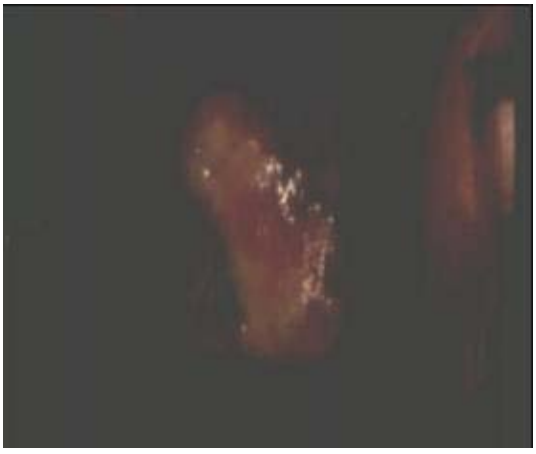
Case 2: Clinical presentation of Genital Ulcer on Day 7

Case 3

This HIV-seropositive patient initially presented with a single, painful purulent ulceration of 4 days duration. His HIV PVL at the initial visit was $3.5x10^5$ copies/ml and $CD4^+$ count was 244 cells/mm^3. He was treated syndromically with antibacterial antibiotics and his condition deteriorated at subsequent follow-up visits. The ulcer size increased from approximately 5 mm in diameter on Day 1 to 15 mm by Day 21. His HIV PVL also increased to $4.7x10^5$ copies/ml on Day 14. The ulceration improved after the initiation of valaciclovir therapy on Day 21.



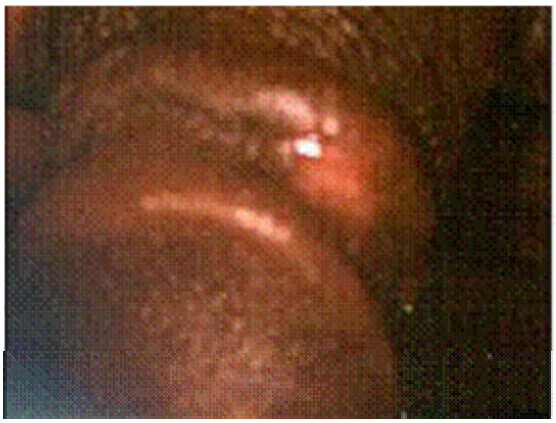
Case 3: Clinical presentation of Genital Ulcer on Day 1



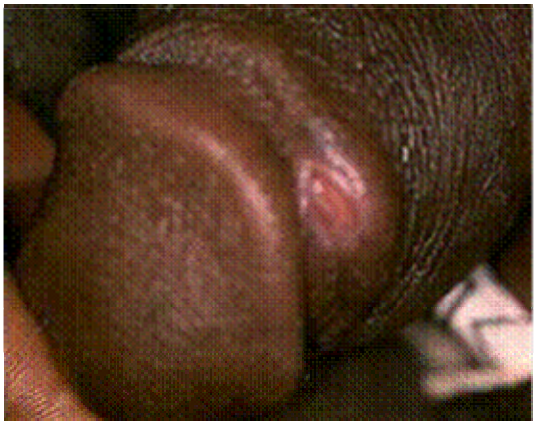
Case 3: Clinical presentation of Genital Ulcer on Day 21

Case 4

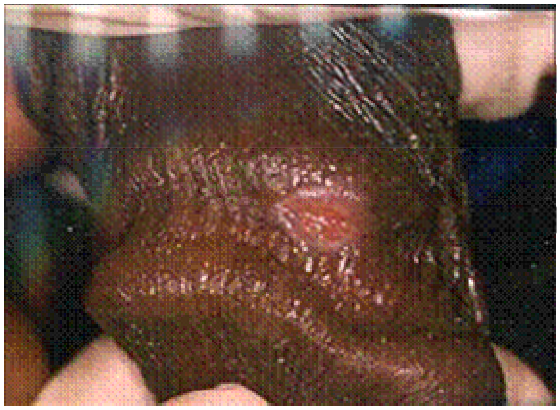
This patient was HIV-seropositive and presented with a single painful ulcer of approximately 6 mm in diameter of 6 days duration. His HIV PVL at the initial visit was $5.6x10^5$ copies/ml and the $CD4^+$ count was 288 cells/mm^3. The patient was treated syndromically using antibacterial antibiotics. No improvement occurred during the first two weeks and valaciclovir therapy was initiated on Day 14. The PVL on Day 7 increased to $1.0x10^6$ and then declined to $4.5x10^5$ copies/ml by Day 14.



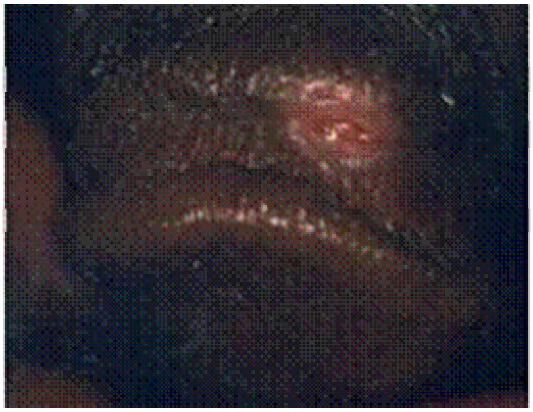
Case 4: Clinical presentation of Genital Ulcer on Day 1



Case 4: Clinical presentation of Genital Ulcer on Day 7



Case 4: Clinical presentation of Genital Ulcer on Day 14



Case 4: Clinical presentation of Genital Ulcer on Day 21

In southern Africa, the classical bacterial causes of GUD, namely primary syphilis, chancroid, granuloma inguinale and LGV have been chronically endemic for many years. The findings of studies conducted during the period 1998 – 2000, showed 49 of 396 patients (12%) presenting with deep, purulent, tender and non-vesicular ulcerations were caused by single infections with HSV. In addition, ulcerations in a further 19 patients (4.8%) with similar presentations were caused by mixed infections involving HSV together with HD and/or TP. The mean $CD4^+$ count of

the 36 HIV-seropositive patients with single HSV infections was 367 cells/mm^3. The majority of these (66%) had persistent ulcerations at Day 7 after they had completed standard syndromic treatment for non-vesicular GUD (i.e. treatment for primary syphilis and chancroid) at enrolment. At their return visit on Day 14, 40% of HIV-seropositive patients with single genital herpes had persistent ulcerations. The treatment outcomes of 270 HIV-seropositive patients enrolled in the study is shown in Figure 4.2.

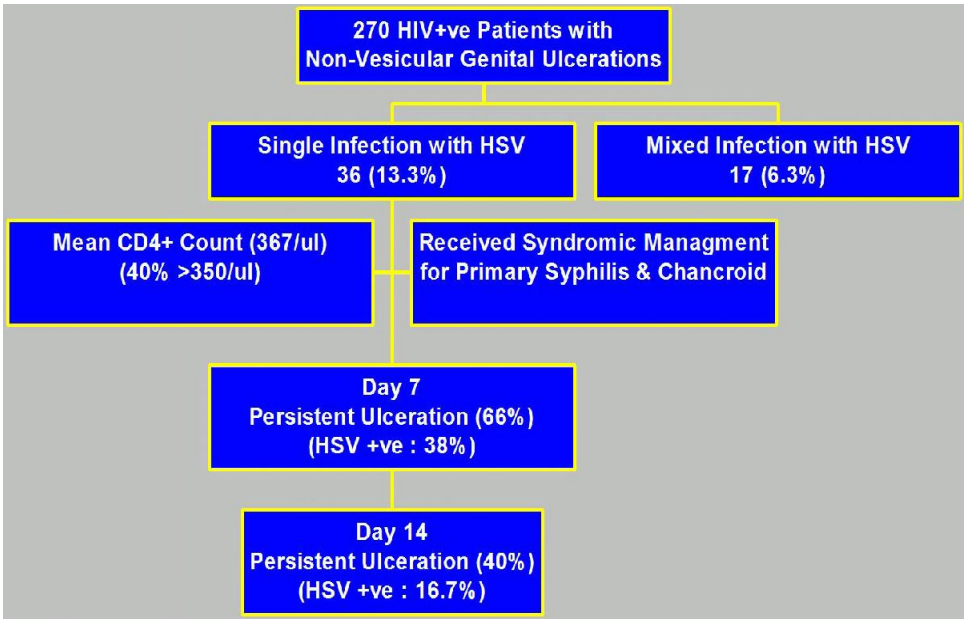


Figure 4.2 Atypical clinical presentation and prolonged ulceration of genital herpes in HIV co-infected miners in the absence of anti-herpes therapy (1998 – 2000)

4.4 Chancroid

The clinical presentations of 264 laboratory-confirmed chancroid cases (either by culture and/or M-PCR) are reviewed in this chapter. Of 264 chancroid patients, only 232 who returned for their two-weekly follow-up visits were included in this review. All patients fulfilled standard clinical criteria and were diagnosed as chancroid at enrolment. Of the 232 subjects, 151 (65.1%) were HIV-seropositive and 48% had CD4$^+$ counts less than 350 cells/mm^3. No atypical presentations were observed among HIV-seropositive patients. Although 54 patients (23.3%) did not achieve clinical cure at Day 14, there was no difference in cure rates between HIV-seropositive and HIV-seronegative patients (22.5% vs. 22.2%, p 0.78). Five of 34 HIV-seropositive patients (14.7%) who exhibited treatment failure at Day14 were subsequently found to be positive by M-PCR

(HSV) compared to 1 of 18 (5.6%) HIV-seronegative patients (p = 0.59). Similar proportions of HIV-seropositive and HIV-seronegative patients who experienced treatment failure at Day 14 were initially positive for M-PCR (HD) alone (38.2% vs. 38.9%). In summary, we did not observe any atypical presentations or unexpected treatment failures among HIV-seropositive patients presenting with chancroid where HD was the only pathogen demonstrated.

4.5. Other STIs (LGV, Donovanosis)

Careful scrutiny of limited information available from the studies conducted in 1994 – 2000, revealed no noticeable changes in clinical presentation of LGV and donovanosis in HIV-seropositive patients.

4.6 Discussion

Since HIV was discovered in 1984, many anecdotal reports have revealed that the natural history, clinical presentation, performance of diagnostic tests and response to conventional therapy of some STIs are altered in patients co-infected with HIV[1-3].

Most atypical presentations of syphilis reported in patients with HIV infection include unusual eye, bone and neurological involvement[4,5]. A more aggressive clinical course of syphilis has also been observed[6]. Changes in performance of routine laboratory tests, particularly RPR test have also been reported[7,8]. In these studies, more frequent biological false-positive results were reported among patients infected with HIV than among the HIV-seronegative population. Other studies have reported that HIV-seropositive patients who received adequate treatment for syphilis appear more likely to serorevert to negative treponemal antibody testing instead of remaining seropositive for their life-time[9]. Studies have also indicated that atypical presentations such as persistent chancres[10], early ocular involvement[11], ulcerative skin lesions[12,13], gummatous disease[14,15], are more commonly found in HIV-seropositive patients with syphilis than HIV-seronegative persons.

The study reported here reviewed the clinical presentation of patients with syphilitic ulceration and their response to conventional therapy. We observed that atypical presentations of primary

syphilitic chancres were common among patients in southern Africa. Patients often presented with multiple painful ulcers, and the overall clinical accuracy of diagnosis was very low (31.7% in HIV-seropositive and 17.6% in HIV-seronegative patients, p=0.4). The majority of primary chancres mimicked the clinical features of chancroid (i.e. purulent, deep, multiple and painful). During our studies, we did not observe any unusual increase in an aggressive form of syphilis such as lues maligna or a higher incidence of secondary syphilis among HIV positive patients. Similarly, there was no significant difference in treatment responses of primary syphilis between HIV-seropositive and HIV-seronegative patients following standard therapy using a single intramuscular dose of benzathine penicillin. Complete healing of ulcers was usually achieved within two weeks after initiation of therapy in both HIV-seropositive and seronegative patients. Even with application of sensitive M-PCR, the clearance of TP from the ulcerations was found as early as Day 7 after therapy in both groups. Similarly, the quantitative RPR titres of those patients at 14 days after therapy were either the same or lower than baseline titres.

No unusual presentations related to abnormal neurological or ophthalmic presentations were observed during general examination of our patients. However, a system-specific clinical examination and additional laboratory investigations were not conducted in the present study. Our study also did not make provision for long-term follow-up of the patients with primary syphilis nor was it designed to perform invasive investigations such as routine lumbar punctures.

Concerns have been expressed over the natural history of syphilis in HIV-infected individuals, particularly the possibility of an accelerated course to neurosyphilis and possible neurorelapses[16,17]. The 1998 CDC STD treatment guidelines recommend conducting more frequent follow-up evaluations of therapeutic responses at three to six-months intervals for two years in HIV-seropositive patients with early or latent syphilis or syphilis of unknown duration[18]. In addition, it recommends CSF examination on failure to show a 4-fold decline in serum nontreponemal (reagin) test titre at specific times (ranging 6 to 24 months depending on the stage of syphilis). Unfortunately, these guidelines are not feasible in developing countries; therefore, the long-term consequences of HIV-infected patients presenting with primary syphilis in these countries remain unknown. More studies are required to explore the long-term impact of

HIV on syphilis co-infection and the effectiveness of currently recommended therapies for various stages of syphilis in HIV-seropositive individuals.

In this chapter, we studied the effect of HIV-co-infection in patients with M-PCR-confirmed primary syphilis on the performance of treponemal and non-treponemal serological tests. Studies reported unusual serological responses in HIV infected individuals with syphilis such as false negative tests or delay in seroconversion[19,20] and falsely positive serological responses[21,22]. In our study, no biological false positive reactions of RPR tests were detected among HIV-seropositive patients. Unlike studies conducted in developed countries, the prevalence of intravenous injecting drug usage was very rare in our patients co-infected with HIV and syphilis. Overall, only 69.3% of patients with M-PCR-confirmed TP infection were found RPR-seropositive. The rate of RPR-positivity among primary syphilis patients co-infected with HIV (57.4%) was significantly lower than those without HIV co-infection (74.1%). Similarly, the RPR seropositivity was significantly lowered in the patients presenting with mixed TP and other GUD aetiologic agents (50.9%) when compared to the rate of patients with single TP infection (79.2%). The sensitivity of RPR test in detecting primary syphilis in HIV-seropositive patients was significantly reduced in those with mixed TP infection compared to patients with single TP infection. Therefore, the utility of RPR test in detecting primary syphilis may be reduced further in areas where both HIV-coinfection as well as GUD with multiple aetiologies are common.

Changes in the natural history and clinical presentation of genital herpes in HIV-infected individuals have been reported since the early days of the HIV epidemic. When AIDS was first reported in 1981, HSV was one of the initial opportunistic infections described in the report. In 1986, Schneiderman et al also reported a case of genital herpes in a man with acquired immunodeficiency syndrome (AIDS), who was receiving chemotherapy for Hodgkin's disease[23]. Typical primary HSV infections present with skin lesions in different stages of evolution including pustules, vesicles, shallow painful ulcers, and crusted lesions. HSV ulcers in people with advanced HIV-1 disease are often large, deep, slow to heal, and, not infrequently, appear in atypical areas of the body.

In southern Africa, the HIV epidemic reached maturity in the late-1990s with an increasing number of immunocompromised patients being recorded. Most patients with genital herpes who were included in the studies reported here presented with large non-vesicular, deep, purulent ulcerations that mimicked chancroid. These patients were treated according to the syndromic STI management alogorithm covering only primary syphilis and chancroid. As a result, the failure of treatment for non-vesicular genital ulcer diseases in southern Africa increased substantially during the mid-1990s. HIV-seropositive patients presenting with atypical genital herpes represented up to 13% of chancroid-like ulcerations in one series and the majority had persistent ulcerations for more than two weeks. Over 16% of patients with HIV and HSV co-infection who were treated with antibacterial agents continued to shed HSV in their genital lesions for more than two weeks. Some patients also experienced extremely high HIV plasma viral loads during these protracted episodes. In these patients, rapid improvement and eventual cure had only been achieved after initiation of specific anti-herpes therapy.

As the HIV-epidemic advanced in southern Africa, the changing GUD situation was reported in other countries in the region. Lack of genital herpes cover would therefore undermine the ultimate goal of syndromic GUD management, namely to achieve a rapid diagnosis and effective treatment in order to control further STI and HIV transmission. In the late-1990s, increasing numbers of patients with genital herpes were presenting with atypical, chronic, erosive lesions with no spontaneous healing. Unfortunately, antiviral drugs for treatment of genital herpes remained expensive and were not universally available in public health care facilities in most southern African countries.

In our study, only limited information was available on frequency of reactivation of genital herpes in HIV-seropositive individuals and the level of subclinical HSV shedding. However, Corey et al have described that HSV-2 reactivation occurs more frequently in HIV-seropositive than HIV-seronegative men while a high frequency of subclinical disease can also be observed in the immunocompromised populations[24]. Both $CD4^+$ counts and HIV PVLs influence HSV-2 reactivation rates. A negative correlation has been documented between $CD4^+$ count and the frequency of HSV reactivation.

Generally, it has been recommended that chancroid patients co-infected with HIV should be closely monitored due to potential treatment failure. Studies previously conducted in Malawi and Kenya reported that healing of chancroid ulcerations was slower among HIV-infected patients[25-26]. Higher failure rates were reported in Kenya with single dose therapy for chancroid[27-29]. In contrast, in Rwanda, researchers found that HIV and the degree of immunosuppression as measured by $CD4^+$ counts had no effect on bacteriological and clinical outcomes and that treatment failures were entirely attributable to resistance of *H.ducreyi* to trimethoprim-sulfamethoxazole (TMP-SMX)[30]. The dosage and duration of the fleroxacin regimen also needed to be increased to treat HIV infected patients in Nairobi[31]. A higher treatment failure rate among HIV infected patients has, however, not been observed by the same Kenyan team in a more recent study using low dose erythromycin or single dose ciprofloxacin[32].

The U.S. Centers for Disease Control and Prevention recommends that "since data on therapeutic efficacy with the recommended ceftriaxone and azithromycin regimens among patients infected with HIV are limited, those regimens should be used among persons known to be infected with HIV only if follow-up can be assured" (CDC, 1997). Some experts suggest using the full dose 7-day regimen of erythromycin for treating HIV infected persons.

In our study, HIV-seropositive patients with chancroid who have shown poor clinical response to routine chancroid therapy, were often co-infected with genital herpes. The antimicrobial resistance patterns of *H.ducreyi* were the same for the isolates obtained from HIV-seropositive and HIV-seronegative patients (unpublished observation). Caution should be exercised in interpretation of the results obtained in earlier studies where laboratory investigations for HSV co-infection were not performed.

4.7 Concluding remarks

In summary, atypical clinical presentations of genital ulcer disease, particularly of genital herpes, have become increasingly common in southern Africa, and have been strongly associated with HIV infection and related immunodeficiency. However, high prevalence of mixed infections associated with genital ulcerations has also contributed to the poor accuracy of clinical diagnosis of GUD in the region. The treatment failures associated with genital herpes have,

HSV as well as HIV. Caution should be exercised in the interpretation of syphilis serological testing in cases of GUD particularly in those who are co-infected with HIV because many cases of primary syphilis may be associated with other causes of GUD.

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CHAPTER 5

HIV shedding from genital ulcerations

Male patients presenting with genital ulcerations were enrolled in these studies to measure HIV shedding from genital lesions and the subsequent effect of treatment on HIV viral shedding. A clinical and microbiological evaluation was performed using standard methods as described in section 2. A novel specimen collection technique for the quantitative measurement of HIV shedding from genital ulcer lesions was devised by the investigator. Follow-up visits were scheduled on Day 7, Day 14 and on Day 21, during which the clinical and microbiological status of lesions was assessed and levels of HIV shedding determined.

5.1 Collection of genital ulcer exudates for HIV viral load measurement

In order to determine quantitatively the number of HIV RNA copies in fixed amounts of genital ulcer exudate, a standard volume of exudate was collected. After a target ulcer had been identified according to standard criteria (see section 2), 10 ml of sterile distilled water was gently expressed onto the target ulcer using a syringe and wide-bore injection needle (Figure 5.1). The washings were collected in a sterile disposable plastic container with a wide opening, taking care that no spillage occurred. The total volume of washings was transferred into a 10 ml plastic tube and kept on ice and placed in storage at − 70C° within 12 hours.

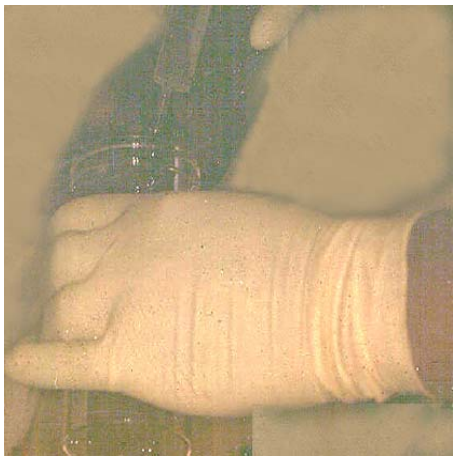


Figure 5.1 Collection of genital ulcer exudates for HIV RNA measurement

5.2 Quantification of HIV viral load in ulcer washings

The quantitative measurement of HIV RNA copies in ulcer washings was conducted at the National Institute for Virology (Johannesburg), using the Roche Amplicor (Cobas Amplicor HIV version 1.0 monitor, Roche Diagnostics, USA) test. Processing of washing fluid followed the standard procedures used for HIV viral load determinations (on plasma specimens) as described in Section 2.3.4.2. The results were expressed in terms of the number of HIV RNA copies per ml of washings fluid collected. A pilot evaluation of the method was conducted before initiation of the study. No significant inhibition of PCR was encountered even following the precautionary step of re-testing all critical washings with low or negative counts before they were reported. The consistency of viral load measurements between batches was also evaluated.

In order to minimize contamination with blood as a result of bleeding which could occur following swabbing of the ulcerations, the washing procedure was conducted before other specimens were collected.

5.3 Patient characteristics

All patients were HIV-seropositive miners seeking treatment at the Carletonville mine STI clinic for genital ulcer disease. The mean age patients was 35 years (range 22 – 50 years). None had clinical symptoms or signs of AIDS at the time of their initial consultation visit.

Of 78 patients enrolled, $CD4^+$ results were available for 70 patients. The mean $CD4^+$ count measured at Day 1 was 398 cells/mm^3 (SD = 233, range 10 – 1500 cells/mm^3). Although no AIDS defining illnesses had been diagnosed previously, seven patients (10%) had a $CD4^+$ count of fewer than 200 cells/mm^3, while 31 (44%) patients had counts of 200 – 350 cells/mm^3 and 32 (46%) counts of >350 cells/mm^3 (Table 5.1).

Table 5.1 Distribution of $CD4^+$ counts of patients at Day 1 (N=70)

	$CD4^+$ Counts (cells/mm^3)	
Median (range)	336 (10 – 1,500)	
$CD4^+$ Group	**Number**	**%**
<200	7	10%
200-350	31	44%
>350	32	46%

The HIV plasma viral load (PVL) at Day 1 was determined for all patients and the median PVL was 115,838 copies/ml (range 400 – 871,617 copies/ml). The majority (97%) of patients had a detectable PVL level (i.e. more than 400 copies/ml) at Day 1 (Table 5.2). Of 74 patients who returned for their Day 7 follow-up visit, repeat PVL results were available for 22 (29.7%) patients.

Table 5.2 Distribution of HIV-PVL among GUD patients at Day 1 (N = 78)

	HIV-PVL (copies/ml)	
Median (range)	115,838 (1086 – 871,617)	
PVL Group	**Number**	**%**
≤10,000	10	13%
>10,000 - 30,000	11	14%
Above 30,000	57	73%

The clinical presentation of ulcers and the results of laboratory investigations are shown in Table 5.3.

Table 5.3 Clinical parameters of genital ulcers (N = 78)

Clinical Parameters of Genital Ulcers	
Duration of Ulcers	
Median	5 days (1 - 30 days)#
Ulcer of < 7 days	80%
Median Ulcer Size*	8 mm (3 - 30 mm) #
Mean Number of Ulcers	3 (1 - 18) #
Clinical Diagnosis	
Chancroid	63 (80.0%)
Herpes	16 (20.0%)
Aetiological Diagnosis (Culture/M-PCR +ve)	
Chancroid	41 (52.6%)
Genital Herpes	31 (39.7%)
Mixed Chancroid & Herpes	6 (7.7%)

* Approximate measurement of the largest ulcer (i.e. target ulcer) diameter in mm
\# Parentheses indicate range

5.4. Patients positive for *H. ducreyi* only at enrolment (N= 41)

A total of 41 patients were found to be positive for *H. ducreyi* alone at the enrolment visit (Day 1). The Day 1 clinical and laboratory findings of 41 patients positive for HD only at enrolment were as follows.

The median ulcer size was 9 mm (range 4 – 30 mm) and the median number of ulcers was 2 (range 1 – 9). The median $CD4^+$ count was 361 cells/mm^3 (range 10 – 1028 cells/mm^3). The majority of patients (95%) had a detectable HIV plasma viral load (PVL) and the median PVL was 98,519 copies/ml (range - 1371 – 871,617 copies/ml). Thirty-four patients (82.9%) had HIV PVL >10,000 copies/ml at Day 1. Thirty-four patients (82.9%) had detectable HIV RNA in their ulcer lavage fluid with a median lesional HIV shedding of 3,782 copies/ml (range 568 – 62,756 copies/ml of lavage fluid). Among patients with PVL >10,000 copies/ml, HIV RNA was detected in the lavage of 33 patients (97.1%) compared to only 1 of 7 patients (14.3%) with a PVL ≤ 10,000 copies/ml.

All patients were treated syndromically using antibiotics known to be effective for both primary syphilis and chancroid. Patients were grouped into three categories based on treatment outcome (i.e. both clinical and microbiological) at Day 7 and Day 14. Group A included 17 patients who had achieved both clinical and microbiological cure at Day 7; Group B included 20 patients who had achieved clinical improvement with or without microbiological cure at Day 7; and Group C included four patients with no clinical improvement with or without microbiological cure at Day 7. Thirty-seven (90.2%) of patients in Groups A and B showed clinical improvement at Day 7 while four patients in Group C experienced clinical failure. Among patients in Groups A and B, the overall reduction in ulcer size ranged from 12.5% to 100% (i.e. completely healed) compared with initial measurements at Day 1. The mean ulcer size reduction at Day 7 was 72.5% of initial ulcer size measured at Day 1.

Overall, 18 patients (43.9%) from Groups A, B and C continued shedding HIV in their lavage fluid on Day 7 with a median number of 4,649 HIV copies detected per ml (range 771 – 53,630 copies/ml). At Day 14, 27 patients (65.9%) achieved a clinical cure (i.e. ulcer completely healed) and continued shedding of HIV was reduced in seven patients (17.1%).

The clinical and microbiological treatment responses seen in patients in Groups A, B and C are shown in Figure 5.2. The patients in Group A had a successful treatment outcome, their ulcerations were completely healed (clinical success) and no aetiological agents were detected in the ulcer exudates (microbiological success) at Day 7. In this group, 13 patients (76.5%) had initial PVLs of >10,000 copies/ml and 12 patients (70.6%) had detectable HIV RNA in their lavages at Day 1. All but two patients stopped shedding HIV RNA in their lavage fluids. HIV shedding rates declined from 70.6% on Day 1 to 11.8% on Day 7. Two patients continued to shed HIV RNA in their washings at Day 7 despite the fact that their ulcers were completely epithelialized; one had a high PVL (259,188 copies/ml) at Day 1, and HSV was still detected from the specimen taken on Day 14. The remaining patient also had a high PVL (227,538 copies/ml) and a very low $CD4^+$ count (54 cells/mm^3). In addition, both had laboratory-confirmed urethritis. All patients in Group A, including two cases that had relapsed clinically (one HD +ve and the other HSV +ve) stopped shedding HIV by Day 14.

The remaining 24 patients in Groups B and C presented with continuing ulcerations on Day 7 although 20 patients (Group B) showed some reduction in ulcer size (i.e. mean ulcer size reduction of 50% of measurements recorded on Day 1). Only two patients exhibited microbiological failure for HD at Day 7. However, both were found HD negative at Day 14. Twelve of 20 patients (60%) had shown a complete clinical and microbiological cure at Day 14. Culture and/or M-PCR for HD were negative in 18 of 20 patients (90%) at Day 14.

In Group B, 18 patients (90%) had initial PVLs of >10,000 copies/ml and all 18 patients had detectable HIV RNA in their lavages at Day1. Two patients with initial PVL of ≤ 10,000 copies/ml did not shed HIV in their ulcer exudates. After treatment, 7 patients (35%) stopped HIV RNA shedding in their lavages by Day 7. HIV RNA was, however, detectable in the lavages of 12 patients, including one patient who did not shed HIV on Day 1, although the HIV shedding rate decreased by 60% among patients on Day 7. Of these 12 patients with detectable HIV RNA in their lavages on Day 7, two exhibited microbiological failure (positive HD by culture and/or M-PCR) and 10 had a microbiological cure.

Of 18 patients who achieved microbiological cure by Day 7, three had microbiological relapses (two became HD and one HSV positive by M-PCR), three had microbiological cure and 12 had both clinical and microbiological cure by Day 14. One of the two patients exhibiting microbiological relapses with HD on Day 14, also resumed HIV shedding, while the other remained a non-shedder for HIV RNA by Day 14.

One patient, who tested HSV positive on Day 14, had an initial PVL of 31,550 copies/ml. *H. ducreyi* culture and M-PCR became negative at Day 7 following anti-chancroid treatment. However, HIV shedding from lesions increased from 3,028 copies/ml on Day 1 to 16,217 copies/ml on Day 7. On Day 14, as mentioned above, HSV was detected in the patient's ulcer exudates while HIV shedding in lavage fluid was maintained at 11,181 copies/ml.
Lesional HIV shedding became undetectable in three patients who achieved microbiological cure at Day 14, despite their ulcers not having healed completely. Of 12 patients who achieved both clinical and microbiological cure at Day 14, three patients continued shedding

HIV on Day 14. Two of these three patients had relatively high PVL, but no other reason could be determined for the continued shedding of HIV on Day 14 in these cases.

Of four patients who experienced clinical failure at Day 7 (Group C), two had the same ulcer size as on Day 1, and the remaining two had an increase in ulcer size. Two patients continued shedding *H. ducreyi* at Day 7 and Day 14 and one patient had a microbiological relapse (positive for HD after initial clearance) at Day 14.

In Group C, three of four patients (75%) had an initial PVL of >10,000 copies/ml and all four continued to shed HIV RNA in their lavages. No clinical improvement was detected among these patients and three of the four were microbiological failures on Day 14.

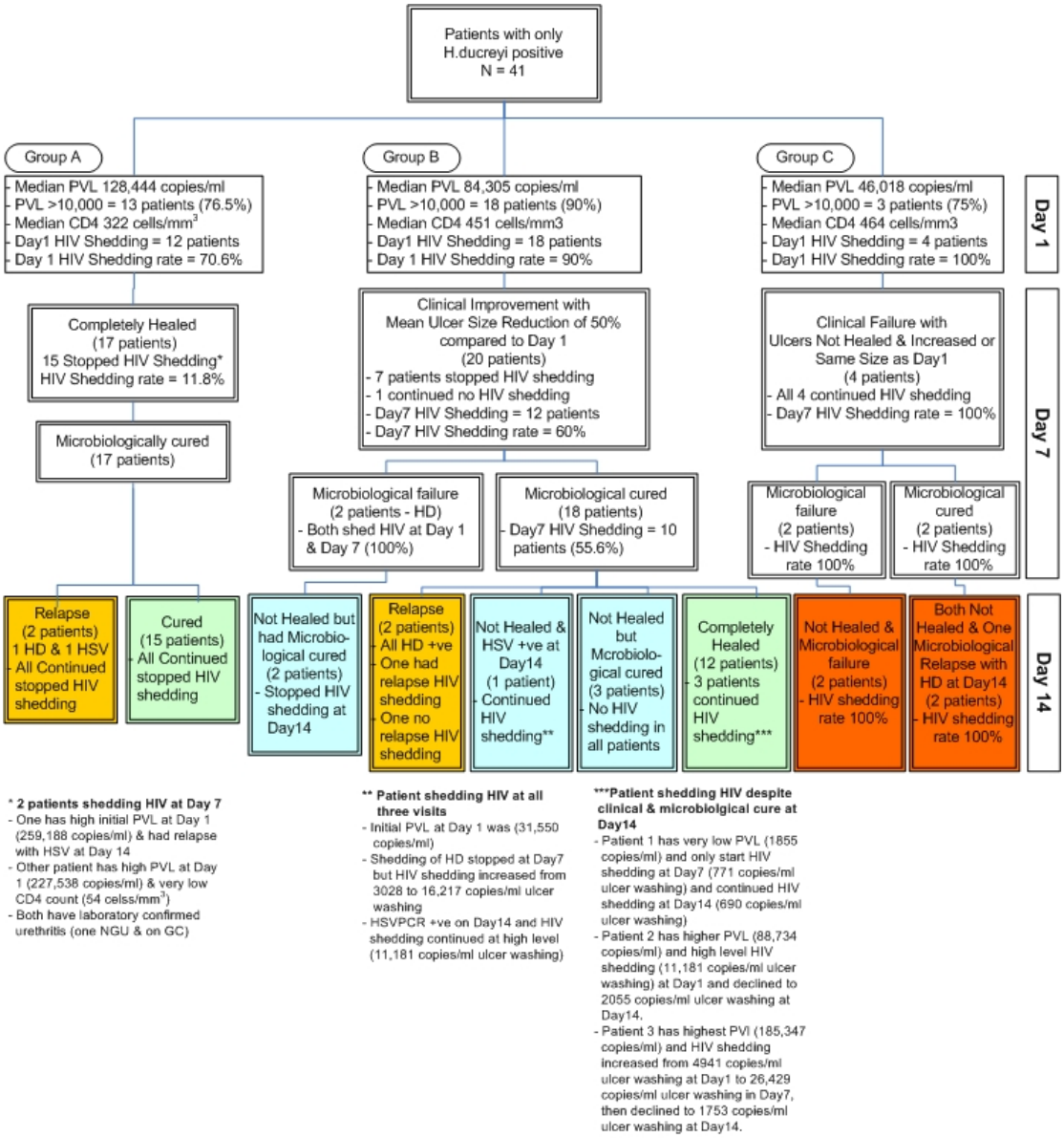


Figure 5.2 Clinical and microbiological course of genital ulcerations and impact of HIV shedding from ulcers among patients with only *H. ducreyi* detected on Day 1

5.5 Patients with mixed herpes and chancroid at enrolment (N=6)

Six patients were positive for HD and HSV at enrolment and their clinical and microbiological findings are summarised in Figure 5.3. At the enrolment visit, the mean ulcer size for these patients was 10.3 mm (range: 6 – 15 mm) and the mean number of ulcers was 4.1 (range: 1 - 18). The median $CD4^+$ count was 225 cells/mm^3 and the median PVL was 258,989 copies/ml (range: 19,030 – 753,240 copies/ml). All patients had PVL of >10,000 copies/ml at Day 1. The majority (93.3%) of patients shed HIV RNA in their lavages at Day 1 with a median HIV RNA

of 6,458 copies/ml (range: 764 – 37,661 copies/ml). The clinical and microbiological outcomes and patterns of HIV shedding in lavage specimens are shown in Figure 5.3.

Following syndromic treatment using antibacterial agents, 3 (50%) continued to shed HIV RNA in their lavages at Day 7 despite having microbiological cure and clinical improvement. One patient was microbiologically cured for HD at Day 7, but continued to be positive for HSV on Day 7 and Day 14. There was no sign of clinical improvement in this patient and he continued to shed HIV RNA on both Day 7 and Day 14 while the remaining patients stopped shedding HIV by Day 14.

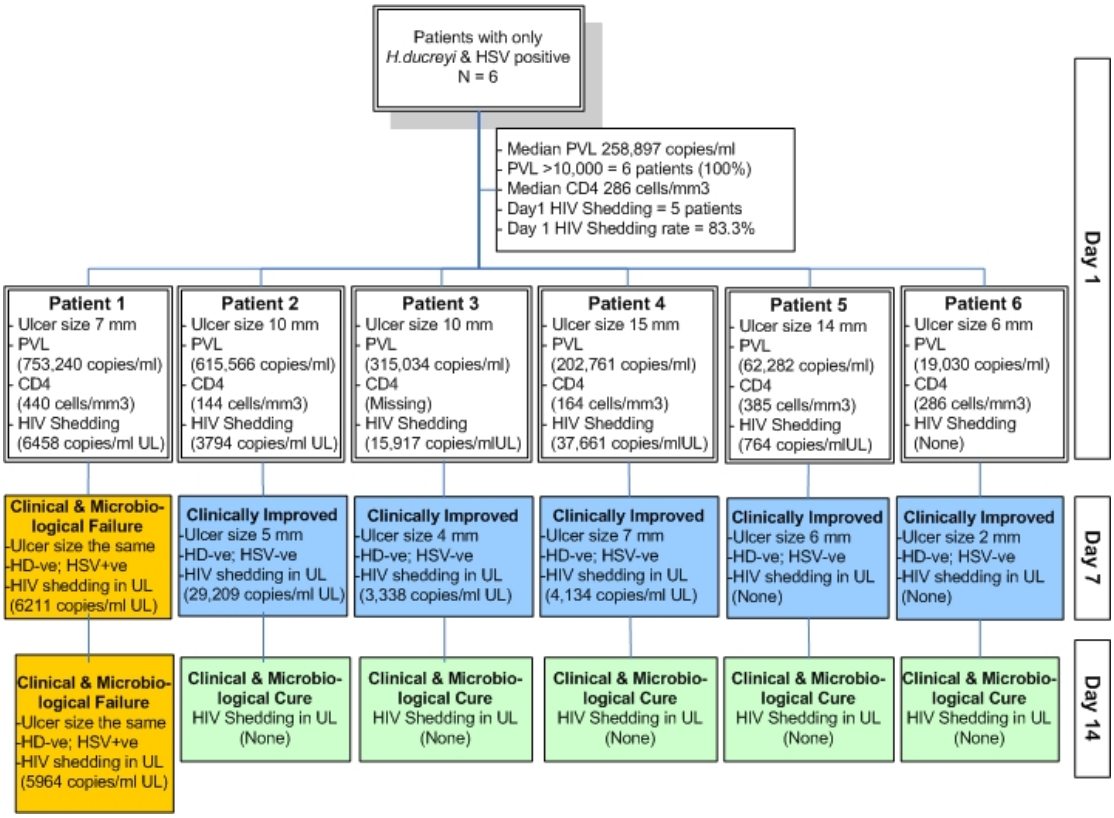
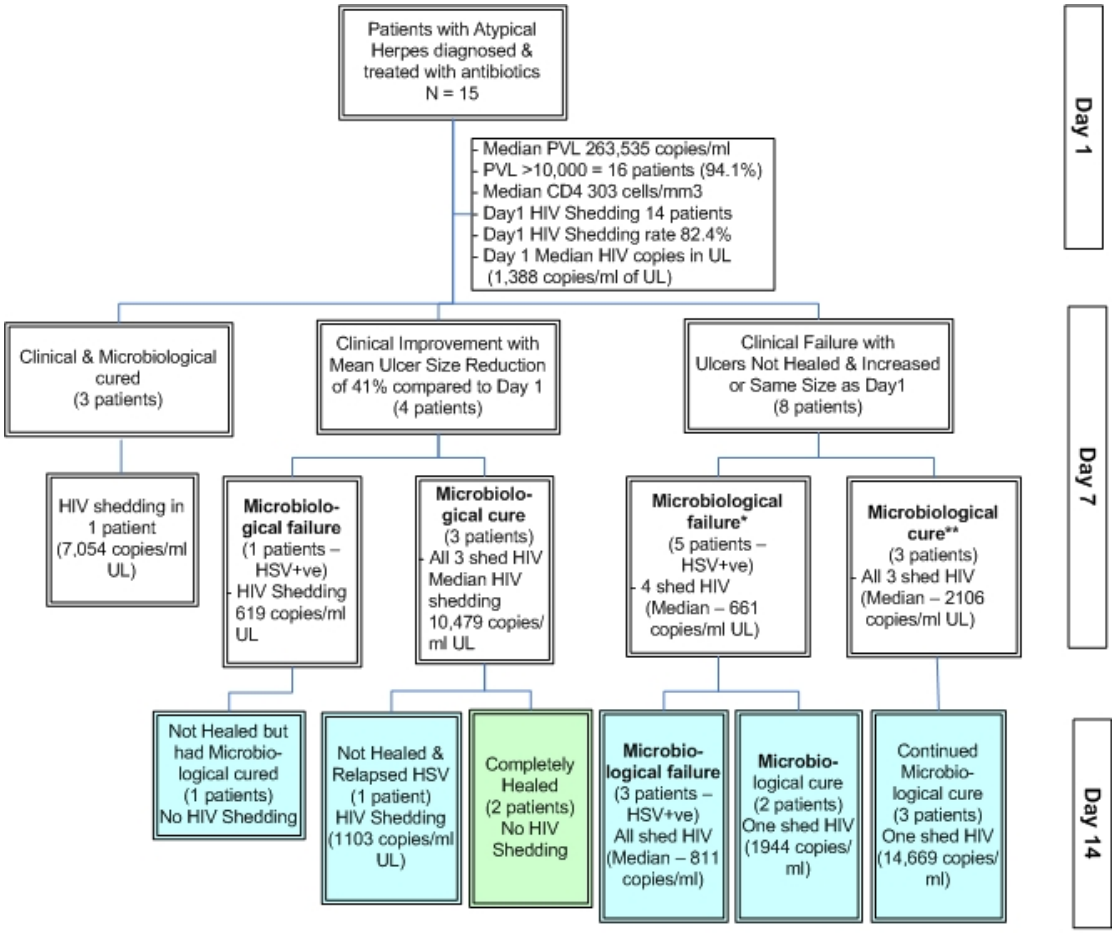


Figure 5.3 Patients with mixed herpes and chancroid at enrolment (N=6)

5.6 Patients with atypical herpes at enrolment (N=15)

At the enrolment visit, 15 (19.2%) HSV-positive patients were wrongly diagnosed as chancroid based on their atypical clinical presentations (i.e. soft, deep, purulent ulcerations without any history of vesicular lesions). All patients received syndromic treatment with standard antibiotic therapy. The clinical, microbiological and HIV shedding pattern of these patients are summarised in Figure 5.4. The mean size and number of ulcers in this group were similar to those of patients with chancroid and mixed chancroid and herpes (mean ulcer size = 10.5 mm; mean ulcer number = 3.8). The median $CD4^+$ count was 288 cells/mm^3 (range: 233 – 787 cells/mm^3) and median PVL was 223,809 copies/ml (range: 2,242 – 871,058 copies/ml) at Day 1. Fourteen patients (93.3%) had PVLs >10,000 copies/ml and 12 patients (80%) shed HIV in their lavages with a median of 3,018 copies/ml (range: 412 – 38,745 copies/ml) at Day 1.

Following syndromic treatment with antibiotics, three patients had clinical and microbiological cure at Day 7. Of the remaining, four patients (26.7%) showed some clinical improvement (i.e. mean ulcer size reduction of 41% compared to Day 1) and eight patients (53.3%) exhibited clinical failure on Day 7. HIV shedding in lavages continued in the majority of patients (80%) with a median HIV shedding of 1,643 copies/ml (range 464 – 9,212 copies/ml) at Day 7. The majority of patients (92%) who shed HIV were those who did not achieve clinical and/or microbiological cure at Day 7. HIV shedding was reduced in five patients (33.3%) on Day 14. Of those, four also shed HSV at Day 14 (Figure 5.4).



* Two of five patients with microbiological and clinical failures at Day7 received Valacyclovir 500 mg BD x 5 days at Day7.
- One patient has decreased in HIV shedding from 1,694 copies/ml UL in Day7 to 432 copies/ml UL in Day14.
- The other patient did not shed HIV at Day7 and continued not shed at Day14.

**Another two patients with clinical failure but microbiologically cure at Day7 received Valacyclovir treatment at Day7.
- One patient had an increase in HIV shedding (9212 to 14,669 copies/ml UL) in Day14 when compared to Day7. Information on Day21 follow-up visit was available and shedding decreased to 1,630 copies/ml UL at Day21. His initial PVL was 871,058 copies/ml.
- For the other patient in this group, the HIV shedding has declined from 2,106 copies/ml UL in Day7 to an undetectable level (≤400 copies/ml). His initial PVL was 561,195 copies/ml.

Figure 5.4 Patients with atypical herpes at enrolment (N=15)

5.7 Patients with typical herpes at enrolment (N=16)

Among patients with GUD, 16 (20.5%) presented with typical genital herpes (i.e. multiple, shallow, non-purulent ulcerations with blisters or history of blisters). All patients received anti-herpes therapy at Day 1 (i.e. valacyclovir 500 mg BD x 5 days). The mean ulcer size at enrolment was 4.6 mm (range: 3 – 10 mm). The median HIV PVL was 72,005 copies/ml (range 1,086 – 750,000 copies/ml) and the median $CD4^+$ count was 376 cells/mm^3 (range: 256 – 1500 cells/mm^3). The majority of patients (87.5%) had a PVL of more than 10,000

copies/ml and HIV shedding was detected in 56.3% of patients at Day 1. The median HIV RNA detected in lavages at Day 1 was 1,890 copies/ml UL (range: 530 – 8,215 copies/ml).

Fourteen patients (87.5%) achieved clinical and microbiological cure at Day 7 and none continued to shed HIV at this visit. Two patients without clinical cure at Day 7, however, continued to shed HIV, although a slight decline in the amount of HIV shedding was observed in both patients. All patients achieved complete clinical and microbiological cure and none shed HIV at Day 14 (Figure 5.5).

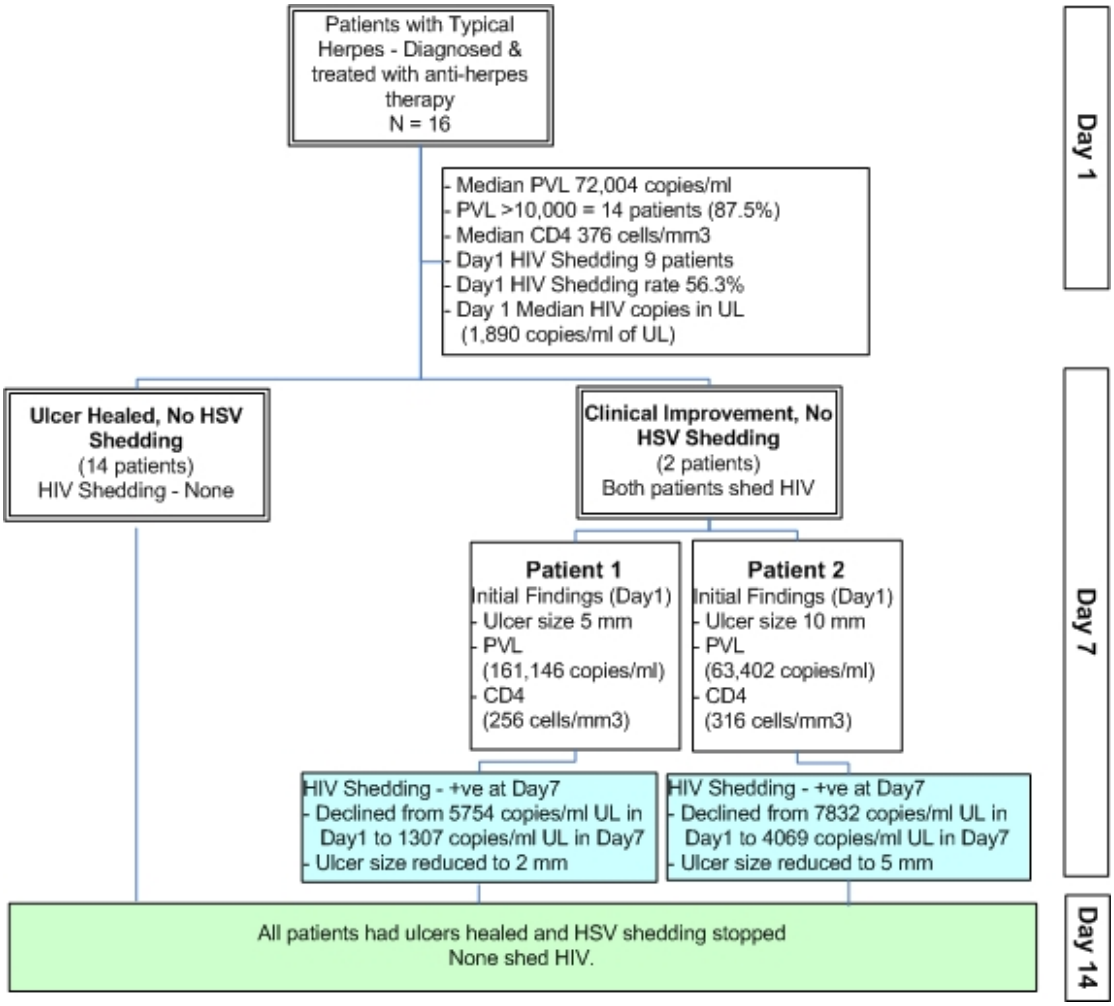


Figure 5.5 Patients with typical herpes at enrolment

5.8 Summary of viral shedding from genital ulcerations

The clinical, microbiological and treatment responses of patients with genital herpes infections of any kind are summarised in Table 5.4. Overall, the mean size of ulcers in patients with classical genital herpes was significantly smaller than in other groups. ($F=4.03$, $p<0.01$) The median PVL of patients with atypical herpes was significantly higher than patients with chancroid (Mann-Whitney U test, $p<0.05$).

Table 5.4 Summary of clinical and microbiological outcomes

	G.Herpes N=16	Chancroid N=41	Atypical G.Herpes N=15	Chancrod & G.Herpes N=6	Sig.
Baseline Clinical Characteristics					
Mean Ulcer Size at enrolment (mm)	4.6* (3 - 10)	11.5 (4 - 30)	10.5 (4 - 27)	10.3 (6 - 15)	Anova F=4.03. p=0.01
Mean Ulcer Number at enrolment	4 (2 - 10)	2.6 (1 - 9)	3.8 (1 - 7)	4.1 (1 - 18)	
Median CD4 + Counts (cells/mm^3)	376 (256 - 1500)	361 (10 - 1028)	288 (233 - 787)	225 (124 - 440)	
Median HIV PVL at enrolment (copies/ml)	72004 (1,086 - 750,000)	98,519[1] (1,371 - 871,617)	223,809[2] (2,242 - 871,058)	258897 (19,030 - 753,240)	Mann-Whitney U test 1 vs 2 (p=0.04)
HIV PVL >10,000 copies/ml at enrolment	34 (82.9%)	14 (87.5%)	6 (100%)	14 (93.3%)	
Patients with HIV shedding in ulcer lavage (UL) in Day1	9 (56.3%)	34 (82.9%)	12 (80%)	5 (83.3%)	
Median HIV RNA detected in ulcer lavage (UL) in Day1	1,890 (530 - 8,215)	3,782 (568 - 62,756)	3,018 (412 - 38,745)	6,458 (764 - 37,661)	
Follow-up visits					
Patients with clinical improvements at Day7	12 (75.0%)	37 (90.2%)	9 (60.0%)	5 (83.3%)	
Mean ulcer size reduction at Day7 (% of ulcer size in Day1)	92.5 (50 - 100)	72.5 (12.5 - 100)	63.7 (16.7 - 100)	57.4 (50 - 66.7)	
Patients with HIV shedding in ulcer lavage (UL) in Day7	2 (12.5%)	18 (43.9%)	12 (80.0%)	4 (66.7%)	
Median HIV RNA detected in ulcer lavage (UL) in Day7	2,688 (1307 - 4069)	4,649 (771 - 53,630)	1,643 (464 - 9,212)	4,134 (3,338 - 29,209)	
Patients achieved clinical cure at Day14	16 (100.0%)	27 (65.9%)	5 (33.3%)	5 (83.3%)	
Patients with HIV shedding in ulcer lavage (UL) in Day14	0 (0%)	7 (17.1%)	6 (40.0%)	1 (16.7%)	

The majority of patients in the various groups had PVLs greater than 10,000 copies/ml at the time of enrolment visit. Although the rate of HIV shedding at enrolment (56.3%) was lower in patients with typical herpes than in other groups (82.3%), this difference was not statistically significant (χ^2 = 3.49, p 0.06). HIV shedding was significantly reduced in this group at Day 7 after patients had received treatment with anti-herpes therapy (56.3% vs 12.5%, χ^2 = 6.79, p 0.009). All patients ceased shedding HIV by Day 14.

Similarly, shedding of HIV decreased significantly following treatment among patients with chancroid alone - from 82.9% on Day 1 to 43.9% on Day 7 (χ^2 = 13.46, p<0.001). A further significant reduction in HIV shedding was found on Day 14 (43.9% vs. 17.1%, χ^2 = 6.96, p<0.001). There was a decrease in HIV shedding from 83.3% on Day 1 to 66.7% on Day 7

among patients with mixed chancroid and herpes. However, this decline was not statistically significant. (83.3% vs 66.7%, p NS) A similar trend was observed on Day 14.

In contrast, the same rate of HIV shedding was observed between Day 1 and Day 7 in patients with atypical herpes who received treatment with antibiotics only on Day 1. The shedding of HIV was reduced on Day 14 although 40% of patients still continued to shed HIV. When comparing the rates of shedding on Day 7 between the different groups, the atypical herpes group had significantly higher shedding than the other groups who had received either correct or partially correct therapy (Figure 5.6).

The majority of patients in all aetiology groups had HIV PVLs of more than 10,000 copies/ml. When comparing the rate of shedding at enrolment, there was an association between HIV PVL and HIV shedding (Table 5.5). The rate of HIV shedding in patients with PVL >10,000 copies/ml was significantly higher than in patients with PVL ≤10,000 copies/ml with an odds ratio of 59 (95% CI – 6.7 – 552.9, p<0.0001).

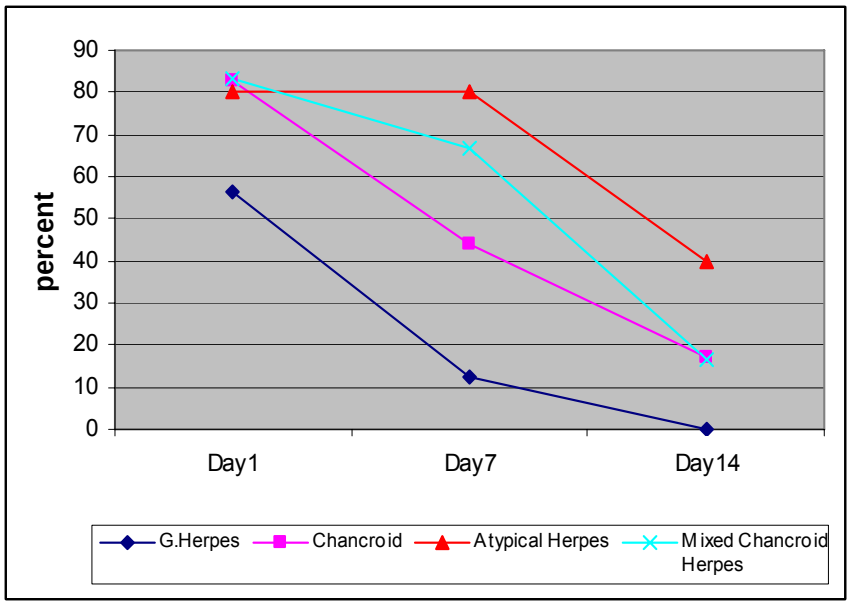


Figure 5.6 Proportion of patients shedding HIV in genital ulceration by ulcer aetiology

Table 5.5 Association between HIV PVL and HIV shedding in lavage fluids

PVL at Day1	HIV Shedding in Lavage Fluid		Total
	≥ 400 copies/ml	< 400 copies/ml	
PVL >10,000 copies/ml	59 (86.8%)	9 (13.2%)	68 (87.2%)
PVL ≤ 10,000 copies/ml	1 (10.0%)	9 (90.0%)	10 (12.8%)
All patients	60 (76.9%)	18 (23.1%)	78 (100.0%)

Odds Ratio = 59.0 (95% CI – 6.7 – 552.9). p<0.0001

5.9 Discussion

Since the beginning of the HIV epidemic, the natural history and mode of transmission of HIV have been extensively studied by researchers. The major modes of transmission of HIV are accepted to be unprotected sexual intercourse with infected individuals, transfusion of infected blood and blood products, parenteral injections using contaminated needles and mother-to-child transmission. Globally, the predominant mode of transmission of HIV varies significantly between regions. UNAIDS/WHO estimated that the global total number of people living with HIV had reached 39.4 million in 2004[1]. Despite the fact that that transmission through sexual intercourse has been reported to be lower than that of transmission through other routes such as sharing contaminated needles, mother to child transmission and transfusion of contaminated blood[2], in most developed countries, this mode of transmission still accounts for 75 to 85 percent of the total global burden of HIV[3].

As described by Anderson and May, the rate of spread of HIV infection depends on the duration of infectiousness of infected individuals and the average rate of sex partner change[4]. Although untreated HIV-infected persons can be expected to be infectious for the rest of their lives, the level of infectiousness through sexual transmission varies with many biological and epidemiological determinants. For example, an individual's infectiousness is known to increase during primary HIV infection and again during the late stage of the disease associated with low $CD4^+$ counts and an AIDS defining illness[2,5-9]. It has been documented that from the time of primary HIV infection, dissemination of the virus to patients' tissue

compartments results in the early establishment of viral reservoirs and shedding into oral and genital fluids[9-10]. It has also been shown that high-level shedding persists in the seminal plasma in some patients, despite effective anti-retroviral therapy resulting in reduction of PVLs[2]. STD-associated local immune activation together with inadequate drug penetration, slower clearance of HIV particles and/or slower decay rates of infected cells within the genital tract have been quoted as possible reasons for this phenomenom. Genital infections documented to be associated with increased detection of HIV in semen include seminal leukocytosis, urethritis, gonorrhoea, and cytomegalovirus infection[11-13]. Successful treatment of acute urethritis has been shown to reduce the rate of detection of HIV in semen[11].

In the studies reported in this chapter, we investigated the level of dissemination of HIV in another compartment, genital ulcer exudates. The HIV RNA concentration in genital ulcer exudates was quantitatively measured at different stages of the disease. Various biological and clinical determinants associated with the level of shedding were also explored.

With the rapid advancement of the HIV epidemic in southern Africa, we have documented the co-existence of a GUD epidemic that may have assisted in driving the epidemic to its highest level. In this setting, successful treatment of GUD in HIV-seronegative patients should reduce the risk of acquisition of HIV. This is even more so when over one third of men and women with GUD admit to having continued their sexual activities despite the presence of ulcerations[14]. When the HIV prevalence among GUD patients reached extremely high levels in the mid-1990s (over 50% of STI clinic patients on the gold mines), HIV shed in genital ulcer exudates could have contributed considerably to the increase in infectiousness of these core HIV transmitters.

During the period 1998 – 1999, we recruited HIV-seropositive GUD patients with chancroid alone (52.6%), genital herpes alone (39.7%) and mixed chancroid/genital herpes infections (7.7%) into our studies. HIV RNA was detected in genital ulcer exudates in more than 80% of 78 patients with large ulcerations which were clinically diagnosed as chancroid at the time of diagnosis. All patients were ART naïve and the majority (87.2%) had PVL >10,000 copies/ml

at the time of enrolment. Patients presenting with a typical clinical presentation of genital herpes (small, superficial, multiple vesicular ulcerations) had the lowest prevalence of HIV shedding (56.3%) at enrolment. The amount of HIV shedding, as measured by the number of HIV RNA copies detected per ml of ulcer lavage, varied considerably between patients. The highest amount of shedding of over 60,000 HIV RNA copies/ml of lavage fluid was detected in one patient with chancroid. The median shedding in the group was 3,782 copies/ml. In these studies, 10 ml normal saline was used to collect the exudates from ulcers in the form of ulcer lavage. The amount of exudates captured from the target ulcers in the 10 ml wash fluid would be relatively small and likely to be considerably less than 1 ml. Therefore, the amount of HIV RNA copies in actual ulcer exudates would be more than 10 times higher than that of the lavage fluid. By taking into account that most patients have multiple ulcerations, the total quantity of HIV copies shed from genital lesions could be several fold higher than the 'per ml' measured in the lavage specimen. Furthermore, the transmission efficiency in STI patients with untreated or inadequately treated genital ulcerations might have significantly increased and therefore contributed to the rapid spread of HIV infection in the community.

Various effective interventions such as overall improvement of GUD management in the mine and surroundings, targeted intervention of high-risk women using peer education and periodic presumptive treatment using azithromycin, brought the epidemic situation of chancroid in southern Africa under control in the early 2000s[15]. Similarly, enhanced screening and treatment of asymptomatic syphilis patients are recognised as factors that led to a rapid decline in syphilis seroprevalence among high-risk populations and this reached its lowest level in the early 2000s. Unfortunately, a decrease in cases of bacterial GUDs was accompanied by a rapid increase in the relative prevalence of genital herpes, particularly among HIV-seropositive patients in recent years (Section 3.4.3). A large proportion of these patients presented with atypical ulcerations that mimicked chancroid. In the absence of anti-herpes therapy in most public sector clinics, all patients with atypical herpes are routinely treated only with antibacterial agents and treatment failures are common; ulcerations often continue for more than two weeks, and shedding of HIV from ulcer exudates also continues. In our study, up to 40% of patients with atypical herpes who did not receive appropriate anti-

herpes treatment had detectable HIV RNA copies in their ulcer exudates at the Day 14 follow-up visit. Of those patients presenting with clinically recognisable genital herpes and who received appropriate antiherpes therapy, none had detectable HIV RNA in ulcer exudates at Day 14.

Our study findings also provide evidence of high level of dissemination of HIV into genital ulcer exudates when co-infected with HD and/or genital herpes. The rate of HIV shedding in genital ulcer exudates was directly associated with patient's PVL. Based on a proxy PVL cut-off level of 10,000 copies/ml and the degree of HIV shedding found in the study, shedding was significantly higher in patients with PVLs >10,000 copies/ml than with lower PVLs with an odds ratio of 59.0 (95% C, 6.7 – 552.9).

The important role of PVL in heterosexual transmission of HIV has been reported in various studies[16-17]. Gray *et al* found that higher plasma viral loads and genital ulcerations were the main determinants of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1 discordant couples in Rakai, Uganda. That study also concluded that the overall probability of transmission per coital act was 0.0011 which is within the range of transmission probabilities per act (0.0001 – 0.0020), found in other countries[18-21]. That study was conducted in Rakai during the period 1994 – 1998, and although their study population was different from ours, it is interesting to observe that the median PVL of the participants in their study was much lower than patients enrolled in our study (i.e. 12,476 copies/ml vs.115,838 copies/ml). The difference might be partially explained by a difference in the type of specimen used between the two studies. In our study, plasma samples were used for viral load measurement, while serum specimens were used in the Rakai study (up to a 21% lower counts have been recorded in serum when compared to plasma specimens, although this difference was not considered statistically significant; p=0.09)[22]. In the Rakai study, Quinn et al also reported that among HIV discordant couples in whom the initially HIV-1 negative partner seroconverted, the mean serum HIV-1 RNA level of the HIV-1 seropositive partner was significantly higher than in couples with seronegative partners who remained seronegative (mean, 90,254 copies per ml vs. 38,029 copies per ml, p 0.01)[17] . A dose response effect was found in the study in

which the rate of transmission increased from 2.2 per 100 person-years to 23.0 per 100 person-years as the serum HIV-1 RNA level increased from less than 3500 copies per ml to 50,000 or more copies per ml (adjusted rate ratio, 11.87). Our study findings also established a similar association between PVL and excretion of HIV RNA in genital ulcer exudates. Our study did not, however, include the measurement of HIV PVL in semen, but highlights the association between PVL and genital ulcer exudates and the role of PVL in dissemination of HIV into genital fluids.

In summary, appropriate diagnosis and treatment of genital ulcerations resulted in a rapid decline of HIV shedding in ulcer exudates, while any treatment failures or inadequate therapies led to delay in healing of ulcerations as well as continued shedding of HIV in ulcer exudates. Our study also highlighted the rapid increase in the relative prevalence of genital herpes among HIV-seropositive patients with genital ulcerations since the late 1990s. Therefore, syndromic management of GUD in southern Africa utilizing antibiotics alone should, theoretically become less effective, leading to a situation of increased treatment failure and continued shedding of both HSV and HIV in the community. Some countries such as Botswana and South Africa have initiated pilot projects to explore the feasibility in inclusion of anti-herpes therapy in the syndromic GUD management protocols. However, the availability and cost of non-curative anti-herpes therapy remain major problems in many developing countries.

In conclusion, effective control of the GUD epidemic in southern Africa is of paramount importance in preventing the continued rapid spread of HIV infection. Reduction or elimination of GUD cases is likely to contribute substantially to the prevention of HIV acquisition among high-risk HIV-seronegative patients and in addition lead to reduced infectiousness of HIV-seropositive individuals.

In 2003, WHO and partner agencies declared a commitment to improve access to anti-retroviral therapy (ART) for all HIV-seropositive patients in developing countries[23]. Wider access to ART in the coming years could ultimately reduce the PVL in the HIV-seropositive

population in southern Africa, although the highest PVLs could be among those with GUD who have not yet seroconverted. It is also possible that the overall immune reconstitution which occurs in treatment naïve population after introduction of ART could provide a positive spin-off effect in reducing the infectiousness of HIV-seropositive individuals in southern Africa by reducing recurrences of genital herpes. Safeguard activities must be implemented to re-enforce promotion of safer sex behaviour among high-risk individuals to prevent potential backlash of “treatment optimism” as seen among MSM population in more developed countries[24-25]. Further studies are needed to monitor these rapidly changing dynamics of HIV and STI epidemiology in southern Africa.

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CHAPTER 6

Interactions between HIV and STIs in women

6.1 Introduction

UNAIDS/WHO has estimated that 37.8 million people (range: 34.6–42.3 million) worldwide were living with HIV by the end of 2003[1]. While in the early days of the HIV epidemic, a disproportionately high number of men were infected with HIV, this proportion has dramatically changed in recent years with the number of women infected by HIV worldwide steadily growing. By the end of 2003, almost half of all people infected (approximately 17 million) were women. The HIV epidemic has also spread geographically; the burden of infection differing in different parts of the world. Although it has just over 10% of the world's population, up to two-thirds of all people living with HIV— approximately 25 million (range: 23.1–27.9 million) are resident in sub-Saharan Africa.

Countries in the southern African region including South Africa, Botswana, Zimbabwe, Malawi, Lesotho, Swaziland, Namibia and Mozambique were hardest hit by the exponential spread of HIV in the mid 1990s and they accounted for 38.3% of all the infections on the continent by 1999[2]**.** In 2003, HIV prevalence among pregnant women in most countries in southern Africa exceeded 20% (Figure 6.1). In South Africa, estimates of the number of women of reproductive age (15 – 49) who were living with HIV at the end of 2003 ranged from 2.5 million to 3.3 million[3].

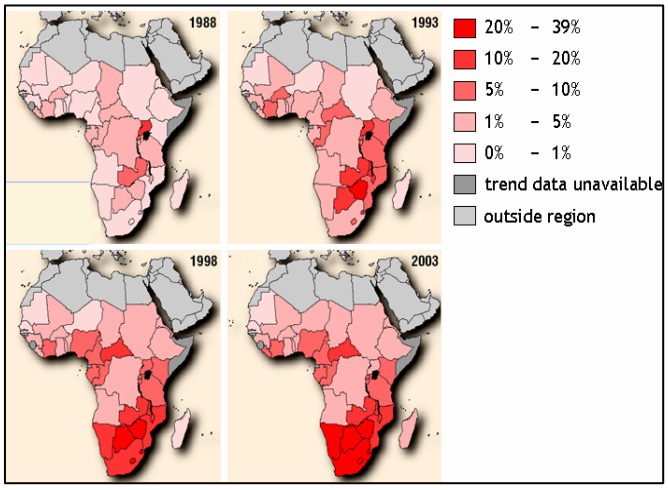


Figure 6.1 Trend of HIV infection in Africa (1988 – 2003)
Source: UNAIDS[3]

Studies of discordant couples in North America and Europe reported a higher male-to-female transmission efficiency than from female-to-male.[4-6] Data also suggest that male-to-female transmission during sex is about twice as likely to occur as female-to-male transmission, if no other sexually transmitted infections are present. Moreover, young women are biologically more susceptible to infection than older women prior to menopause[1]. Women's increased vulnerability to HIV is not only due to biological determinants. Many other social and economic determinants also play a significant contribution. In many societies, sexual decision-making remains solidly in the hands of men and therefore, male partners' sexual behaviour is often the most important risk factor for HIV acquisition among women.

Other important factors associated with HIV dissemination have been mentioned by Quinn in 1994[7]. These include population migration (both internal and cross-border), urbanization, social disruption, poor medical services, declining economies or inequitable distribution of wealth, low social status of women, and rates of infection with conventional STIs. These macro factors, in addition to those of behaviour, combine to shape the course of the HIV epidemic in southern Africa. It has been suggested that, in South Africa, various factors linked to social inequality have combined to shape the pattern and growth of the HIV/AIDS epidemic and it is therefore necessary to focus on all social dimensions in order to fully understand and combat the epidemic[8]. Biological and epidemiological interactions between STI and HIV in women are also very important and require the implementation of evidence-based interventions.

Many studies have documented these interactions in different countries largely during the early phase of the epidemic[9 – 12]. As in men, STI/HIV interactions in women are also bi-directional in nature. Interactions between HIV and STIs caused by human papilloma viruses (HPVs), herpes simplex virus (HSV), bacterial vaginosis (BV), candida vaginitis, trichomoniasis (TV), gonorrhoea (GC) and chlamydial (CT) infections have all been noted. Owing to the presence of physical, physiological and hormonal complexities involved with the female reproductive system, there are many limitations to studies performed previously[13 -15].

In our studies, we have undertaken to specifically explore the association between HIV and more conventional reproductive tract infections including HPV among South African women in Johannesburg, South Africa.

6.2 Study sites, population and methods

6.2.1 Background information: study site

The epidemiological and microbiological studies on HIV and STI interactions among women were conducted at the Esselen street STI clinic and the HIV clinic at the Hillbrow Hospital both serving an inner-city area of greater Johannesburg, South Africa which has a population of close to three million[16]. During the study period, major transformation of the city administration took place. Five previously-independent metropolitan areas were merged in 2000 to form the current Johannesburg "Unicity".

According to the Greater Johannesburg Metropolitan Council, the estimated size of the Hillbrow area, in which both the clinic and hospital are situated, is 35 hectare and the population density (100 or more per hectare) is 615. The area is the most densely populated area in Greater Johannesburg with significant inner city decay owing to lack of essential services and poorly maintained buildings[17].

Overall, the economic growth of the city has been unable to keep pace with population growth. The loss of low-skilled employment opportunities as a result of the decline in the mining and manufacturing sectors in the area has further exacerbated the unemployment situation. The decentralization of economic development to the north of Johannesburg has impacted on the low-income households living in the southern areas of the city including Hillbrow (Figure 6.2). An influx of illegal immigrants has also had an influence on employment possibilities for residents of the area.

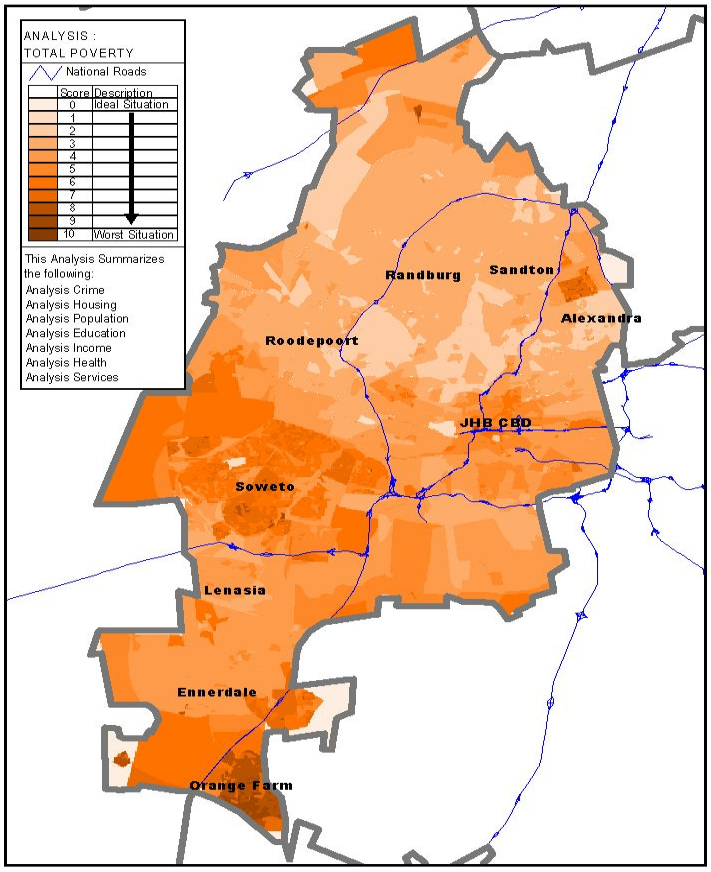


Figure 6.2 Analysis of total poverty in Johannesburg
(Source: http://ceroi.net/reports/johannesburg/csoe/html/nonjava/Poverty/analysis.htm)

6.2.2 Study objectives

The study was undertaken to investigate the patterns of vaginal and cervical infections in HIV-seropositive and HIV-seronegative women and their role in genital shedding of HIV infection among HIV-seropositive women.

The microbiological flora of the vaginal and cervix among HIV-seropositive and HIV-seronegative women was investigated and demographic and clinical indicators identified to predict cervical infection among women presenting with vaginal discharge. The magnitude of the problem of pelvic inflammatory disease (PID) among HIV-seropositive and HIV-seronegative women with vaginal discharge was also investigated. With respect to viral STIs, we assessed the prevalence of HPV and HSV infections. In addition, the association between HPV, HIV and abnormal cervical cytology and determinants of shedding of HIV among HIV-seropositive women were also studied. The study focused on the role of vaginal and cervical

infections on cervico-vaginal shedding of HIV and the effect of treatment of these infections on HIV shedding.

6.2.3 Study population

The study population comprised women attending the Esselen Street STI Clinic, Hillbrow, Johannesburg with symptoms of vaginal discharge. Non-menstruating women with vaginal discharge and no recent history of antibiotics usage were included in the study. Women who did not give consent for a speculum examination were excluded. However, the demographic and sexual history of these women was recorded for comparative analysis with those who were included in the study. A sub-group of HIV-seropositive women were recruited in order to explore the impact of vaginal and cervical infections on genital shedding of HIV. Women were included in this sub-group who were found to be HIV-seropositive after agreeing to voluntary HIV counseling and testing.

The majority of women attending the STI clinic were middle or low-income residents of inner city areas although some residents from remote townships who were working in or around the inner city also attended. Anecdotal evidence indicated considerable population movement in the area due to migration from other provinces within South Africa, or neighbouring countries (personal observation). Both an informal and formal sex trade was thriving in the area and a substantial number of STI patients were working as sex workers. Based on a study conducted by Reproductive Health Research Unit (RHRU) at the same clinic, around the same time period,[18] the majority (93%) of clients of sex workers are South African, with a mean age of 31 years (range 19-53). Most clients (53%) are single men who had never married. Sex workers indicated that they were having a mean of 2 sexual partners per day at the time of interview (range 1-8). Fifteen percent of clients said openly that they would offer more money to sex workers for sex without a condom.

The HIV Clinic at Hillbrow Hospital serves as a tertiary referral clinic for HIV-seropositive patients from Johannesburg and surrounding townships. The clinic population is largely

composed of mixed socioeconomic groups but came mostly from low or middle-income families.

6.2.4 STI services in study areas

Following the restructuring of health services in the city, the Esselen Street Clinic was administered under Region 8 and covered areas including Hillbrow, Berea, Yeoville, Jeppestown, Berea and downtown Johannesburg. The estimated number of people reached by the Region 8 health services was approximately 169,000[19].

The routine clinic protocol for management of female STI patients includes the collection of demographic and personal details, clinical history taking, genital examination including speculum and bi-manual examination, serological screening for syphilis and the offer of VCT for HIV, syndromic diagnosis and management, using a standard treatment algorithm catering for counselling, provision of contact slips and condom promotion. When indicated clinically, additional laboratory investigations (e.g. cervical smears for cytological examination and laboratory tests for vaginal/cervical infections) and referrals are provided routinely.

6.2.5 Methodology (specific to STI and HIV interactions in women)

6.2.5.1 The cross-sectional prevalence study

A pre-coded questionnaire was developed based on experience gained from earlier studies. The questionnaire was pre-tested using interviewers at the clinic where the studies were being conducted. Dedicated interviewers conducted the interviews in a private room. Following the interview and completion of the questionnaire, the investigator examined the patient and recorded the clinical findings. The sample questionnaire is shown in Annexure 6.1.

During the speculum examination, a high vaginal swab was taken for pH testing, an amine test, wet mount microscopy and examination of Gram-stained smear. A further two high vaginal swabs were taken and immediately inoculated into culture media for the culture of *Trichomonas vaginalis, Ureaplasma urealyticum, Mycoplasma hominis* and *Candida* species.

Any abnormalities of the cervix and vaginal canal observed during the examination were recorded. Ten ml of sterile normal saline was used to flush out the cervix with a 20 ml syringe. The cervicovaginal lavage (CVL) fluid was allowed to pool in the posterior fornix for 2 – 3 minutes. The CVL was then aspirated using a sterile 10 ml disposable pipette.

After wiping the cervix with sterile gauze, cervical specimens were collected for gonococcal, chlamydial culture and PCR testing for HPV and HSV. A Cytobrush® (Histobrush, Hardwood Products Company, USA) was used to collect specimens from the endocervix and the transformation zone and fixed immediately on a slide for cytological examination. Two ectocervical specimens (one cotton swab for HSV PCR and one Digene Hybrid Capture specimen collection kit for HPV testing) were also collected. PCR for HSV and HPV (MY 9/11) were conducted using the methods outlined in Sections 2.3.3.7 and 2.3.3.8.

After removal of the speculum, potential cervical excitation was elicited on bimanual examination and recorded by using a subjective scoring system. Blood was then taken from patients for syphilis, chlamydial and type-specific herpes serology. A small amount of serum was separated, coded and kept for future unlinked anonymous HIV testing. To maintain anonymity, sera were kept in the –70° C freezer until completion of the study. Thereafter, the record of identity of the patient was destroyed and the sera tested for HIV antibody. A first catch urine specimen was also collected for ligase chain reaction (LCR) for chlamydial and gonococcal infections. The aetiology of vaginal and cervical infections was assessed through the application of standard laboratory methods as described in Section 2.3.3.

After collection of specimens, patients were treated according to the standard syndromic management algorithm for vaginal discharge (Annexure 6.4). Other presenting syndromes, if present, were treated concurrently, using appropriate algorithms. Patients were requested to return for a follow-up visit after 14 days. The contact address of the patient was recorded for active follow-up if, necessary.

During the follow-up visit, available laboratory and cytology results were considered and additional treatment provided where necessary. A recommended protocol was followed for women with abnormal cervical cytological reports. Attempts were made to contact patients who defaulted by mail or telephone. If they were not available at a given address, evidence of their identity was removed and destroyed from case report forms and their sera were subsequently tested anonymously for HIV antibodies.

6.2.5.2 HIV shedding and impact of STI treatment

At the Esselen Street clinic, a further sub-group of women who requested voluntary HIV testing for various reasons was included for HIV shedding studies. In collaboration with the Outreach Program of the Greater Johannesburg Metropolitan Council and the Reproductive Health Research Unit, female sex workers voluntarily requesting HIV testing were also included in the studies. All participants were given an explanation of the study and written informed consent was obtained. Thereafter, pre-test and post-test counselling for HIV was conducted by trained counsellers.

Interviews were conducted to obtain information on demography, sexual practices and STD symptoms. A thorough clinical and gynaecological examination was conducted, including speculum examination. Vaginal and cervical specimens, including cervico-vaginal lavage specimens, were collected as previously described. Through the vaginal speculum, 10 ml of sterile normal saline was slowly and gently applied to the cervix using a syringe. After approximately 2 to 3 minutes, all fluid pooled in the posterior fornix was collected using a 10 ml sterile disposable pipette and placed in a sterile plastic tube. All specimens were stored at -70ºC until processed to measure HIV RNA copies using a standard protocol as described in Section 2. All participants with STD signs and symptoms were treated immediately using the appropriate syndromic STD management protocols.

Two endocervical specimens (using sterile cotton-tipped swabs) and a first-catch urine specimen were collected to detect *C. trachomatis* and *N. gonorrhoeae* by culture and LCR testing (See Section 2.3.3). Finally, a specimen was collected from the squamo-columnar

junction of the cervix and a Papanicolaou smear prepared. Plasma specimens were also collected but tested for HIV viral load measurement only if the patient was found to be HIV-seropositive.

Blood was collected and tested for syphilis serology (RPR and FTA-ABS), HIV antibody status and chlamydial and herpes serology. The rapid HIV test was used to screen patients and all positive specimens were confirmed by two different ELISA tests. The SAIMR and South African National HIV testing protocol was followed for all specimens. CD4 counts and HIV plasma viral loads were determined for all HIV-seropositive women.

Appointments were made for a follow-up visit after 7 days. All participants were given their HIV results and post-test counselling at the follow-up visit. The significance of CD4 results was explained to all HIV-seropositive women and they were referred to the Hillbrow Hospital HIV clinic for further management. A further general and gynaecological examination was conducted at the follow-up visit. At this visit, the vaginal, cervical and blood specimens were repeated from HIV-seropositive women as were all other laboratory investigations, except for syphilis serology, HPV and Papanicoloau smear testing.

6.2.6. Data entry and analysis

Each subject was given a unique study number which was recorded on all interview and examination sheets and on all specimens. The name, address and clinic number of each participant was recorded next to the study number in a separate register that was used for the follow-up of subjects. This register also included laboratory results. The register was kept in a confidential file at the Reference Centre for STI (RCSTI).

All demographic, sexual behavioural and clinical information together with laboratory results were entered in the customized SPPS® (SPSS Inc., Chicago, IL.) database and stored at the RCSTI. Patient identifiers were removed permanently after results were provided to the patients at their follow-up visit. Descriptive statistics of pre-coded demographic and clinical variables were analysed and presented. The prevalence of vaginal and cervical infections and

positive syphilis serology was calculated and the associations between HIV and other reproductive tract infections explored.

6.2.7. Ethical considerations

The protocol was reviewed and approved by the Ethics Committee of the University of Witwatersrand, and by a joint RCSTI-Gauteng Transitional Metropolitan Council panel.

Informed written consent was obtained from all subjects (Annexure 6.2 and 6.3). Subjects who refused to participate were assured that they would not be penalized in any way and would have a right to be examined and treated in the same way as study participants. Any abnormalities detected during examination were addressed or referred to the appropriate service. All participants were invited to return for the results of their tests on a certain date where results were explained and acted upon if necessary. While waiting to be examined, a health education session, including demonstration of condom use, was conducted for all STI patients.

6.2.8 Data Collection Instruments

6.2.8.1 Study questionnaires (Annexure 6.1)

6.2.8.2 Patient information sheet (Annexure 6.2)

6.2.8.3 Informed consent (Annexure 6.3)

6.2.8.4 Treatment algorithm for vaginal discharge (Annexure 6.4)

Annexure 6.1 Study questionnaires

CLINIC CODE [ ] PATIENT CODE [ ] DATE D D M M Y Y

1. AGE (years) — 1/Age

2. Marital Status — 2/Marital
   1 = Never Married, 2 = Married (Customary/Lawful)
   3 = Divorced/Separated 4 = Others (Specified)
   married goto No. 4
   Mario

3. Do you have any current sexual partner? — 3/Partner

4. How long have you been with this partner? (months/weeks) — 4/Pdura32 (Month / Week)

5. Are you living with your partner/husband? — 5/Live

6. How long have you been living together with this partner? (months) — 6/Ldura42 (Month / Week)

7. What work does your partner/husband do ? — 7/Poccup

8. Does he work away from home ? — 8/Paway

9. How often do you see him? — 9/Pfreq
   1 = Daily, 2 = Weekly 3 = Monthly 4 = >Monthly

10. What standard did you pass at school? — 10/Edu (Post matric)

11. What is your occupation ? — 11/Occup

12. Are you currently employed? — 12/Employ

13. (if no) Who support you financially? — 13/Support

14. Do you use contraceptives? — 14/CContra
    1 = Pills, 2 = Injection, 3 = IUD, 4 = Other, 5 = Never

    14.1 (if Yes) For how long? (Months) — 141/Cduracon (Month / Week)

15. Have you ever used the following contraceptives? — 15/Pcontra
    1 = Pills, 2 = Injection, 3 = IUD, 4 = Other, 5 = Never

    15.1 (if Yes) For how long? (Months) — 151/Pduracon (Month / Week)

16. When was your last menstrual period? — 16/ LMP (D D M M Y Y)

17. (if period is overdue) Are you pregnant? — 17/ Preg.
    (1 = Yes, 2 = No, 3 = Not sure)

18. Do you have any menstrual irregularities? (e.g : Menorrhagia/ Metrorrhagia etc.) — 18/ Irregu.

19. How many times have you been pregnant? — 19/ Gravida

20. How many times did you have the following problems? — STD
    (Enter 0 for None)

Ectopic Preg.	Still Birth	Abortion	Neonatal Death	Gynae. Problem

21. Have you ever had a history of post-coital bleeding ? (i.e. Bleeding after sex) — *21/ PC Bleed* [1 | 2]

22. What age did you start having sex? — *22/ Age at sex* [ ]

23. Number of sexual partners ; in lifetime — *23/ Partner1* [ ]

last year — *Partner2* [ ]

24. Have you ever used condom ; in lifetime — *24/ Comdom1* [1 | 2]

last year — *Condom2* [1 | 2]

25. How many times did you have any of the following problems since last easter?
PVD = PV Discharge, LAP = Lower Abd. Pain, BOM = Burning Urine,
Sores = Genital Sores, Syp = Syphilis (Fill 0 for None) — *25/PSTD*

PVD	LAP	BOM	Sores	Syp	Wart

26. Have you seen or suspected that your partner has the 'drop' or sores or genital warts or swelling of his private parts? (1 = Yes, 2 = No, 3 = Not sure) — *26/PartSTD*

Drop	Sore	Warts	Swelling

27. When did you see? — *27/PartSTD1* [ ]

28. Could you tell me what problem do you have at present?
(1 = Yes, 2 = No, 3 = Not sure)

	1 or 2	Dura (days)
251 Pain/Burning when passing urine ?		
252 Abnormal discharge?		
253 Itchiness of the private parts?		
254 Sores on the private parts?		
256 Pain in the lower abdomen?		
257 Genital warts/ Abnormal growth?		

29. Do you smoke currently? — *CSmoke* [1 | 2]

(if yes) How many cigarettes do you smoke a day and for how long? — *CDura(months)* [ ] *CAmt* [ ]

(if no) Have you ever smoked before? — *PSmoke* [1 | 2]

(if yes) How long did you smoke and how many cigarettes did you smoke a day? — *PDura (months)* [ ] *PAmt* [ ]

30. Do you drink alcohol currently? — *Cdrink* [1 | 2] *CAmtdrink* [ ]

(if yes) How many days in a week do you drink?

(if no) Have you ever drink alcohol before? — *Pdrink* [1 | 2]

(if yes) How long did you drink and how many days in a week did you drink? — *PDuradrink (months)* [ ] *PAmtdrink* [ ]

31. EXTERNAL GENITALIA | 1 | 2 |

25.1 Vulval Rash	1	2
25.2 Pruritus Vulvae	1	2
25.3 Anogenital Warts	1	2
25.4 Other abnormal growth	1	2
25.5 Vulval Leucoplakia	1	2

32. GENTIAL SORES | 1 | 2 |

33. CLINICAL DX | 1 | 2 | 3 | 4 | 5 | 6 |
(1=Chancroid,2=Syphilis ,3=G, Herpes, 4=LGV, 5=G.I, 6=Other)

34. GLANDS | 1 | 2 |

28.1 Site	Uni	Bi
28.2 Matted	1	2
28.3 Only inguinal	1	2
28.4 Size (mm)		

35. Lower abdominal tenderness | 1 | 2 |

36. Vaginal discharge

36.1 Frothy & profuse

36.1 Curdlike | 1 | 2 |

			Severity Grade
30.3 Discharge from Cx	1	2	
30.4 Cx Erosion &/or Easy bleed	1	2	
30.5 Cx Excision Test	1	2	

37. If CET is positive, please assess the severity?
(1=Mild, 2=Moderate, 3=Severe)

1	2	3

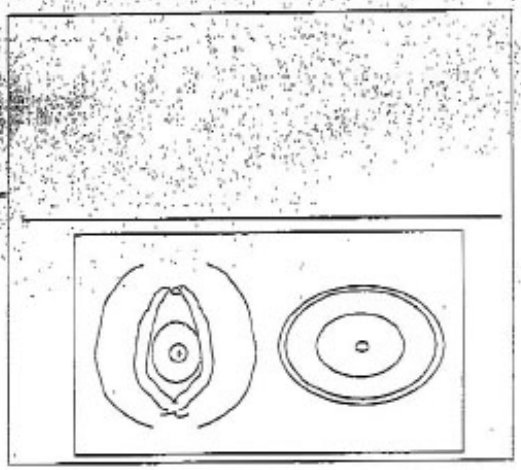
38. Do you think cervix is normal looking? | 1 | 2 |

39. (If not) PLEASE DESCRIBE;

40. Other Diseases

35.1	
35.2	
35.3	
35.4	
35.5	
35.6	

41. OTHER IMPORTANCE CLINICAL NOTES



42. Photo taken | 1 | 2 |

43. Photo Num.

TO

CLINIC CODE: | | | PATIENT CODE : | | | | | | LABORATORY

GENITAL ULCER

1	2

1. MICROSCOPY

	Collected	Positive	Negative	Contaminated/ Missing
1.1) Dark-Field		1	2	3
1.2) Giemsa/Dono. Bodies		1	2	3
1.3) Urethral smear		1	2	3

2. CULTURE (Ulcer Specimen)

	Collected	Positive	Negative	Contaminated/ Missing
2.1) Ulcer *H.ducreyi*		1	2	3
2.2) Ulcer *H. simplex*		1	2	3
2.3) Ulcer *C. trachomatis*		1	2	3

3. CULTURE (Bubo Aspirate)

	Collected	Positive	Negative	Contaminated/ Missing
3.1) Bubo *H. ducreyi*		1	2	3

VAGINAL DISCHARGE

1	2

Wet Mount Microscopy

	Collected	Positive	Negative	Contaminated/ Missing
4.1) Vaginal pH		1	2	3
4.2) Amine test		1	2	3
4.3) Clue cells		1	2	3
4.5) Yeast		1	2	3
4.6) Trich.		1	2	3

5. CULTURE (Post. fornix / Cx)

	Collected	Positive	Negative	Contaminated/ Missing
5.1) *N. gonorrhoeae*		1	2	3
5.2) PPNG		1	2	3
5.3) *C. trachomatis*		1	2	3
5.4) *H. simplex*		1	2	3
5.5) *M. hominis*		1	2	3
5.5) *T. vaginalis*		1	2	3
5.6) Candida species		1	2	3

6. SEROLOGY

	Collected	TITER				
6.1) RPR (0 =Neg)						
6.2) FTA-ABS (0 =Neg)						
	1	2	3			
6.3) Chlamydial MIF						

7. LIGASE CHAIN REACTION (LCR) Cervical Swabs

1	2

	Collected	Positive	Negative	Contaminated/ Missing
7.1) *N. gonorrhoeae*		1	2	3
7.2) *C. trachomatis*		1	2	3

8. LIGASE CHAIN REACTION (LCR) Urine

1	2

	Collected	Positive	Negative	Contaminated/ Missing
8.1) *N. gonorrhoeae*		1	2	3
8.2) *C. trachomatis*		1	2	3

9. PCR Genital Sores

1	2

	Collected	Positive	Negative	Contaminated/ Missing
9.1) *Herpes simplex*		1	2	3
9.2) *H. ducreyi*		1	2	3
9.3) *T. pallidum*		1	2	3

10. PCR Cervix

1	2

	Collected	Positive	Negative	Contaminated/ Missing
10.1) *Herpes simplex*		1	2	3
10.2) *H. ducreyi*		1	2	3
10.3) *T. pallidum*		1	2	3

11. PCR Vaginal Washing

1	2

	Collected	Positive	Negative	Contaminated/ Missing
11.1) *Herpes simplex*		1	2	3
11.2) *H. ducreyi*		1	2	3
11.3) *T. pallidum*		1	2	3

12. HUMAN PAPILLOMA VIRUS

	Collected	Positive	Negative	Contaminated/ Missing
HPV				

13. PAP SMEAR

	Collected						
3.1 Squamocolumnar junction							
3.2 Endocervix							
3.3 Cytology Report	0	1	2	3	4	6	9

[0=Normal, 1=CIN1 2=CIN2, 3=CIN3, 4=Invasive, 5=Indequste, 9=Missing)

Detail Report (Cytology, HPV and Colposcopy)

1.
2.
3.
4.
5.

14. Multiplex PCR — Genital Swab

1	2

	Collected	Positive	Negative	Contaminated/ Missing
11.1) *Herpes simplex*		1	2	3
11.2) *H. ducreyi*		1	2	3
11.3) *T. pallidum*		1	2	3

14. Multiplex PCR — Vag. Washing

1	2

	Collected	Positive	Negative	Contaminated/ Missing
11.1) *Herpes simplex*		1	2	3
11.2) *H. ducreyi*		1	2	3
11.3) *T. pallidum*		1	2	3

Annexure 6.2 Patients information sheet

Appendix (4)

Subject Information and Consent Form

Based on the history and clinical examination, we believe that you have contracted a sexually transmitted agent. You can get more than one sexually transmitted disease as these agents infect through the same mode of transmission. Therefore, mixed infection with different agents in a patient is very common. Some of the sexually transmitted agents can cause serious complications like swelling of upper genital tract organs, such as womb, tubes etc. It is also believed that chronic infection due to certain STD agents can lead to cervical cancer in women.

The National Reference Centre for STD (NRC/STD), S.A. Institute for Medical Research (S.A.I.M.R.) is studying the common causes of vaginal and cervical infections among women with vaginal discharge and the associated complications, such as upper genital tract infection, cancer of cervix and HIV infection in South Africa. We are asking you to participate in this research study which will help us to develop more appropriate prevention and management strategies for these serious complications due to STD in South Africa.

If you decide to participate, we will conduct an interview to ask some relevant questions related to your personal and sexual history. Then, we will conduct a routine speculum examination to check your lower genital tract and swabs will be taken from your vagina and cervix for laboratory tests. We will test for all possible bacterial, fungal, parasite and viral sexually transmitted agents from these swabs. A PAP smear will be taken to check the presence of any abnormal cells which may progress to cancer of cervix in the future. The blood will be collected for the routine syphilis and chlamydial serology. A voluntary HIV test will be made available on request from you after pre-test counselling is provided. We will check your urine for some bacterial infections. All the results will be made available to you without any extra cost during your return visit. The necessary treatment and/or referral to specialist will also be arranged.

After aforementioned procedures have been completed, your identification (name, address etc.) will be removed from your record. Then, the stored blood will be tested for HIV antibody anonymously (without knowing identity of the patient). The information obtained from the interview and laboratory tests will be kept in a secured place at NRC/STD, SAIMR under lock and key. Confidentiality will be maintained throughout the study period.

If you are willing to continue participating in the study, we will include you in our follow-up study, where you will get regular six-monthly check-up for genital tract infections and cervical cytology. Similar procedures will be carried out during the follow-up visits.

There is no harmful effect due to the speculum examination and specimen collection. You will get benefit from a proper genital examination, laboratory back-up diagnosis and frequent screening for cancer of cervix. All participants will contribute to the essential knowledge resulting from proper diagnosis and management of serious STD, their complications and genital cancer in women.

Participation in this study is voluntary and you are free to refuse to participate or to withdraw your consent and to stop participation at anytime. Such refusal or discontinuance will not affect your regular treatments or medical care in any way.

I have fully explained the procedures, laboratory tests to be done and their purpose. I have asked whether any questions have arisen regarding the procedures and have answered these questions to the best of my ability.

Date ; _____ Doctor : _____

I have been fully informed about the procedures mentioned above. In signing this consent form, I agree to have my genital and blood specimens taken for investigational purposes. I understand that I am free to refuse to participate or to withdraw my consent in this study at anytime. I understand also that if I have any questions at anytime, they will be answered.

Date : _____ Patient : _____

or Guardian/Next of Kin : _____

_____

National Reference Centre for STD
Dept. of Microbiology, School of Pathology
S.A. Institute for Medical Research & Univ. of Witwatersrand

Annexure 6.3 Informed consent

HIV & STD INTERACTION STUDY (SAIMR)

Patient Consent Form

I (full name) _____ hereby confirm that I have read the patient information sheet (or it has been read out loud to me) and understand what the study is all about. I understand that I will take part in the study for a period of two weeks (two clinic visits), and am fully aware of the requirements and consequences of my participation in the study.

I understand that my decision to take part in this study is voluntary and that I may withdraw from the study at any time.

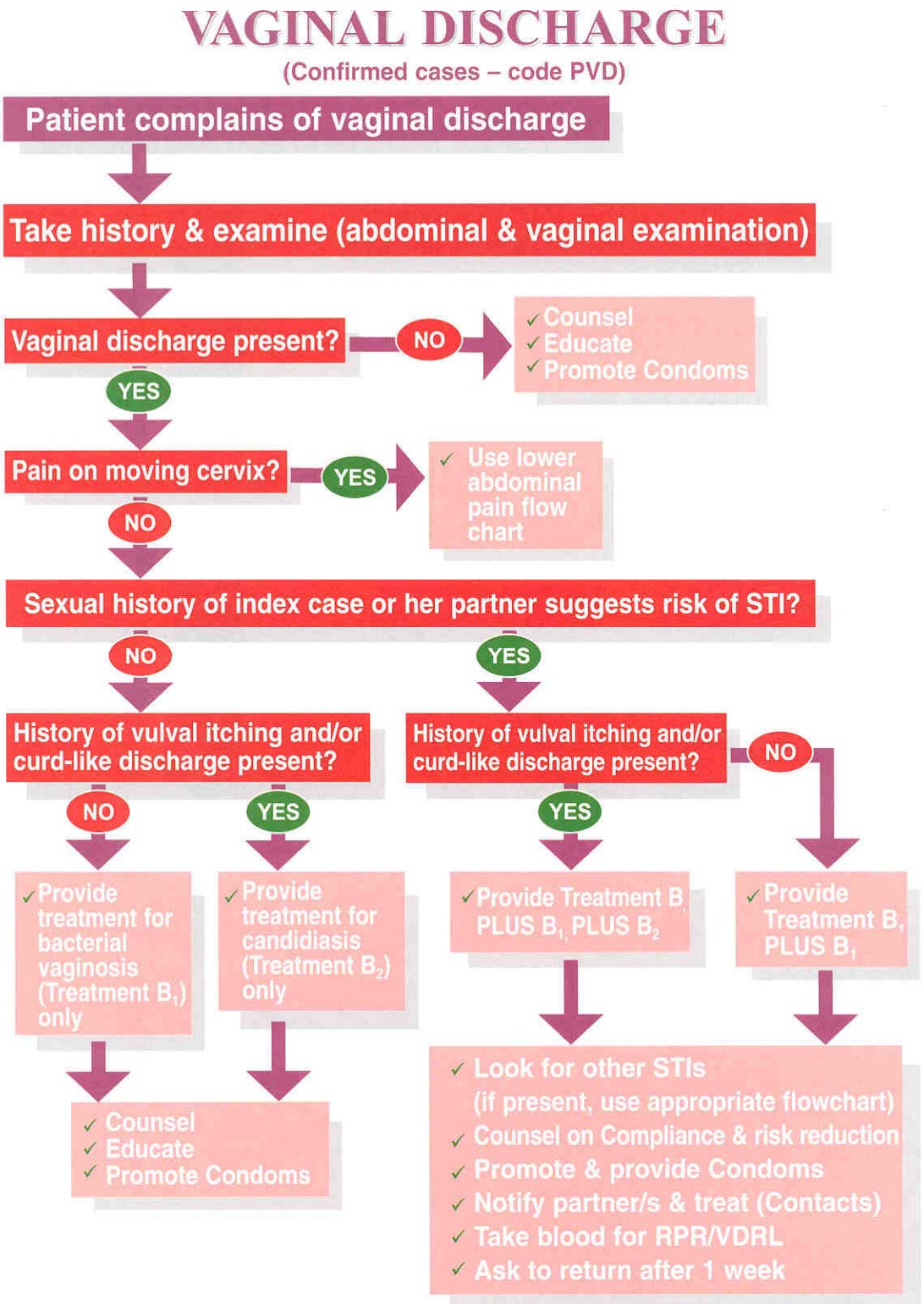
Patient Signature: _____

Date: _____

Place: _____

Witness: _____

Annexure 6.4 Treatment algorithm for vaginal discharge



TREATMENT OF VAGINAL DISCHARGE (PVD)

TREATMENT OPTIONS – B

Treatment for Gonorrhoea

Ciprofloxacin 500 mg p.o. stat OR

Ofloxacin 400 mg p.o. stat OR

*Ceftriaxone 125 mg i.m. stat OR

*Spectinomycin 2g i.m. stat

PLUS

Treatment for Chlamydia

Tetracycline or *Erythromycin 500 mg p.o. qid for 7 days OR

Doxycycline 100 mg p.o. bd for 7 days OR

*Azithromycin 1g p.o. stat

TREATMENT OPTIONS – B_1

Treatment for Trichomoniasis and Bacterial vaginosis

Metronidazole 400 mg p.o. bd for 7 days OR

Metronidazole 2 g p.o. stat OR

(less effective than multidose therapy for bacterial vaginosis)

Tinidazole 500 mg p.o. bd for 5 days OR

*Ampicillin 500 mg p.o. qid for 7 days OR

(active against bacterial vaginosis only)

Clindamycin 300 mg p.o. bd for 7 days

TREATMENT OPTIONS – B_2

Topical Treatment for Candidiasis

*Clotrimazole 200 mg pessaries nightly for 3 nights OR

(also active against *T.vaginalis*)

*Clotrimazole 500 mg pessary stat on retiring OR

*Miconazole 200 mg pessaries nightly for 7 nights OR

*Nystatin pessaries twice daily for 14 days OR

*Econazole 150 mg depot ovule stat on retiring

Provide appropriate antifungal cream for cases of pruritis vulvae

Treatments marked * are safe for use during pregnancy/breastfeeding

6.3 Results

6.3.1 Interactions between HIV and STIs in women

6.3.1.1 Demographic and socioeconomic status of patients

A total of 274 women presenting with vaginal discharge during the period May 1996 – December 1996 were enrolled in the study. The overall HIV seroprevalence among those enrolled was 48.2% (95% CI – 42.3% - 54.1%). Of these, 257 patients agreed to provide details on demographic and sexual behaviour-related issues relevant to the study. The associations between these parameters and HIV infection are presented here.

The mean age of 257 patients from whom information was available, was 24 years (range – 15 – 59 years). More than 80% of patients were under 30 years of age and 39 of 257 (15.2%) were in the 15 – 19-year age group (Figure 6.3).

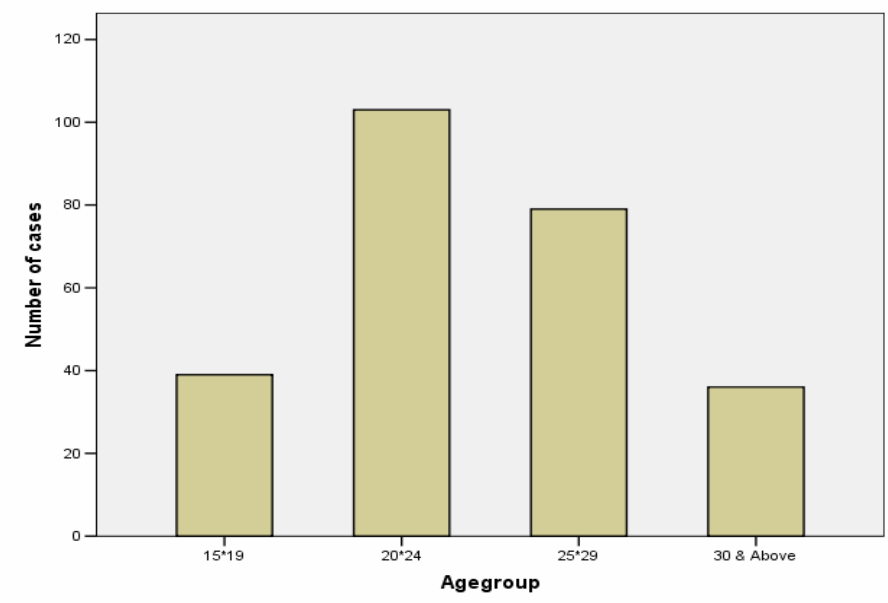


Figure 6.3 Age distribution of women presenting with STI symptoms, Johannesburg (May 1996 - December 1996) (N= 257)

The majority (70%) of patients resided in Hillbrow and surrounding areas while the remaining patients came from different residential areas (townships) outside Johannesburg. Two hundred and twenty-eight patients (89.1%) were never married but 96.1% had current sexual partner(s). The median duration of a sexual relationship with a current partner was 24 months (range: 1 – 180 months). Of 254 patients with current sexual partners, 42.9% were

living with their partners for a median duration of 21 months (range: 1 – 468 months). Although only 3% of patients were educated to a post-matriculation level (completed high school), the majority had at least a primary or secondary school education with a median years-of-schooling duration of 8 years schooling. Of 159 patients who acknowledged that they required external financial support, 93 (58.5%) received support from family members (i.e. parents, husband, sisters etc.) and 41.5% from boyfriends.

The association between socioeconomic and demographic determinants and HIV serostatus is shown in Table 6.1. HIV seropositivity was significantly associated with a short duration of partnership with the current sexual partner – less than 12 months (Odds ratio 2.2; 95% CI 1.3 – 3.7, p 0.005); being an unemployed or a school leaver at secondary school level (Odds ratio 2.7, 95% CI 1.2 – 6.1, p 0.02) and economically dependent on a boyfriend (Odds ratio 2.8, 95% CI 1.4 – 5.3, p 0.002) (Table 6.1).

6.3.1.2 Sexual behaviour and reproductive health

Overall, the median age at first sexual intercourse was 17 years (range: 13 – 24 years), and the median number of life-time sexual partners was 3 (range: 1 – 35). Women who reportedly had only one sexual partner during their life-time had a significantly lower prevalence of HIV than those with more than one partner (33.3% vs. 52.1%, Odds ratio 2.2, 95% CI 1.0 – 4.6, p 0.04). The HIV seroprevalence was significantly higher among women who had their sexual debut at less than 15 years of age compared with those whose sexual debut occurred when they were older (81% vs. 45.7%, Odds ratio 5.1; 95% CI 1.6 – 15.5, p 0.005). Twenty percent of patients had more than five life-time sexual partners. However, the mean number of sexual partners during the past six months was 1.4 (range – 0 - 17) with 23% of patients having more than one sexual partner during the past six months. Contraceptive usage was also low with only 47.5% of patients ever having used any form of contraception. Pills (14.4%) and injectables (25.7%) were the most frequently used methods of contraception among patients. Condom use was very low with only 40.9% ever having used a condom in the past six months. A high proportion, (45.1%) of women noticed that their current sexual partner had STI-associated signs such as “drop”, sores or genital swellings during their current

relationship (Table 6.1). Multivariate analysis indicated that only recent sexual partnerships (i.e. those <12 months duration) and economic dependency on a boyfriend were independently associated with a high risk of HIV-seropositivity (Adjusted Odds ratio for recent sexual partnership 3.7; 95% CI 1.7 – 7.9, p 0.001, and for economic dependency on boyfriends – 2.6; 95% CI 1.2 – 5.7, p 0.013).

Table 6.1 Association between demographic, sexual and reproductive determinants and HIV infection in women with STIs

Variable	n (%)	HIV (%)	OR	95% CI	*P*
Demographic characterisitcs					
Age	n = 257				
15 - 19	39 (15.2)	53.8	1		
20 - 24	104 (40.1)	52.4	0.9	0.5 - 2.0	
25 - 29	79 (30.7)	45.6	0.7	0.3 - 1.5	
30 & Above	36 (14.0)	38.9	0.5	0.2 - 1.4	
Martial status	n = 256				
Married	28 (10.9)	32.1	1		
Never married	228 (89.1)	50.4	2.1	0.9 - 4.9	
Current sexual partner present	n = 256				
No	10 (3.9)	40.0	1		
Yes	246 (96.1)	48.8	1.4	0.4 - 5.2	
Duration of sexual partnership	n = 246				
12 months	161 (65.4)	42.2	1		
<12 months	85 (34.6)	61.2	2.2	1.3 - 3.7	0.005
Highest education level	n = 254				
Secondary & above	176 (69.3)	48.3	1		
Primary	78 (30.7)	48.7	1.0	0.6 - 1.7	
Occupation	n = 256				
Student	42 (16.4)	33.3	1		
Unemployed/School leavers	53 (21.9)	57.1	2.7	1.2 - 6.1	0.02
Domestic or similar jobs	65 (25.4)	49.2	1.9	0.9 - 4.3	
Other employment	93 (36.3)	49.5	2.0	0.9 - 4.2	
Main economic support	n = 159				
Family (Parents/Husband etc.)	93 (58.5)	34.4	1		
Boyfriend	66 (41.5)	59.1	2.8	1.4 - 5.3	0.002
Sexual & Reproductive History					
Age at first sex	n = 256				
15 & Above	232 (91.7)	45.7			
Under 15	21 (8.3)	81.0	5.1	1.6 - 15.5	0.005
Current contraceptive use	n = 257				
None	135 (52.5)	46.7	1		
Non-hormonal devices	19 (7.4)	31.6	0.5	0.2 - 1.5	
Hormonal (pills/injectable)	103 (40.1)	48.2	1.3	0.8 - 2.2	
Number of life-time sexual partners	n = 251				
One	36 (14.3)	33.3	1		
More than one	215 (85.7)	52.1	2.2	1.0 - 4.6	0.04
Number of sexual partners in past 6 months	n=255				
One	196 (76.9)	46.4	1		
More than one	59 (23.1)	55.9	1.5	0.8 - 2.6	
Condom use in past six-months	n = 254				
No	150 (59.1)	45.3			
Yes	104 (40.9)	52.9	1.4	0.8 - 2.2	
STI symptoms in current sexual partner	n = 255				
No	140 (54.9)	43.6			
Yes	115 (45.1)	54.8	1.6	1.0 - 2.6	

6.3.1.3 Clinical presentation and HIV infection

The majority of patients (68.6%) reported a past history of STI symptoms. Over one third of patients with STI symptoms had more than one episode of vaginal discharge during the past six months while 18.9% had genital sores, 2.9% genital warts, 29.1% burning on urination, and 33.3% lower abdominal pain. No significant association between the presence of past history of STI symptoms and HIV infection was found (Table 6.2).

A summary of presenting STI signs and symptoms is shown in Figure 6.4.
On examination, genital ulcerations were found in 27 cases (10.5%). The HIV seroprevalence rate among these women were significantly higher than those without genital ulcerations (74.1% vs. 45.0%, Odds ratio 3.5; 95% CI 1.4 – 8.6, p 0.006). Majority (98.9%) of patients examined in this series were found to have an abnormal vaginal discharge.

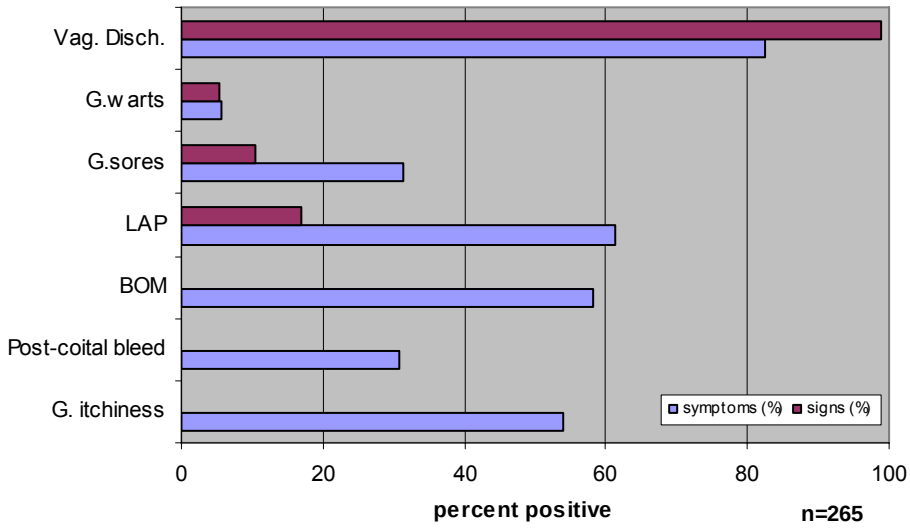


Figure 6.4 Presenting STI signs and symptoms

A significant association between HIV seropositivity and “profuse” vaginal discharge was found (55.0% vs. 42.1%, Odds ratio 1.7; 95% CI 1.03 – 2.8, p 0.04). Abnormal cervical lesions were frequently observed and cervical mucopus was detected in 43.5%, and cervical ectopy or erosion in 51.8%. Cervical excitation tenderness was elicited in 17% of patients, indicating a mild to moderate degree of PID. Although no significant association between HIV seropositivity and the presence of the aforementioned clinical signs was found, HIV

seroprevalence among those with any type of cervical lesions (i.e. erosions/warts/ulcerations) was significantly higher than those without cervical lesions (60.4% vs. 40.3%, Odds ratio 2.3; 95% CI 1.36 – 3.8, p 0.002) (Table 6.2).

Multivariate analysis showed that there was a significant association between HIV-seropositive status and the presence of genital sores on examination (Adjusted odds ratio 3.3; 95% CI 1.2 – 3.5, p 0.017) and any abnormal cervical lesions (Adjusted odds ratio 2.0; 95% CI 1.2 – 3.5, p 0.008).

Table 6.2 Association between STI symptoms and signs and HIV infection

Variable	n (%)	HIV (%)	OR	95% CI	*P*
STI Symptoms					
Past history of STI symptoms	n = 255				
No	80 (31.4)	46.3			
Yes	175 (68.6)	49.7	1.1	0.7 - 2.0	
Past history of vaginal discharge	n = 175				
Never	32 (18.3)	56.3			
Once	74 (42.3)	47.3	0.7	0.3 - 1.6	
More than once	69 (39.4)	49.7	0.8	0.3 - 1.8	
Past history of genital sores	n = 175				
Never	97 (55.4)	46.4			
Once	45 (25.7)	55.6	1.4	0.7 - 2.9	
More than once	33 (18.9)	51.5	1.2	0.6 - 2.7	
Past history of genital warts	n = 175				
Never	163 (93.1)	48.5			
Once	7 (4.0)	57.1	1.4	0.3 - 6.5	
More than once	5 (2.9)	80.0	4.3	0.5 - 38.9	
Past history of burning micturation	n = 175				
Never	74 (42.3)	48.6			
Once	50 (28.6)	50.0	1.1	0.5 - 2.2	
More than once	51 (29.1)	51.0	1.1	0.5 - 2.2	
Past history of lower abdominal pain	n = 174				
Never	58 (33.3)	55.2			
Once	58 (33.3)	50.0	0.8	0.4 - 1.7	
More than once	58 (33.3)	43.1	0.6	0.3 - 1.3	
Clinical Signs (on examination)					
Genital ulcerations	n = 258				
No	231 (89.5)	45.0			
Yes	27 (10.5)	74.1	3.5	1.4 - 8.6	0.006
Profuse vaginal discharge	n = 255				
No	126 (49.4)	42.1			
Yes	129 (50.6)	55.0	1.7	1.03 - 2.8	0.039
"Curd-like" vaginal discharge	n = 255				
No	199 (78.0)	51.3			
Yes	56 (22.0)	39.3	0.6	0.34 - 1.1	
Cervical mucopus	n = 255				
No	144 (56.5)	47.2			
Yes	111 (43.5)	50.5	1.1	0.69 - 1.9	
Cervical ectopy/erosion	n = 255				
No	123 (48.2)	46.3			
Yes	132 (51.8)	50.8	1.2	0.73 - 2.0	
Cervical excitation tenderness	n = 247				
No	205 (83.0)	48.3			
Yes	42 (17.0)	52.4	1.2	0.61 - 2.3	
Any cervical lesions (Erosion/Warts/Ulcers)	n = 255				
No	149 (58.4)	40.3			
Yes	106 (41.6)	60.4	2.3	1.36 - 3.8	0.002

6.3.1.4 Interactions between vaginal infections and HIV

Of 274 patients complaining of vaginal discharge and other related symptoms, 223 (81.4%) had at least one identifiable cause of their discharge (i.e. bacterial vaginosis, candidiasis or trichomoniasis). The majority of patients (50.5%) had a single aetiology of their discharge while 25.8% had two and 5.3% had three identifiable aetiologies that could have accounted for the vaginal discharge. Among patients with a single vaginal infection, 58 had bacterial

vaginosis (42.6%), 50 had candidiasis (36.8%) and 28 had trichomoniasis (20.6%). Mixed infections of all three main aetiological conditions were common. The rate of HIV infection was also significantly higher in patients suffering from multiple vaginal infections (any two aetiologies) compared with those without vaginal infections (26.3% vs. 18.6%, Odds ratio 2.4; 95% CI 1.2 – 5.1, p 0.02).

More detailed descriptions of each cause of vaginal discharge and its association with HIV are also discussed in the specific section on each disease.

6.3.1.4.1 Bacterial vaginosis and HIV

Clue cells with typical morphology indicative of bacterial vaginosis were present in 133 of 274 (48.5%) patients. All except one patient had vaginal pH readings of >4.5, and 75.2% of clue cell-positive patients were also amine test positive. No significant association between the presence of BV and any of the following: age, age at sexual debut, numbers sexual partners in past six months and condom use was detected. A significantly lower risk of BV was, however, found to be associated with a history of hormonal contraceptive use (i.e. either oral or injectable contraceptives) (40.8% vs. 54.8%, Odds ratio – 0.57, 95% CI 0.34 – 0.95, p 0.03). All patients with BV were confirmed as having an abnormal vaginal discharge on examination but no significant association was found between BV and early signs of pelvic inflammatory disease (PID) as diagnosed by the presence of cervical excitation tenderness. Although patients with BV had a higher HIV prevalence rate than HIV-seronegative patients, this was not statistically significant (52.6% vs 44.0%, $\chi^2 = 2.1$, p 0.15).

6.3.1.4.2 Vaginal candidiasis and HIV

One hundred and thirteen patients (41.2%) were found to be positive by culture for Candida species. Unlike BV, there was no association between hormonal contraceptive use and the detection of yeasts using the culture (46.6% in users vs. 39.3% among non-users, p 0.2). A significant proportion of patients with vaginal candidiasis presented with vaginal itchiness (49.6% vs. 31.9%, $\chi^2 = 8.2$, p 0.004). A significantly higher proportion of patients with a

typical clinical presentation of vaginal candidiasis (i.e. curd-like vaginal discharge) were positive for yeasts on culture (66.1% vs. 34.7%, $\chi^2 = 17.7$, p<0.0001).

There was an association between HIV and vaginal candidiasis. Of 113 patients with a positive culture for yeasts, 66 (58.4%) were HIV-seropositive compared to 66 (41%) of patients without candidiasis The HIV prevalence among patients with vaginal candidiasis was significantly higher than those without candidiasis (58.4% vs. 41.0%, Odds ratio 2.0; 95% CI 1.2 – 3.3., p 0.005) (Table 6.3). Alternatively, a significantly higher proportion of HIV-seropositive patients presented with vaginal candidiasis compared to HIV-seronegative patients (50% vs. 33%, Odds Ratio 2.02, 95% CI – 1.24 – 3.29, p 0.005).

6.3.1.4.3 Trichomoniasis and HIV

A total of 78 patients (28.6%) were confirmed positive for *T. vaginalis* (TV) on direct microscopic examination and/or culture. No association between demographic data, contraceptive use and sexual behavioural determinants and trichomoniasis was found. An abnormal vaginal discharge was observed in all patients with TV and a significantly higher proportion had a profuse vaginal discharge (42.2% in TV-positive vs. 14.3% in TV-negative, Odds ratio 4.4, 95% CI 2.4 – 8.1, p<0.0001).

A similar HIV prevalence rate was detected among TV-positive and TV-negative patients (46.2% vs. 49.2, $\chi^2 = 0.2$, p 0.6).

6.3.1.5 Cervical Infections and HIV

Of those interviewed, 156 (61.4%) complained of lower abdominal pain; however, only 42 (17%) were confirmed by the clinician to have cervical excitation tenderness on bi-manual gynaecological examination. During speculum examination, the following abnormalities were found: 111 patients (43.5%) exhibited cervical discharge; 132 (51.8%) cervical erosions and 16 (5.5%) had cervical warts. Up to 41.6% of patients had at least one or more abnormal cervical lesions such as cervical erosions, ulcerations, or warts, and these lesions were significantly associated with HIV infection. HIV prevalence was 60.4% in those with positive

cervical abnormalities compared to 40.3% among those without abnormalities (Odds ratio 2.3; 95% CI 1.36 – 3.8, p 0.002).

Overall, gonococcal and/or chlamydial cervical infections were detected in 119 (43.4%) of patients. Sixty-one patients (22.3%) were positive for *N. gonorrhoeae* alone, 33 for *C. trachomatis* alone (12.0%), and 25 for both (9.1%).

6.3.1.5.1 Gonococcal infection in women

The prevalence of gonococcal cervicitis (GC) among STI patients was 31.4%. There was no difference in mean age between GC-positive and GC-negative patients (Mean age 24 ± SD 6 years in GC-positive group vs. 24.7 ± SD 5.4 years in GC-negative group, t-test, p 0.56). Although the difference was not statistically significant, the age-specific prevalence rates were higher among younger women with the highest rate of 36.9% found in among those in the 20-24 year age group. The prevalence was also high in the 15 – 19 year age group with 33.3% being GC-positive. No significant association between positive GC status and other socioeconomic and demographic determinants was detected.

During clinical history taking, 115 patients (45.1%) reported that their current sexual partner was suffering from STI symptoms such as discharge, sores, warts or genital swelling. There was no association between the presence of any STI symptoms among sexual partners and GC-positive status (36.5% in patients positive history vs. 30.0% in patients with no history, χ^2 = 1.2, p 0.27). However, the GC-positivity rate was significantly higher among those who reported that their partner was suffering from genital discharge (i.e. ‘drop’) compared to those without such a history (45.6% vs. 23.9%, Odds Ratio 2.7, 95% CI – 1.2 – 6.1, p 0.02).

During clinical examination, the physical appearance of the cervix was recorded in 255 cases. It was found that 111 (43.5%) patients had a cervical discharge and 132 (51.8%) had cervical erosion. The presence of abnormal cervical discharge was significantly associated with gonococcal infection (46.8% vs. 22.2%, Odds Ratio 3.1, 95% CI – 1.8 – 5.3, p<0.0001). Similarly, 38.6% patients with cervical erosions were GC-positive compared to 26.8% among

patients with no cervical erosions (Odds Ratio 1.7, 95% CI – 1.0 – 2.9, p 0.045).

During bi-manual examination, the cervical excitation test (CET) was conducted in 247 patients. Of these, 42 patients (17.0%) had a positive CET which was significantly associated with gonococcal infection (50.0% vs. 30.2%, Odds Ratio – 2.3, 95% CI – 1.8 – 4.5, p 0.014).

Multivariate analysis showed that the presence of cervical mucopus was the only symptom independently associated with gonococcal cervicitis (Table 6.3).

Table 6.3 Multivariate analysis on potential predictors of cervical GC infection

Criteria	Adjusted OR	95% CI		P
Sexual partner with urethritis ('drop')	2.3	1.0	5.6	0.06
Cervical mucopus	3.7	1.5	9.3	0.006
Cervical ectopy/erosion	1.0	0.4	2.5	0.99
CET-positive	1.5	0.5	4.6	0.48

There was no association between the presence of specific vaginal infections or the number of mixed infections and the gonorrhoea status of the patients (Table 6.4).

Table 6.4 Association between vaginal and gonococcal cervicitis

Number of Vaginal Infections Detected	Gonococcal Infection		Total
	Positive	Negative	
No Vaginal Infection	13	38	51
	25.5%	74.5%	
One Vaginal Infection	44	92	136
	32.4%	67.6%	
Two Vaginal Infections	26	46	73
	36.1%	63.9%	
Three Vaginal Infections	3	12	15
	20.0%	80.0%	
Total	86	188	274
	31.4%	68.6%	

χ^2 = 2.5, p 0.47

The HIV seroprevalence among patients with gonococcal infections was higher (52.3%) than those without gonococcal infection (46.3%). However, this difference was not found to be statistically significant. (χ^2 = 0.87, p 0.35)

6.3.1.5.2. Chlamydial infection in women

Of 274 patients, 58 (21.2%) were positive for *C.trachomatis* (CT), and 33 (12.0%) had a single infection with CT. The mean age of CT-positive patients was 23.4 years (SD ± 3.8 years) and there was no significant difference when compared to that of CT-negative patients. (23.4 years vs. 24.9 years, p 0.08) The highest prevalence rate was found in the 20-24 age group (30.1%) and the prevalence among teenagers (i.e. 15-19 age group) was 17.9%.
No significant association was found between CT-positive status and other socioeconomic and demographic characteristics. Unlike gonococcal infection, there was no significant difference in CT prevalence between women who reportedly observed STI symptom(s) in their partners and those did not. The rate of chlamydial infection was similar among women who reported that their partner had a urethral discharge and those did not notice urethral discharge among their partners. (27.9% vs. 21.7%, χ^2 = 0.55, p 0.46)

Although the rate of CT infection was higher among patients with cervical discharge compared to those without cervical discharge, this difference was not statistically significant (27.9% vs.18.8%, χ^2 = 3.0, p 0.08). Similarly, neither cervical erosion (25.8% vs 19.5%, χ^2 = 1.4, p 0.24) nor a positive CET test (21.4% vs. 23.4%, χ^2 = 0.77, p 0.78) showed a significant association with chlamydial infection. HIV prevalence was virtually identical among those with and without CT infection (48.3% vs, 48.1%, p NS) (Table 6.5).

Table 6.5 Association between vaginal infection and chlamydial cervicitis

Number of Vaginal Infections Detected	Chlamydial Cervical Infection		Total
	Positive	Negative	
No Vaginal Infection	11	40	52
	21.6%	78.4%	
One Vaginal Infection	27	109	136
	19.9%	80.1%	
Two Vaginal Infections	17	55	72
	23.6%	76.4%	
Three Vaginal Infections	3	12	15
	20.0%	80.0%	
Total	58	216	274

$\chi^2 =$ 0.41, p 0.94 (NS)

6.3.1.6 HPV and HIV in women

6.3.1.6.1 Results of HPV PCR (MY 9/11)

Of 274 patients enrolled in the study, the overall prevalence of HPV detected by HPV PCR using a consensus primers (MY9/11) was 73%. No significant difference in the mean age of HPV PCR-positive when compared to PCR-negative women was found (24.4 years vs. 24.8 years, t-test p=0.68). No significant differences in socioeconomic, demographic and selected sexual behavioural determinants between HPV PCR-positive and negative patients were found (Table 6.6). However, patients who reported that their partners had current symptoms of STIs had significantly higher rate of HPV infection diagnosed by PCR (MY 9/11) (80% vs. 65%, Odds Ratio 2.2; 95% CI = 1.2 – 3.8, p 0.008).

A lower HPV prevalence was observed among HIV-seronegative patients in all age groups compared with their HIV-seropositive counterparts. The HPV prevalence rates were significantly higher among the HIV-seropositive women in the 20 – 24 and 25 – 29 groups than their HIV-seronegative counterparts (85.2% vs. 65.3%, Odds ratio 3.1; 95% CI 1.2 – 7.9 and 77.8% vs. 55.8%; 95% CI 2.8; 95% CI 1.03 – 7.5) respectively. However, no significant differences were found in the 15 – 19 and 30 years and above age groups (Figure 6.5).

Table 6.6 Association between demographic and behavioural risk factors and HPV (MY 9/11)

Variable	n (%)	HPV (%)	OR	95% CI	*P*
Demographic characterisitcs					
Age	n = 257				
15 - 19	39 (15.2)	71.8	1		
20 - 24	103 (40.1)	75.7	1.2	0.5 - 2.8	
25 - 29	79 (30.7)	65.8	0.8	0.3 - 1.7	
30 & Above	36 (14.0)	75.0	1.2	0.4 - 3.3	
Martial status	n = 256				
Married	28 (10.9)	67.9	1		
Never married	228 (89.1)	72.4	1.2	0.5 - 2.9	
Current sexual partner present	n = 256				
Yes	246 (96.1)	71.5	1		
No	10 (3.9)	80.0	1.6	0.3 - 7.7	
Duration of sexual partnership	n = 246				
?12 months	161 (65.4)	66.5	1		
<12 months	85 (34.6)	81.2	2.2	1.2 - 4.1	0.015
Highest education level	n = 254				
Secondary & above	176 (69.3)	72.2	1		
Primary	78 (30.7)	71.8	1.0	0.5 - 1.8	
Occupation	n = 256				
Student	42 (16.4)	71.4	1		
Unemployed/School leavers	53 (21.9)	75.0	1.2	0.5 - 3.0	
Domestic or similar jobs	65 (25.4)	76.9	1.3	0.6 - 3.2	
Other employments	93 (36.3)	66.7	0.8	0.4 - 1.8	
Main economic support	n = 159				
Family (Parents/Husband etc.)	93 (58.5)	73.1	1		
Boyfriend	66 (41.5)	74.2	1.1	0.5 - 2.2	
Sexual & Reproductive History					
Age at sex	n = 253				
15 & Above	232 (91.7)	71.6	1		
Under 15	21 (8.3)	71.4	1.0	0.4 - 2.7	
Current contraceptive use	n = 257				
None	135 (52.5)	73.3	1		
Non-hormonal devices	19 (7.4)	57.9	0.5	0.2 - 1.3	
Hormonal (pills/injectable)	103 (40.1)	72.8	1.0	0.5 - 1.7	
Number of life-time sexual partners	n = 251				
One	36 (14.3)	63.9	1		
More than one	215 (85.7)	73.0	1.5	0.7 - 3.2	
Number of sexual partners in past 6 months	n=255				
One	196 (76.9)	70.9	1		
More than one	59 (23.1)	74,6	1.2	0.6 - 2.3	
Condom use in past six-months	n = 254				
No	150 (59.1)	67.3	1		
Yes	104 (40.9)	77.9	1.7	0.96 - 3.0	
STI symptoms in current sexual partner	n = 255				
No	140 (54.9)	65.0	1		
Yes	115 (45.1)	80.0	2.2	1.21 - 3.8	0.01

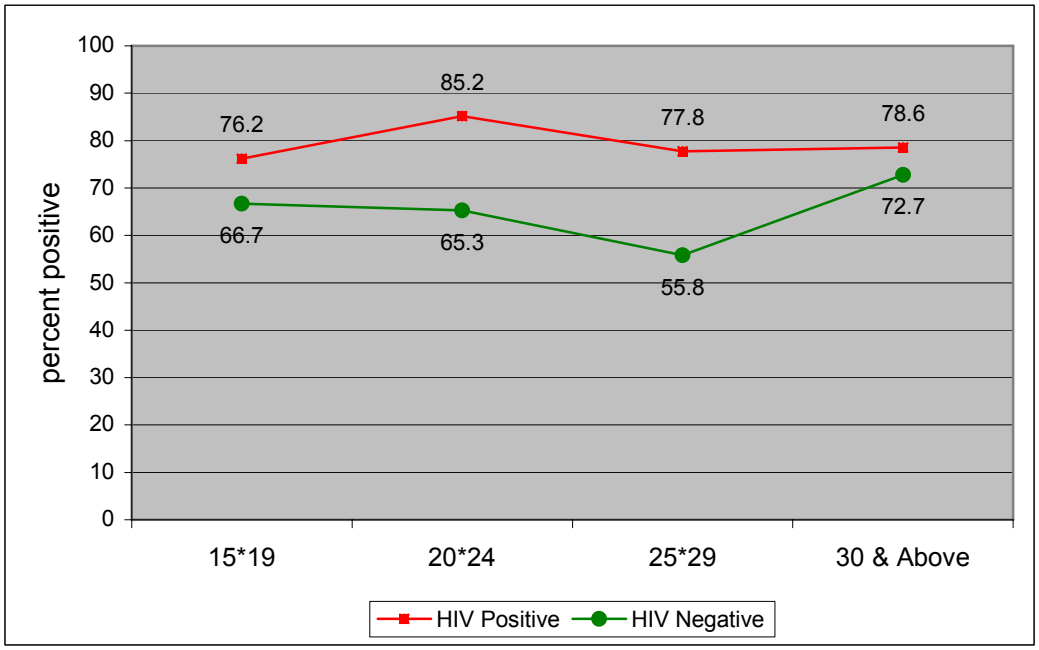


Figure 6.5 Age-specific prevalence of HPV by HIV status using PCR (MY9/11)

6.3.1.6.2 High & low-risk types detected by Hybrid Capture Tube (HCT) method

When using the hybrid capture technique to detect and type HPV infection, a total of 84 patients (30.7%) were positive for low- and/or high-risk HPV types. Of these, 17 patients (6.2%) were positive for both low- and high-risk HPV types. The overall prevalence of low-risk HPV types was 8.8% (95% CI; 5.6 – 12.8%) and that of high-risk HPV was 28.1% (95% CI; 22.9 – 33.8%) The highest prevalence of HPV (low- and high-risk types) was found in the 15 – 19 years age group (43.6%), followed by the 20-24 years age group (32.0%), the 25-29 years age group (24.1%) and the 30 years and above group (25.0%). The difference in age-specific prevalence was however not statistically significant (Figure 6.6).

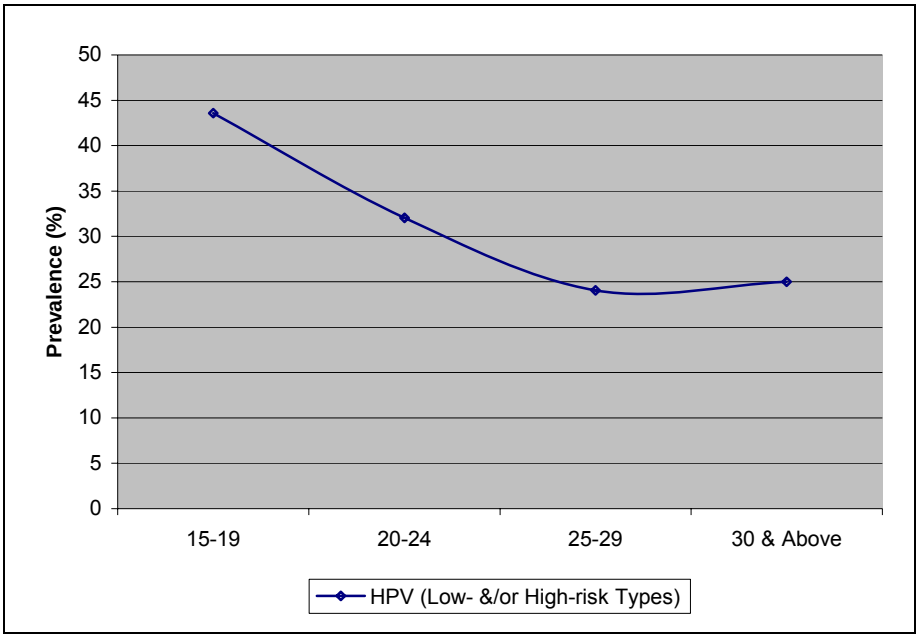


Figure 6.6 Age-specific prevalence rate of HPV (Low- &/or High-risk types)

Overall, the prevalence of low- and/or high-risk HPV was significantly higher among HIV-seropositive women than among HIV-seronegative women (40.2% vs 21.8%, Odds Ratio 2.4, 95% CI -1.4 – 4.1, p 0.001). In HIV-seropositive women, the highest prevalence of HPV infection was also found in the 15-19 age group (52.4%). The prevalence rates among older age groups were slightly lower, except for the 30 and above age group. In contrast, the age-specific HPV prevalence rate among HIV-seronegative women steeply declined in the older age groups. In these women, the highest prevalence (33.3%) was found in the 15-19 age group while the lowest prevalence (9.1%) was found in the 30 & above age group. However, these differences were not found to be statistically significant (Table 6.7 & Figure 6.7).

Table 6.7 Trend in Age-specific prevalence of low- and/or high-risk HPV infections in HIV-seropositive and HIV-seronegative patients

HIV Status	n	Prevalence of HPV (%) *(High- & Low-risk types)*	Odds Ratio	95% CI		P
HIV-negative	**132**					
15 - 19	18	33.3	1			
20 - 24	49	24.5	0.6	0.2	2.1	
25 - 29	43	16.3	0.4	0.1	1.4	NS
30 & Above	22	9.1	0.2	0.03	1.2	
HIV-positive						
15 - 19	21	52.4	1			
20 - 24	54	38.9	0.6	0.2	1.6	
25 - 29	36	33.3	0.5	0.2	1.4	NS
30 & Above	14	50.0	0.9	0.2	3.5	

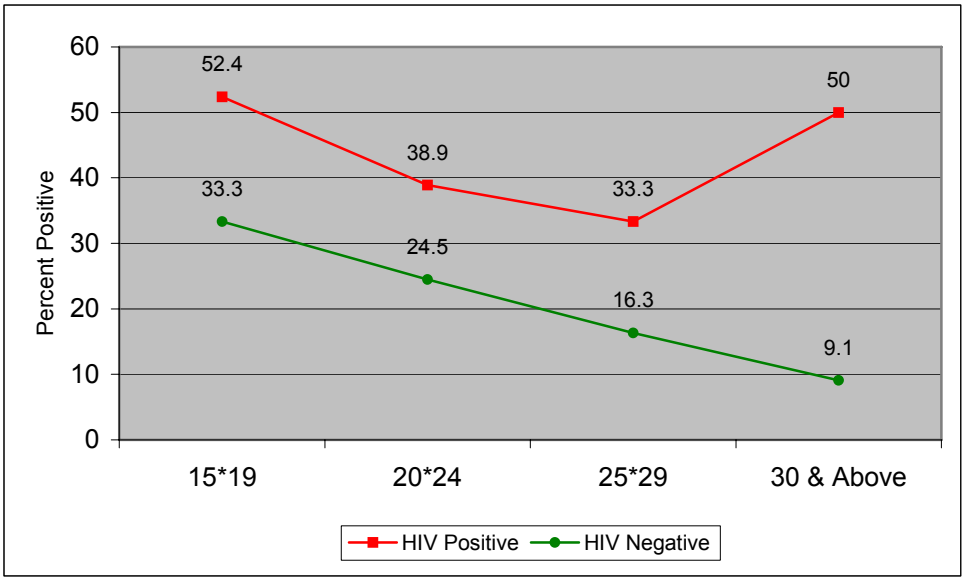


Figure 6.7 Trend in age-specific prevalence of low- and/or high-risk HPV infections in HIV-seropositive and HIV-seronegative patients

The demographic and socioeconomic risk factors for the low-risk HPV types are shown in Table 6. 8.

Table 6.8 Association between demographic and behavioural risk factors and HPV (low-risk types) infection

Variable	n (%)	HPV (%)	OR	95% CI	*P*
Demographic characterisitcs					
Age	n = 257				
15 - 19	39 (15.2)	17.9	1		
20 - 24	103 (40.1)	7.8	0.4	0.1 - 1.1	
25 - 29	79 (30.7)	7.6	0.4	0.1 - 1.2	
30 & Above	36 (14.0)	5.6	0.3	0.1 - 1.4	
Martial status	n = 256				
Married	28 (10.9)	14.3	1		
Never married	228 (89.1)	8.3	0.5	0.2 - 1.7	
Current sexual partner present	n = 256				
Yes	246 (96.1)	8.5	1		
No	10 (3.9)	20.0	2.7	0.5 - 13.4	
Duration of sexual partnership	n = 246				
?12 months	161 (65.4)	5.6	1		
<12 months	85 (34.6)	14.1	2.8	1.1 - 6.9	0.28
Highest education level	n = 254				
Secondary & above	176 (69.3)	10.8	1		
Primary	78 (30.7)	5.1	0.4	0.1 - 1.4	
Occupation	n = 256				
Student	42 (16.4)	11.9	1		
Unemployed/School leavers	53 (21.9)	12.5	1.1	0.3 - 3.6	
Domestic or similar jobs	65 (25.4)	6.2	0.5	0.1 - 1.9	
Other employments	93 (36.3)	7.5	0.6	0.2 - 2.0	
Main economic support	n = 159				
Family (Parents/Husband etc.)	93 (58.5)	10.8	1		
Boyfriend	66 (41.5)	9.1	0.8	0.3 - 2.4	
Sexual & Reproductive History					
Age at sex	n = 253				
15 & Above	232 (91.7)	8.6	1		
Under 15	21 (8.3)	9.5	1.1	0.2 - 5.1	
Current contraceptive use	n = 257				
None	135 (52.5)	9.6	1		
Non-hormonal devices	19 (7.4)	15.8	1.8	0.5 - 6.9	
Hormonal (pills/injectable)	103 (40.1)	6.8	0.7	0.3 - 1.8	
Number of life-time sexual partners	n = 251				
One	36 (14.3)	11.1	1		
More than one	215 (85.7)	8.8	0.8	0.2 - 2.4	
Number of sexual partners in past 6 months	n=255				
One	196 (76.9)	9.7	1		
More than one	59 (23.1)	6.8	0.7	0.2 - 2.1	
Condom use in past six-months	n = 254				
No	150 (59.1)	10.0	1		
Yes	104 (40.9)	7.7	0.8	0.3 - 1.8	
STI symptoms in current sexual partner	n = 255				
No	140 (54.9)	6.4	1		
Yes	115 (45.1)	12.2	2.0	0.8 - 4.8	

Although the prevalence of low-risk HPV infection was significantly lower than that of high-risk HPV infection, there was a stronger association between the low-risk HPV and HIV infection compared to the high-risk HPV-HIV association. The prevalence of low-risk HPV infection in HIV-seropositive patients was 15.2% compared to only 2.8% in HIV-seronegative patients (Odds Ratio 6.2; 95% CI – 2.1 – 18.5, p 0.001). The highest prevalence was found in the

younger age groups in both HIV-seropositive and HIV-seronegative groups (Table 6.9 and Figure 6.8).

Table 6.9 Trends in age-specific prevalence of low-risk HPV infections in HIV-seropositive and HIV-seronegative patients

HIV Status	n	Prevalence of HPV (%) *(Low-risk types)*	Odds Ratio	95% CI		P
HIV-negative	**132**					
15 - 19	18	5.6	1			
20 - 24	49	2.0	0.4	0.02	6.0	
25 - 29	43	2.3	0.4	0.02	6.8	NS
30 & Above	22	0.0	-			
HIV-positive	**125**					
15 - 19	21	28.6	1			
20 - 24	54	13.0	0.4	0.11	1.3	
25 - 29	36	13.9	0.4	0.11	1.5	NS
30 & Above	14	14.3	0.4	0.07	2.4	

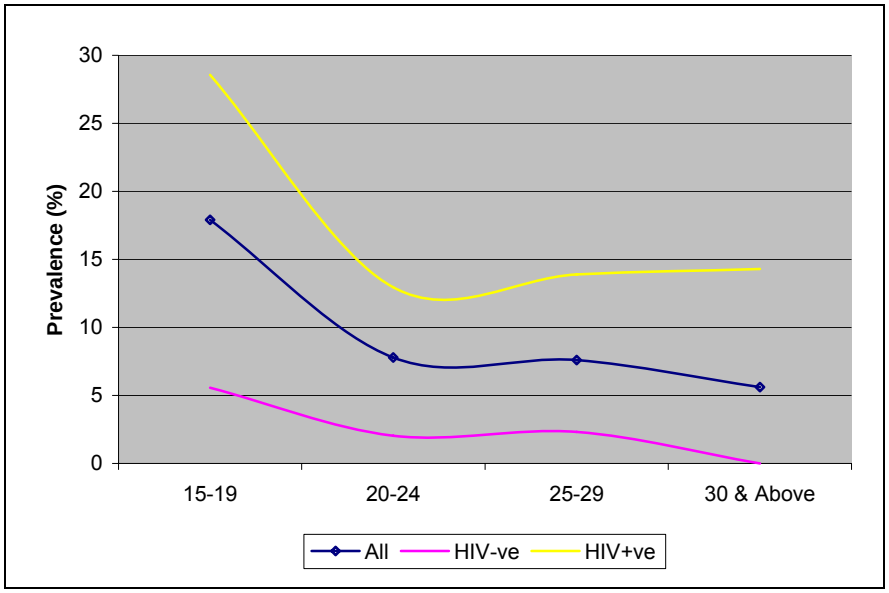


Figure 6.8 Age-specific prevalence rates of HPV (low-risk types) by HIV sero-status

For high-risk HPV infections, the prevalence in HIV-seropositive patients was 36.4% compared to 20.4% in HIV-seronegative patients (Odds Ratio 2.2, 95% CI – 1.3 - 3.8, p 0.003). Similar age-specific prevalence trends were found among patients with high-risk HPV types and low-risk HPV types (Table 6.10 and Figure 6.9).

Table 6.10 Trends in age-specific prevalence of high-risk HPV infections in HIV-seropositive and HIV-seronegative patients

HIV Status	n	Prevalence of HPV (%) *(High-risk types)*	Odds Ratio	95% CI		P
HIV-negative	**132**					
15 - 19	18	27.8	1			
20 - 24	49	22.4	0.8	0.22	2.6	
25 - 29	43	16.3	0.5	0.14	1.9	NS
30 & Above	22	9.1	0.3	0.04	1.5	
HIV-positive	**125**					
15 - 19	21	42.9	1			
20 - 24	54	35.2	0.7	0.26	2.0	
25 - 29	36	30.6	0.6	0.19	1.8	NS
30 & Above	14	50.0	1.3	0.34	5.2	

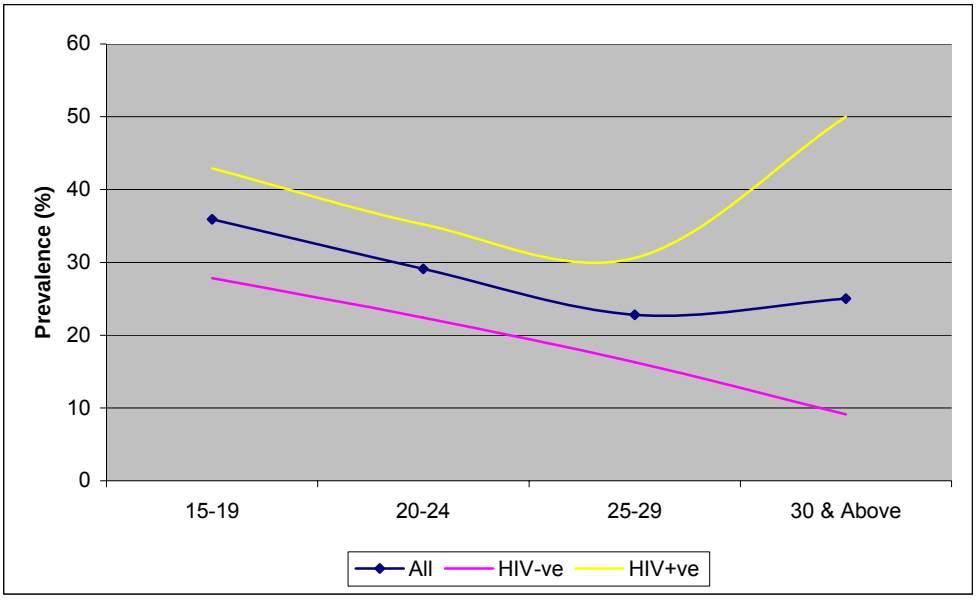


Figure 6.9 Age-specific prevalence rate of HPV (high-risk types) by HIV sero-status

The demographic and socioeconomic risk factors for high-risk HPV types are shown in Table 6.11.

Table 6.11 Association between demographic and behavioural risk factors and HPV (high-risk types)

Variable	n (%)	HPV (%)	OR	95% CI	*P*
Demographic characterisitcs					
Age	n = 257				
15 - 19	39 (15.2)	35.9	1		
20 - 24	103 (40.1)	29.1	0.7	0.3 - 1.6	
25 - 29	79 (30.7)	22.8	0.5	0.2 - 1.2	
30 & Above	36 (14.0)	25.0	0.6	0.2 - 1.6	
Martial status	n = 256				
Married	28 (10.9)	25.0	1		
Never married	228 (89.1)	28.1	1.2	0.5 - 2.9	
Current sexual partner present	n = 256				
Yes	246 (96.1)	27.2	1		
No	10 (3.9)	40.0	1.8	0.5 - 6.5	
Duration of sexual partnership	n = 246				
?12 months	161 (65.4)	24.8	1		
<12 months	85 (34.6)	31.8	1.4	0.8 - 2.5	
Highest education level	n = 254				
Secondary & above	176 (69.3)	27.8	1		
Primary	78 (30.7)	26.9	1.0	0.5 - 1.7	
Occupation	n = 256				
Student	42 (16.4)	33.3	1		
Unemployed/School leavers	53 (21.9)	25.0	0.7	0.3 - 1.6	
Domestic or similar jobs	65 (25.4)	35.4	1.1	0.5 - 2.5	
Other employments	93 (36.3)	21.5	0.5	0.2 - 1.2	
Main economic support	n = 159				
Family (Parents/Husband etc.)	93 (58.5)	31.2	1		
Boyfriend	66 (41.5)	21.2	0.6	0.3 - 1.2	
Sexual & Reproductive History					
Age at sex	n = 253				
15 & Above	232 (91.7)	26.3	1		
Under 15	21 (8.3)	33.3	0.7	0.3 - 1.9	
Current contraceptive use	n = 257				
None	135 (52.5)	28.9	1		
Non-hormonal devices	19 (7.4)	26.3	0.9	0.3 - 2.6	
Hormonal (pills/injectable)	103 (40.1)	26.2	0.9	0.5 - 1.6	
Number of life-time sexual partners	n = 251				
One	36 (14.3)	16.7	1		
More than one	215 (85.7)	28.8	2.0	0.8 - 5.1	
Number of sexual partners in past 6 months	n=255				
One	196 (76.9)	27.6	1		
More than one	59 (23.1)	27.1	1.0	0.5 - 1.9	
Condom use in past six-months	n = 254				
No	150 (59.1)	28.7	1		
Yes	104 (40.9)	26.0	1.1	0.7 - 2.0	
STI symptoms in current sexual partner	n = 255				
No	140 (54.9)	25.0	1		
Yes	115 (45.1)	30.4	1.3	0.8 - 2.3	

6.3.1.6.3 Semi-quantitative measurements of low- & high-risk HPV by HCT assay

Semi-quantitative measurements of HPV viral burden in cervical specimens were assessed using the Relative Light Unit (RLU)/Positive Controls (PCs) ratio (i.e. amount of light emission expressed as relative light units equalling or exceeding the mean of positive controls consisting of 10 pg/ml HPV DNA).

In 26 patients with positive HC-probe A (low-risk types), the median RLU/PCs ratio was 5.13 (range 1.1 – 154.3). The median RLU/PCs ratio was lower in HIV-seronegative patients compared to HIV-seropositive patients (5.13 vs. 1.68, p NS). The viral burden (RLU/PC ratio) decreased with age with a more precipitous decline observed among HIV-seronegative patients (Figure 6.10).

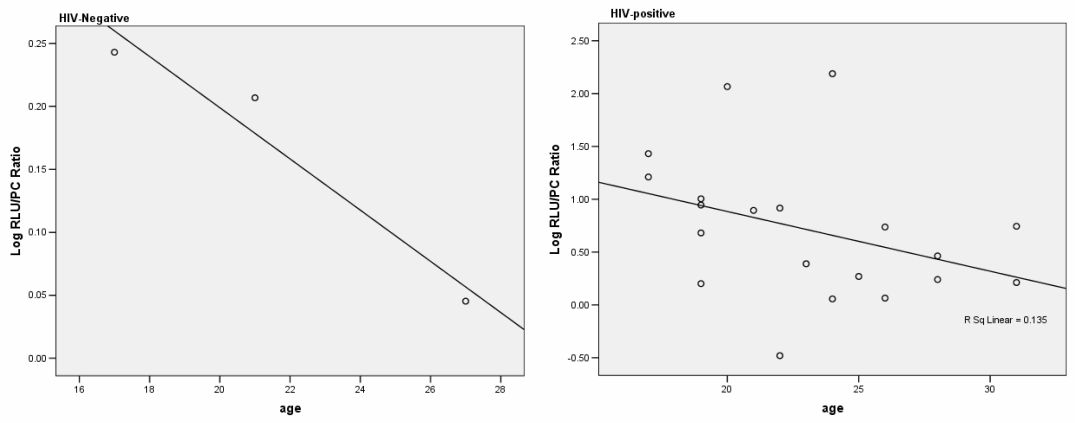


Figure 6.10 Scatter diagram of age and log RLU/PCs ratio for HPV low-risk types in HIV-seronegative and HIV-seropositive patients

In 80 patients who were positive for HC-probe B (high-risk types), the median RLU/PCs ratio was 5.63 (range 1.02 – 166.1). Forty-eight patients (60%) were co-infected with HIV. The median RLU/PCs ratio was significantly higher in HIV-seropositive patients than that of HIV-seronegative patients (8.96 vs. 3.03, Mann-Whitney U, p 0.018). The viral burden as measured by the log RLU/PCs ratio tended to remain high in older HIV-seropositive women while a steady decline with age was observed among HIV-seronegative patients (Figure 6.11).

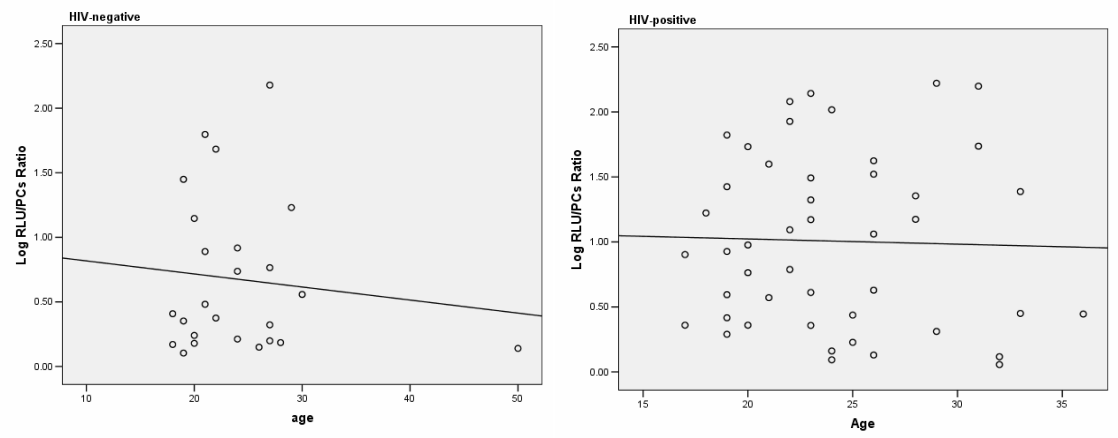


Figure 6.11 Scatter diagram of age and log RLU/PCs ratio for HPV high-risk types in HIV-seronegative and HIV-seropositive patients

6.3.1.6.4 Pap smear results

Of 185 patients on whom Pap smear findings were available, 168 (90.8%) were found to have normal results. Low grade squamous intraepithelial lesions (LSILs) were found in 13 patients (7.0%) and high grade SIL (HSIL) in 4 (2.1%). The prevalence of abnormal Pap smears (i.e. LSIL or HSIL) was significantly higher among HIV-seropositive patients than HIV-seronegative patients (16.7% vs. 3.0%, Odds Ratio 6.5, 95% CI – 1.8 – 23.6, p 0.001). All patients with LSIL were under the age of 30 years while all patients with HSIL were in the 30 and above age group.

A significantly higher proportion of women with low- and/or high-risk HPV infections compared with uninfected patients had an abnormal Pap smear (19.0% vs. 4.7%, Odds Ratio 4.7, 95% CI 1.7 – 13.5, p 0.002). This association was found to be highest in patients with mixed low- and high-risk HPV infection (Odds Ratio 10.1, p 0.002) (Table 6.12). A normal Pap smear was however detected in 44 of 56 (78.6%) women with high-risk HPV.

Table 6.12 Association between HPV infection and abnormal Pap smears

HPV Infection (Low- &/or High-risk)	n	Normal Pap Smear (%)	Abnormal Pap Smear (%)	Odds Ratio	95% CI		P
None	127	121 (95.3)	6 (4.7)	1			
Single (Low or High-risk types)	46	39 (84.8)	7 (15.2)	3.6	1.1	11.4	0.03
Mixed (Low & High-risk types)	12	8 (66.7)	4 (33.3)	10.1	2.4	43.1	0.002

Overall, the presence of abnormal Pap smear was associated with HPV infection and HIV-seropositivity. In multivariate analysis, a significant association was found between abnormal PAP smear status and positive status for high-risk HPV and HIV infection (Table 6.13).

Table 6.13 Multivariate analysis of risk-determinants in patients with abnormal PAP smears

Risk Determinants for Abnormal Pap Smears	Adjusted Odds ratio	95% CI		Sig.
		Lower	Upper	
Low-risk HPV (Pos)	1.3	0.32	5.25	0.71
High-risk HPV (Pos)	3.8	1.23	11.94	0.02
HIV Positive	4.7	1.24	18.01	0.02
Age (30 & Above)	1.4	0.33	5.67	0.66

6.3.1.7 Genital herpes in women with vaginal discharge

Of 274 women presenting with vaginal discharge, 27 (10.5%) were found to have concomitant genital ulcerations on clinical examination. Nine patients (33.3%) were clinically diagnosed as chancroid, 16 patients (59.3%) with genital herpes and two patients (7.4%) with primary syphilis. Of 27 patients with clinical ulcerations, clinical specimens for *H. ducreyi* (HD) culture and HSV-PCR were collected from 19 patients. Seven patients (36.8%) were positive for HD culture while 8 (42.1%) were positive on HSV PCR testing. No aetiological agent was found in four cases (21%). No mixed infection involving HD and HSV was encountered.

Swabs for HSV-PCR were collected from the ectocervix in 274 cases. Of these, 18 (6.6%) were positive on HSV-PCR and five patients had HSV-positive external genital ulcerations. Ten of these patients (55.6%) were also positive for HSV-2 type specific antibody, suggesting recurrent herpes infection. A significantly higher proportion of patients with cervical HSV infection was associated with cervical erosion compared to those without cervical HSV infection (77.8% vs. 49.6%, Odds Ratio 3.6, 95% CI 1.1 – 11.1, p 0.02). The rate of HIV infection detected among patients with cervical HSV infection was significantly higher than that detected among those without HSV (72.2% vs. 46.0%, Odds Ratio 3.1; 95% CI 1.1 – 8.8, p 0.03).

A subset of patients was tested to detect HSV-2 type specific antibody. Of 198 patients with available results, 105 (53%) were HSV-2 antibody positive. No difference in rates of HSV-2 sero-positivity was detected among HIV-seropositive when compared to HIV-seronegative patients (53.5% vs. 52.5%, p 0.9).

6.3.1.8 Syphilis serology

Of 283 patients, 40 (14.1%) were found to be RPR-positive confirmed by a positive FTA-ABS test. Of these, 19 patients had RPR titres ≥ 1:8. In addition, a further 53 patients (18.7%) were found to be RPR-negative but positive by the FTA-ABS. No significant difference in rates of HIV infection between RPR-seropositive and RPR-seronegative patients was detected (45% vs. 48.7%, p 0.6).

6.4 Discussion

In southern Africa, reproductive tract infections (RTIs) and their complications contribute significantly towards morbidity and mortality among women. As documented extensively in the literature, the sexual behaviour of women explains only partially the high burden of RTIs/STIs in southern Africa. More complex gender-specific issues are involved such as gender-based socio-economic inequality in the society and gender differences in HIV transmission efficiency including increased susceptibility of common infection as a result of physiological/hormonal influences, effectiveness of early detection of STIs, health care-seeking behaviours and outcome of partner management[20].

In South Africa, the availability of epidemiological data and reliable information on RTI/STI disease burden among women during the early phase of the HIV epidemic was limited. Some investigators however, had previously discussed the complexity of the diagnosis and management of RTI/STIs in women in southern Africa and explored the application of syndromic management as well as the role of rapid methods for diagnosis to increase the specificity of management of STIs in the 1980s[21]. With the development of the HIV epidemic in southern Africa, more STI aetiological studies were conducted to improve the diagnosis and management of STI syndromes as recommended by WHO[22]. Schneider et al. reported a high prevalence of RTIs/STIs among women attending a family planning clinic in a rural area of South Africa in 1994. She and her co-workers found prevalence of *C. trachomatis* of 12%, of *N. gonorrhoeae* 3% and of *T. vaginalis* and bacterial vaginosis 18% and 29% respectively[23]. In contrast, the present studies were conducted in a major urban area among symptomatic women and therefore higher prevalence rates of RTI/STI were observed with CT (20.1%), GC (31.8%), TV (28.6%) and BV (47.3%). Similarly high prevalence rates were found among symptomatic women attending the STI clinics in other major urban areas such as Cape Town and Durban (Figure 6.12)[24].

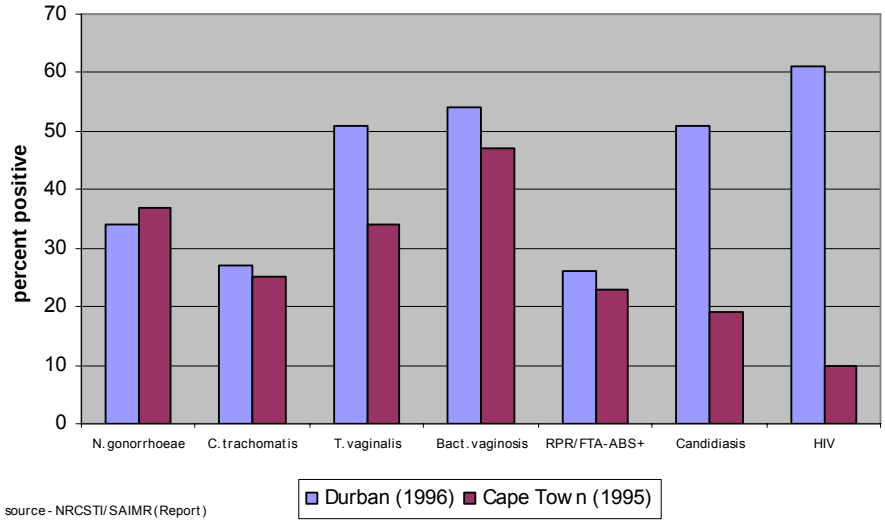


Figure 6.12 Prevalence of vaginal and cervical infections in major cities of South Africa

Unfortunately, no comprehensive aetiological data were available for women attending STI clinics in Johannesburg during the early 1990s. Anecdotal evidence however, indicated that the prevalence of STIs in the study population was high during that time and could have

served as an early indication of imminent spread of HIV in mid-1990s. An aetiological study conducted in Cape Town in 1995 showed that there was a high prevalence of GC, CT and BV prevalence among women presenting with vaginal discharge, despite having a lower HIV prevalence rate compared to other major urban centres in South Africa (e.g. 10% in Cape Town vs. 48.2% in Johannesburg). Sentinel surveillance data however, showed that HIV prevalence among pregnant women in Cape Town has been increasing steadily in recent years, from 1.66% in 1995 to 12.4% in 2002[25].

Despite the very high prevalence of HIV (48.2%) in our study population in Johannesburg, the HIV prevalence in a lower risk population group such as pregnant women in Gauteng Province was 18.7% in 1996. Meanwhile, epidemiological evidence indicated that an accelerated spread of HIV among high-risk population groups in Johannesburg in the early-1990s was imminent. Such early indications were provided by the findings of the HIV/RPR sentinel surveillance system implemented at the Esselen Street STI clinic and the Urban Health Centre located at inner-city of Johannesburg (Figure 6.13). Unfortunately, HIV infection spread rapidly into the general population and reached 31.6% in the sentinel pregnant women population by 2002.It is clear therefore, that epidemiological trends of HIV and STIs provide early warning indications of potential accelerated spread of HIV in both high-risk and low-risk populations in South Africa.

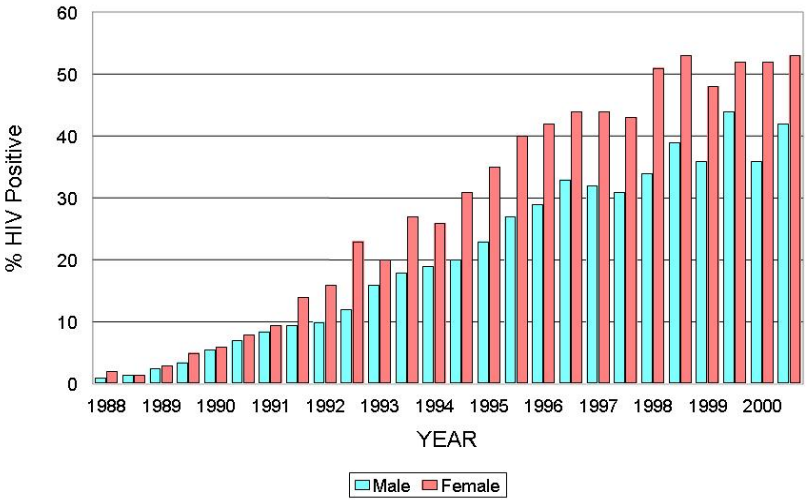


Figure 6.13 HIV/RPR sentinel surveillance among STI patients in Johannesburg (Esselen Street and Urban Health Centre Clinics)

Our study has shown that over 80% of women in Johannesburg presenting with STI symptoms had at least one or more laboratory-confirmed vaginal infection, namely, bacterial vaginosis, candidiasis and/or trichomoniasis. In addition, almost half (43.4%) of the patients were infected with sexually-acquired gonococcal and/or chlamydial infections and up to 17% were confirmed to have some degree of ascending infection or pelvic inflammatory disease (PID), as diagnosed by the CET test. Of great concern are the high levels of infection due to viral agents such as HPV, particularly those sub-types associated with cancer of cervix, especially among women who are co-infected with HIV. Based on HSV-2 type-specific serology, more than half of patients (53%) were found to have been infected with HSV-2, and 6.6% of all patients were shedding HSV-2 from their cervical lesions at the time of consultation. Based on these findings, the STI burden in women of reproductive age in Johannesburg in 1996 was very high.

Many STI/HIV experts have elaborated on the ongoing changes in human ecology and behaviour and their major impact on infectious diseases, including STIs[26]. Holmes has emphasized that any changes in the burden STIs are likely to be linked to a continuum of several determinants. These could be identified as (1) the most remote and least modifiable historical stages of economic development; (2) more proximate factors such as the changing patterns of the environment, demographic transition, migration, or war etc. and (3) the most recent or modifiable events such as new technology (e.g. contraceptives), condom availability, illicit drug use and effective STI treatment services.

In our study areas in Johannesburg, a mixed pattern of recent and historical determinants conducive to rapid spread of STIs, as outlined above, was present. These determinants include chronic poverty, political instability, the existence of large socially/economically marginalised population groups, high- levels of internal and cross-border migration, crime, prostitution, alcohol/drug abuse, gender inequality and lack of early and effective behavioural change interventions. A demographic and health survey conducted by the South African Department of Health (1998) revealed that most urban women (91%) who participated in the survey responded that they could avoid AIDS by using condoms and 89.9% that they could

be protected by staying faithful to their respective partners[27]. In stark contrast, only 12.2% of 3,718 African urban women respondents indicated that they used a condom when they last had sex with a casual acquaintance. Among Gauteng residents attending STI clinics in Johannesburg, 9.6% of 2311 women who ever had sex, responded that they had ever been forced or persuaded to have sex against their will. Our study also found that 48% of patients had never used condoms before. Unfortunately, our study findings on their own could not quantify the social and behavioural risk factors associated with the high STI prevalence present in the study population. Triangulation of information obtained from various studies and our findings however, reveal that complex socio-economic and behavioural factors exist, which led to the rapid spread of STIs and HIV in the area.

Co-factor effects of individual STIs on the transmission efficiency of HIV (i.e. increase in infectiousness of or susceptibility to HIV infection) are complex, particularly when multiple potential co-factors are present in an individual. In our study population, multiple vaginal and cervical STI agents were frequently detected in the same patient. Moreover, there were many other potential physiological (e.g. menstrual cycle, hormonal etc.)[28-29] immunological (e.g. viral load, immune deficiency, partial immunity etc.)[30-31] and cultural determinants (e.g. use of vaginal drying agents, circumcision etc.) which may have influenced the infectiousness of, or susceptibility to, HIV[32-35].

Owing to the cross-sectional nature of our studies, we could only detect associations between various STI agents and HIV but it was not possible to determine increases in susceptibility to or the frequency of transmission of HIV associated with individual infections. Of the three common vaginal infections, vaginal candidiasis had the highest prevalence among HIV-seropositive women. Bacterial vaginosis was overall the most common vaginal infection with a prevalence of 48.5%. Although bacterial vaginosis and candidiasis are not traditionally considered STDs[36], both have been associated with increased acquisition of HIV. Studies suggest that a vaginal milieu with a high pH and a lack of H_2O_2-producing lactobacilli found in bacterial vaginosis, enhances susceptibility to HIV by a factor of 1.4 – 2 [37-39]. It has also been

found that the presence of bacterial vaginosis is associated with an increased prevalence of other STDs[40].

In our studies, a significant association between vaginal candidiasis and HIV infection was detected. A significantly higher proportion of HIV-seropositive patients than sero-negative patients had vaginal candidiasis with an odds ratio of 2.0 (95% CI – 1.24 – 3.29). No significant association was found between HIV and bacterial vaginosis or HIV infection and trichomoniais respectively. The presence of one or more vaginal infections in women was a good predictor of HIV-positivity in our study population. As the majority of women had either one or more vaginal infections, we could not find a significant association with HIV for specific vaginal infections in the study.

In addition to the high prevalence of vaginal infections, almost half of our patients with a vaginal discharge had cervical lesions such as cervical ectopy or erosion/s of the cervix. Over 15% of women had clinical signs indicative of early PID. These cervix-related clinical signs were significantly associated with gonococcal infection in our study population. We did not however, find a significant association between gonococcal infection and HIV infection. The prevalence of chlamydial infection was slightly lower than infection caused by gonococci while a similar HIV prevalence was found between CT-positive and CT-negative patients. Despite the lack of an association between gonococcal infection and/or chlamydial cervicitis and HIV, the extremely high prevalence of GC &/or CT cervical infections (43.4%) in women is a major concern. Studies have reported that women co-infected with HIV and cervical infections have a higher risk in developing PID and, when it develops, it is more severe in HIV-seropositive women[41 – 45].

Although longitudinal studies provide conflicting findings on the effect of STI co-infection on HIV infectiousness[46-47], it is biologically plausible that the infectiousness of HIV could be higher as a result of STI-coinfection. For example, GC, CT and BV infections have been shown to be associated with the presence of cervical interleukin-10 which reportedly enhances HIV replication in macrophages[48]. Studies also have reported that the presence of

cervical GC and/or CT significantly increased the number of $CD4^+$ cells in endocervical specimens[49] and the detection of HIV in cervical secretion has been associated with the presence of cervical mucopus or inflammation[50-51].

In South Africa, an extremely high proportion of women with RTIs/STIs were co-infected with HIV. Unfortunately, in our study population, the interaction between specific RTIs/STIs and HIV has been compromised by the high rate of mixed infections detected. In addition, the cross-sectional design of the study negated differentiation between "cause and effect" of the interaction. For example, the association between vaginal candidiasis and HIV infection could be due to either increased susceptibility to HIV among women with candida vaginitis or an increase in the incidence of candida infection known to be associated with HIV induced immunodeficiency.

The high prevalence of STIs in both HIV-seropositive and negative women in the mid-1990s could serve as a surrogate indicator of the existence of accelerated HIV transmission among women. This phase of the dual epidemic was soon followed by a rapid increase in HIV prevalence among pregnant women in South Africa (Figure 6.14). UNAIDS estimated that some 2.9 million (range 2.5 – 3.3 million) sexually- active women aged 15 – 49 years were living with HIV/AIDS in South Africa by the end of 2003[52].

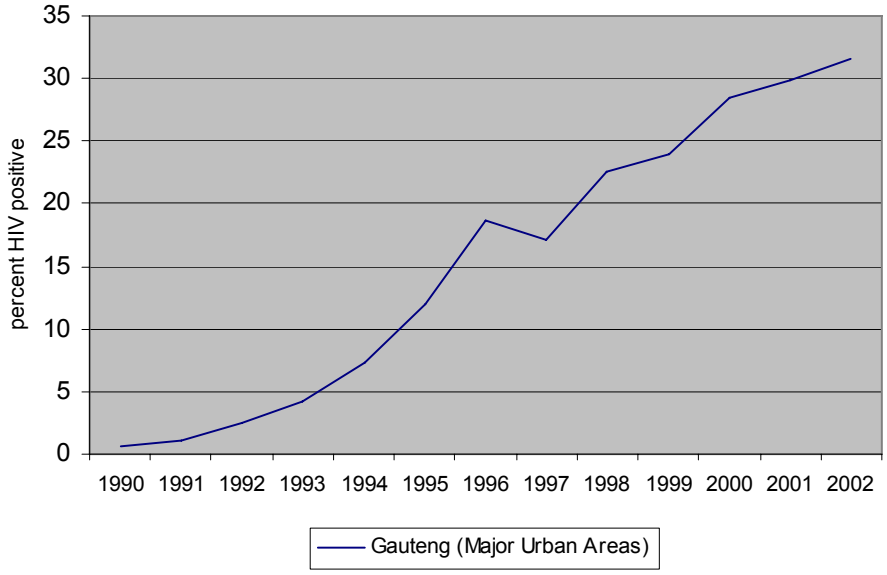


Figure 6.14 HIV prevalence among pregnant women in major urban areas of Gauteng Province (1990 – 2002) – source: UNAIDS

In the early 2000s, the HIV epidemic in South Africa reached the stage where it was estimated to become the leading cause of premature death in women, contributing 46.3% to the total burden of premature death[53] (Figure 6.15).

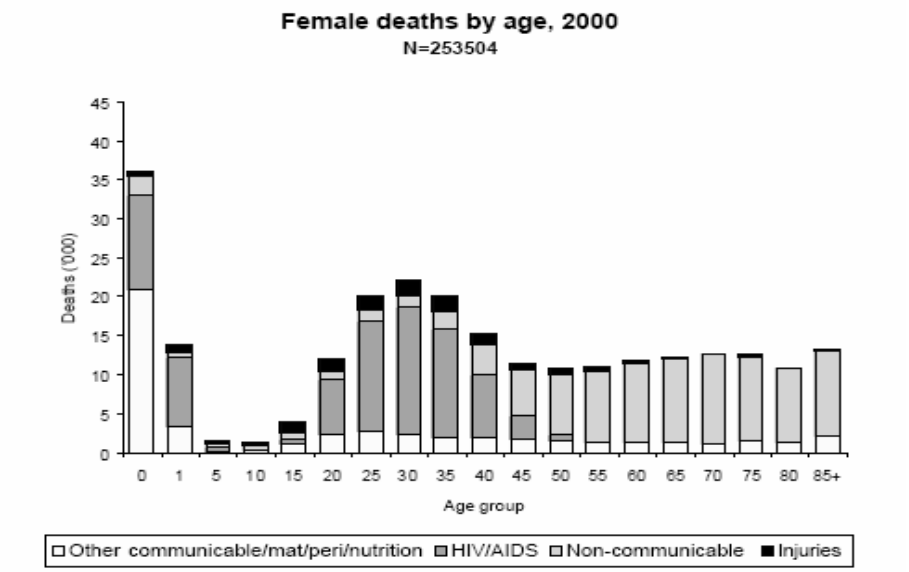


Figure 6.15 Estimates of premature mortality in women. South Africa
(Source: Bradshaw *et al.,* Initial burden of disease estimates in South Africa, 2000)[53]

In the present studies, no significant association between HIV and bacterial causes of vaginal and cervical infections could be detected. However, a significant association was found between viral infections of the cervix, namely cervical HPV and HSV infections, and HIV. Overall, HPV infection was extremely common in our study population with over 70% of patients found to be positive by HPV PCR (MY 9/11). Human papillomaviruses are members of the papovaviridae family of DNA viruses and cause benign and malignant epithelial proliferations[54]. More than 100 different types of HPV have been described, including more than 30 anogenital types, of which approximately 15 are oncogenic[55-56]. The most serious complication of genital HPV is invasive cervical cancer which is the second most common cancer among women worldwide[57]. In South Africa it is the most common cause of cancer among females of African and mixed race origin[58] (Figure 6.16).

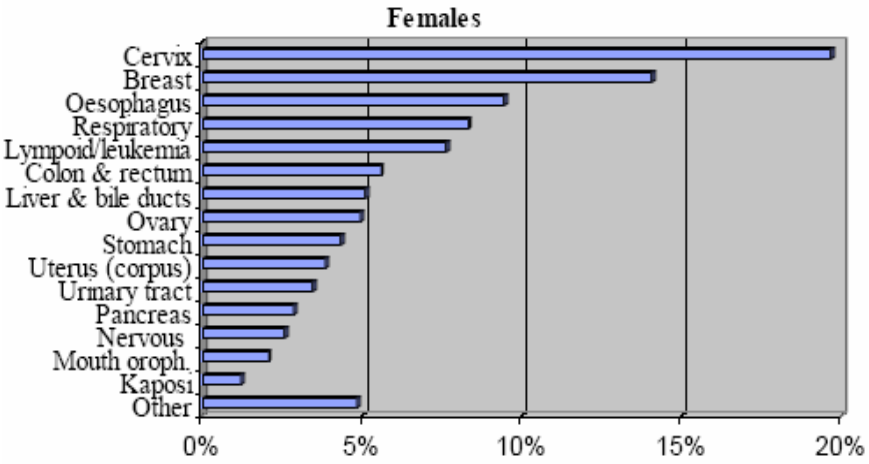


Figure 6.16 Proportion of causes of female cancers in South Africa
(Source: National Cancer Registry, South Africa, 1995)

In 1992, the National Cancer Registry of South Africa reported 25,413 female cancer cases and cervical cancer was cited as the most common cancer among women with 4467 new cases. Of these, 3,390 (75.8%) affected black women and the incidence ranked highest in the world[59]. During 1993 – 1995, the age-standardized incidence rates for cancer of cervix among females of African origin was 26.47 per 100,000 population. Cervical cancer was among the top 20 specific causes of the premature mortality burden (years of life lost, abbreviated as YLL) among women in South Africa in 2000 when it contributed approximately 15% to premature mortality[53]. In response, the Department of Health developed national guidelines for the screening of cervical cancer, offering three free PAP smears every ten years to women over 30 years of age[60].

The evidence for a causative relationship between HPV and cancer of cervix is compelling[61]. Multi-centre studies coordinated by the International Agency for Research on Cancer (IARC) and conducted in 22 countries reported that HPV DNA was identified in almost all (99.7%) cancer cases.[62] The HPV types which are generally characterized as “high-risk” types (16, 18, 18, 31, 33, 35, 45, 51, 52 and 56**)** are associated with low- and high-grade squamous intraepithelial lesions (LSIL and HSIL) and invasive cancer, while “low-risk” types (6, 11, 42, 43 and 44) are associated with genital warts, LSIL, and recurrent respiratory papillomatosis[63-66]. In our study, the probes A and probe B of the Hybrid Capture Tube (HCT) assay system were used to detect low-risk HPV types and high-risk HPV types respectively. The

prevalence of high-risk HPV types was higher than that of low-risk HPV types in our population (28.3% vs. 9.2%). Mixed infections of low- and high-risk types were also common with 6.4% of 283 patients being positive for both risk types. During the mid-1990s and late-1990s, the prevalence of high-risk HPV types was high in South Africa. Wright et al reported that the high-risk HPV types was detected in 21.1% of 1415 women who were participating in routine a cervical cancer screening programme in Cape Town[67]. However, a more sensitive (i.e. detection threshold of 1 pg/ml HPV DNA or 5000 HPV genome copies per test) second generation hybrid capture assay (HC-II) was used in their study. The assay we used was an older HCT assay with a detection threshold of 10 pg/ml which did not include RNA probes for HPVs 39, 58, 59, and 68 in the probe-B cocktail. The prevalence rates found in the two studies are not comparable owing to differences in the populations studied and the location.

Similar to HPV-PCR (MY9/11) results, a significant association between the prevalence of low- and high-risk HPV types and HIV infection was detected in our study population. Among HIV-seropositive patients, the prevalence of high-risk HPV types was as high as 36.4%. Overall, the prevalence of low- and/or high-risk HPV types was significantly higher in HIV-seropositive women. A stronger association was however seen in patients with low-risk HPV types (OR 6.2, 95% CI – 2.1 – 18.5) than in those with high-risk HPV types (OR 2.2., 95% CI – 1.3 – 3.8). Generally, the HPV prevalence was lower in older women with a peak rate detected in women under 25 years of age and this trend could be due to the natural clearance of virus over time, mediated by an effective immunological response as described previously[68-72].

In our study, prospective serial measurements of HPV positivity could not be performed as the study population was highly mobile and adhered extremely poorly to their post-treatment follow-up visits. We observed that HIV prevalence was significantly lower in the older women compared to those in the 15- 19 age group. However, no significant decline in the prevalence of both low- and high-risk HPV types was detected in older women with HIV co-infection, while a significant decrease was found in those who were HIV-seronegative. A similar trend was seen when semi-quantitative measurement of HPV viral burden was compared between

different age groups, using log RLU/PC ratios as proxy markers of viral burden. We also found that the median RLU/PCs ratio for high-risk HPV types was significantly higher in HIV-seropositive compared to HIV-seronegative patients.

Unlike other prospective studies, our study design did not allow for the assessment of the effect of possible persistent HPV infection in HIV-seropositive women enrolled in the study. However, Branca et al. reported that HIV-seropositive patients have a higher rate of new HPV infections during follow-up periods (Odds Ratio 8.8, 95% CI – 1.19 – 64.61) while the clearance of existing HPV infection was markedly poorer than in HIV-seropositive women when compared with their HIV-seronegative counterparts (Odds Ratio 0.33; 95% CI 0.16 – 0.67)[73]. The rate of progression of HPV infection was also higher in HIV-seropositive women[73-75].

A body of evidence suggests that infection with high-risk types of HPV appears to be "necessary" for the development of cancer of cervix (i.e. not "sufficient)". A significant proportion of HPV-infected women do not progress to cancer and other risk-determinants such as prolonged hormonal use, smoking, other genital tract infections, and, more importantly, immunodeficiency due to HIV infection may play a role[76-77]. Epidemiological information obtained from many developed countries suggests that the incidence of cervical cancer is much higher among HIV-seropositive women and it is included as one of AIDS-defining illnesses in the CDC 1993 AIDS case definition[78]. Studies also reported that women with persistent high-risk type HPV infection are at greater risk of developing cervical intra-epithelial neoplasia (CIN) which also tends to persist[79-81]. Based on this evidence, it is biologically plausible that HIV-infected women are more likely to have persistent high-risk types HPV infection and, as a result, a greater chance of progression to invasive cancer than their HIV-seronegative counterparts who have a better capacity for clearance of HPV. In the population studied here, the high prevalence and persistence of high-risk types of HPV among women with HIV co-infection may result in a massive increase in the burden of cervical cancer in South Africa.

In the majority of countries of southern Africa, anti-retroviral therapy is neither widely available nor affordable. International efforts to improve the availability of antiretroviral therapy (ART) in resource poor settings should result in significantly prolonging the life-expectancy of HIV-seropositive individuals. WHO/UNAIDS have set the target to provide universal access to antiretroviral therapy for all persons living with HIV/AIDS and it has declared a 3 by 5 target which aims to provide treatment for 3 million people by 2005. Historically, little attention has been paid to the interaction between HPV and HIV infection as well as the role of HIV-infection in increasing the risk of cervical cancer. A possible reason for this lack of attention may be that the majority of HIV-infected women with high-risk types of HPV infection and SIL are more likely to die of other AIDS-related conditions long before they develop invasive cancer[82-83].

However, if Highly Active Anti-retroviral Therapy (HAART) becomes the standard of care for HIV infections in women in the region, it is possible that the magnitude of the problem of cervical cancer in southern Africa may be a major concern requiring a reassessment of current screening programmes. Clearly, prevention strategies should take into consideration the rapidly changing knowledge of and insights into the natural history, diagnostic methods, and primary/secondary prevention of these viral infections. Screening algorithms should be reviewed regularly to improve the cost-effectiveness, sensitivity, specificity and predictive values of strategies and methods. Recent achievements in vaccine development should be closely monitored and applied appropriately to contain the rapid spread of HPV infection and its sequaelae[84-86].

In contrast to HPV, herpes simplex virus, type 2 (HSV-2) appears to have a significant bi-directional interaction with HIV. Many researchers have reported the presence of biological and epidemiological associations between genital herpes and HIV[87-89]. In a meta-analysis of relevant published information, it was reported that infection with HSV-2 doubled the risk of HIV acquisition through sexual transmission[90]. Furthermore, several studies included in the analysis found that when HSV-2 infection occurred before HIV acquisition, the risk of such acquisition was estimated to be 2.1 (95% CI, 1.4-3.2)[90]. The percentage of HIV transmission

attributable to HSV-2–positive individuals has been estimated to be 52%, with a population attributable risk of 19% in populations with a 22% HSV-2 prevalence increasing to 47% in a population with a 80% HSV-2 sero-prevalence.[90] The likely mechanisms of interaction include (1) the role of HSV as a cause of ulcerations or inapparent micro-ulcerations which provide a portal of entry for HIV, (2) increased lesional and/or mucosal shedding of HIV during episodes of reactivation of HSV-2[88]**.** In our study reported here, 18 of 274 patients (6.6%) were shedding HSV from their cervix and 55.6% of these were type-specific HSV-2 antibody positive. Moderate to severe cervical erosions were observed in the majority (77.8%) of cervical HSV-2 PCR-positive patients and the HIV prevalence was 72% in HSV-2 positive patients. The level of shedding of HIV from HSV-2 positive patients and response to anti-herpes treatment is discussed elsewhere in this thesis (Section 7.4).

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CHAPTER 7

HIV shedding in women with vaginal discharge and impact of therapy

7.1 Introduction

Epidemiological studies have shown that the efficiency of HIV transmission is higher from male-to-female than from female-to-male (See Section 6.1). In their study involving HIV sero-discordant couples in Rakai, Uganda, Gray et al. reported a higher transmission probability per coital act from men to women than from women to men, although this was not statistically significant (0.0013 vs. 0.0009, p 0.34)[1]. The microbiological correlates of HIV shedding in the female genital tract were studied extensively during the period 1996 – 2002[2 – 10]. Among these investigations, the effect of coinfection with genital infections and their treatment on female genital HIV shedding was investigated in studies conducted in both Côte d'Ivoire and Kenya[8-10]. A better understanding of the role of microbiological and other correlates of HIV shedding in the female genital tract among South African women could enhance the prospects of implementing evidence-based intervention programmes in the region.

7.2 Objectives

The studies described here are focused on the role of sexually transmitted vaginal and cervical infections in cervico-vaginal shedding of HIV and the effect of the treatment of these infections on cervico-vaginal shedding of HIV. The association between other potential determinants including host immunity on genital shedding of HIV has also been explored in this chapter.

7.3 Study population and methods

7.3.1 Study population

These studies were conducted at the Esselen Street STI clinic in Johannesburg, South Africa during the period July 1997 to August 1998. A detailed profile of the clinic and study population was described in Section 6.2. For these studies, a sub-group of patients attending the clinic who requested voluntary HIV testing for various reasons were approached and,

pending informed consent, enrolled into the study. In collaboration with the Outreach Programme of the Greater Johannesburg Metropolitan Council and the Reproductive Health Research Unit, female sex workers who voluntarily requested for HIV testing were also included. Following a one-on-one explanation of the study objectives and procedures and explanation of the risks and benefits of participation, pre-test HIV counselling was conducted by the trained counsellors.

7.3.2 Clinical and laboratory method

In each case, an interview was conducted to obtain information related to demographic and socioeconomic characteristics of patients including sexual practices as well as STD symptoms. Following the interview, clinical and gynaecological examinations including a speculum examination were performed. Vaginal and cervical specimens were collected for microbiological and virological investigations as described in sections 6.2.5.1 and 2.3.3. A first catch urine specimen was collected to detect *C. trachomatis* and *N. gonorrhoeae* by LCR testing. Finally, a specimen was collected from the squamo-columnar junction of the cervix and Pap smears were prepared.

In addition to the procedures described in section 6.2.5.1, cervico-vaginal lavage specimens were collected to assess shedding of HIV in genital fluid. After insertion of a vaginal speculum, 10 ml of sterile normal saline was slowly and gently applied onto the cervix using a syringe. After approximately 2 – 3 minutes, all fluid that had pooled in the posterior fornix was collected using a 10 ml sterile disposable pipette and transferred to a sterile plastic tube. All specimens were subsequently stored at -70ºC until processed to measure HIV RNA copies using a standard protocol as described in section 2.3.4.2.

Blood was collected and tested for the syphilis serology (on-site RPR) and subsequent FTA-ABS tests, HIV antibody testing and chlamydial and herpes serology. A rapid HIV serological test was performed to screen patients and all positive tests confirmed with two different ELISA tests. The SAIMR and National HIV testing protocols were followed for all patients. $CD4^+$ counts and HIV plasma viral loads were determined on all participants of the

study. Participants with signs and/or symptoms of STIs were treated immediately using the syndromic STD management approach.

All patients were requested to return for follow-up visits on Day 7 when a general and gynaecological examination was conducted. On their return, vaginal, cervical and blood specimens including a cervicovaginal lavage specimen were collected for repeat laboratory investigations which were identical to those performed at the initial visit except for syphilis serology, HPV testing, Pap smear and CD4 counts which were not repeated. Participants were provided with their HIV results and given post-test counselling. The CD4 results were also provided, and patients were referred to the Hillbrow Hospital HIV clinic for further management.

7.3.3 Data entry and analysis and ethical considerations

Procedures outlined in sections 6.2.6 and 6.2.7 were followed. Modifications of the survey instruments used for the cross-sectional prevalence studies were also used in this study (See section 6.2.8 and Annexures 6.1 to 6.3).

7.4 Results

A total of 68 HIV-seropositive women were enrolled in the study during the period July 1997 – August 1998. The ages of participants ranged from 17 to 34 years of age, with the majority of participants (62.1%) under 25 years of age with a median age of 23 years (±SE 0.5). Most patients were complaining of vaginal discharge (69.1%), vulval itching (44.1%), burning on micturation (BOM) (32.3%) and lower abdominal pain (43.3%). Some presented with genital ulcerations (26.5%) or genital warts (9.0%) (Table 7.1). A history of post-coital bleeding was a prominent feature involving 32.4% of the participants.

Table 7.1 Demographic characteristics and presenting symptoms of 68 HIV-infected women

Age	
Median	23 years
Range	17 - 34 years
Symptoms	
Burning on micturation (BOM)	22 (32.3%)
Abnormal vaginal discharge	47 (69.1%)
Vulvovaginal itchiness	30 (44.1%)
Genital sores	18 (26.5%)
Lower abdominal pain	29 (42.6%)
Abnormal lumps or warts	6 (8.8%)
History of post-coital bleeding	22 (32.4%)
Current contraceptive use	36 (52.9%)
Injectable contraceptive (e.g. Depo)	31 (45.6%)
Oral contraceptive pills	3 (4.4%)
Other methods (e.g. IUD)	2 (2.9%)
Median duration of usage (range)	24 months (1 - 144 months)

Thirty-six patients (52.9%) were using contraceptives at the time of enrolment and the majority employed injectable contraceptives (86.1%); oral contraceptives were used by 8.3%, while other contraceptive methods were used by 5.6%. Fifty-four patients recalled the first day of their last menstrual period (LMP). On average, enrolment occurred approximately two weeks after the first day of their LMP (Median 15 days, range – 1 – 71 days). On examination, genital ulcerations were detected in 9 patients (13.4%) and an abnormal vaginal discharge was observed in 61 (91.2%) patients. The majority (41.9%) of patients had a profuse discharge while 17.7% had a curd-like discharge. Twenty patients (29.4%) had a mucopurulent discharge from the cervix and 24 (35.3%) had cervical erosions. Four patients had clinically visible cervical warts. During bimanual gynaecological examination, 21 patients (30.9%) had cervical excitation tenderness (CET). A repeat examination was conducted on Day 7. The findings obtained at the two visits are presented in Table 7.2.

Table 7.2 Clinical signs detected at enrolment and follow-up visits

	Enrolment visit	Follow-up visit
Abnormal findings in external genitalia	10 (14.7%)	11 (16.2%)
Vaginal rash	4 (5.9%)	9 (13.2%)
Genital warts	5 (7.4%)	3 (4.4%)
Genital ulcer(s)	9 (13.2%)	3 (4.4%)
Abnormal vaginal discharge	62 (91.2%)	40 (58.8%)
Profuse discharge	26 (41.9%)	4 (5.9%)
Curd-like discharge	11 (17.7%)	16 (23.5%)
Cervical discharge	20 (29.4%)	13 (19.1%)
Cervical erosion	24 (35.3%)	18 (26.5%)
Cervical excitation tenderness	21 (30.9%)	7 (10.3%)

$CD4^+$ cell counts at enrolment were obtained in 64 cases and the mean $CD4^+$ count was 531 cells/mm^3 (± SE 39.5, range: 12 – 1513). The majority (68.8%) of patients had $CD4^+$ counts of 350 cells/mm^3 or more. Seven patients (10.9%) had $CD4^+$ counts fewer than 200 cells/mm^3 (Figure 7.1).

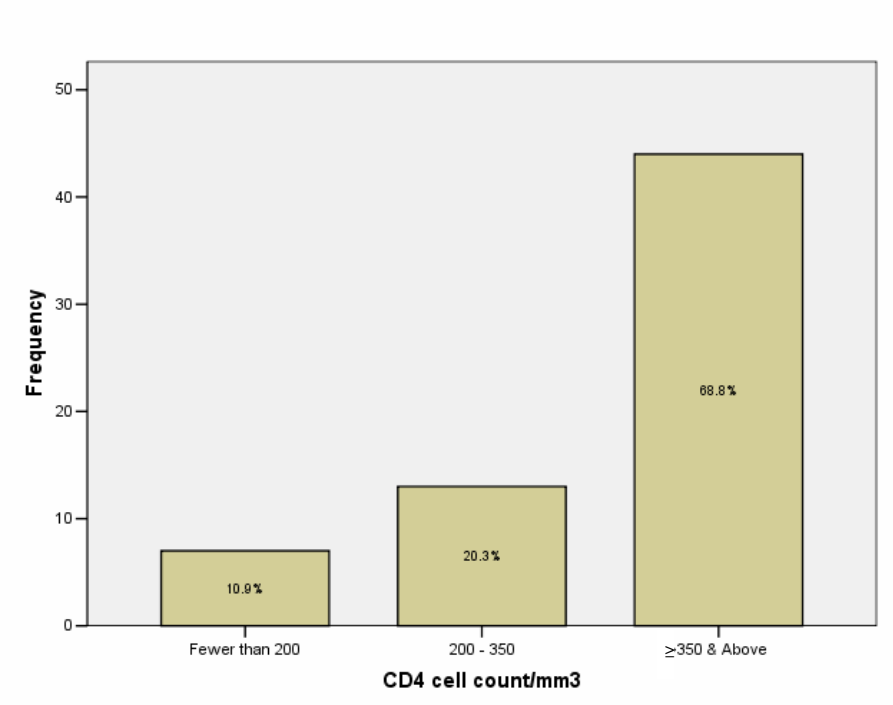

Figure 7.1 Distribution of $CD4^+$ counts at the enrolment visit

The HIV plasma viral load (PVL) was measured at the time of enrolment for all patients and the median PVL was 19,024 copies/ml (range: 450 – 590,203 copies/ml). The median log PVL at the initial visit was 4.27 (±SE 0.1, range: 2.65 – 5.77). The PVL at enrolment was less than 20,000 copies/ml in 34 patients (50%). No patient had received anti-retroviral therapy and the PVL was generally higher in those with low $CD4^+$ counts (Figure 7.2).

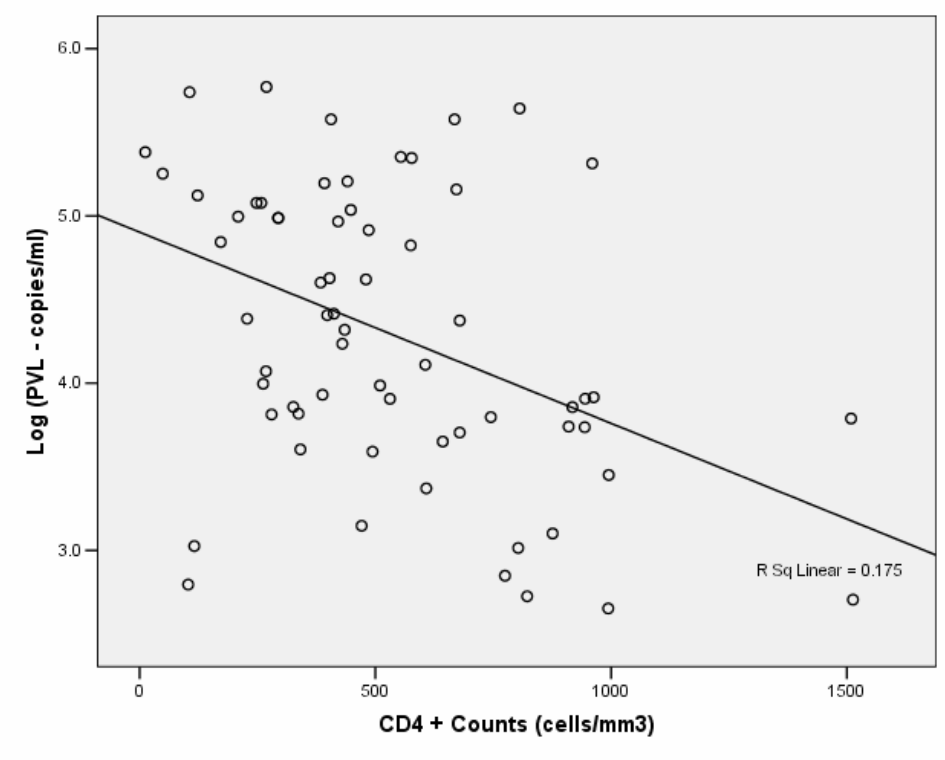

Figure 7.2 Association between $CD4^+$ counts and log PVL measured at enrolment

Forty-six patients (67.6%) were positive for bacterial vaginosis diagnosed by the presence of typical clue cells on Gram stained vaginal smears. All patients had pH>4.5 and 72.7% had a positive amine test. Yeasts were detected in specimens from 44 patients (64.7%) and *T. vaginalis* (TV) in 20 (30.9%). Gonococcal infection was diagnosed in 29 patients (42.6%) and chlamydial infection in 17 patients (25.0%). Overall, 35 patients (52.2%) were positive for gonococcal and/or chlamydial cervical infections. In addition, five patients (7.4%) had a positive HSV-PCR from cervical specimens, and one patient was HSV-PCR positive from genital ulcers. Low-risk HPV infection was detected at enrolment in 23 cases (33.8%) and high-risk HPV in 50 patients (73.5%) (Figure 7.3). The median log Relative Light Unit/Positive

Control ratio (RLU/PC) for low-risk HPV was 1.1 (±SE 0.21, range 0.04 – 3.1) and for high-risk HPV 1.7 (±SE 0.14, range 0.1 – 3.3). Syphilis serology was positive (i.e. RPR confirmed by an FTA-ABS test) in 11 patients (16.2%).

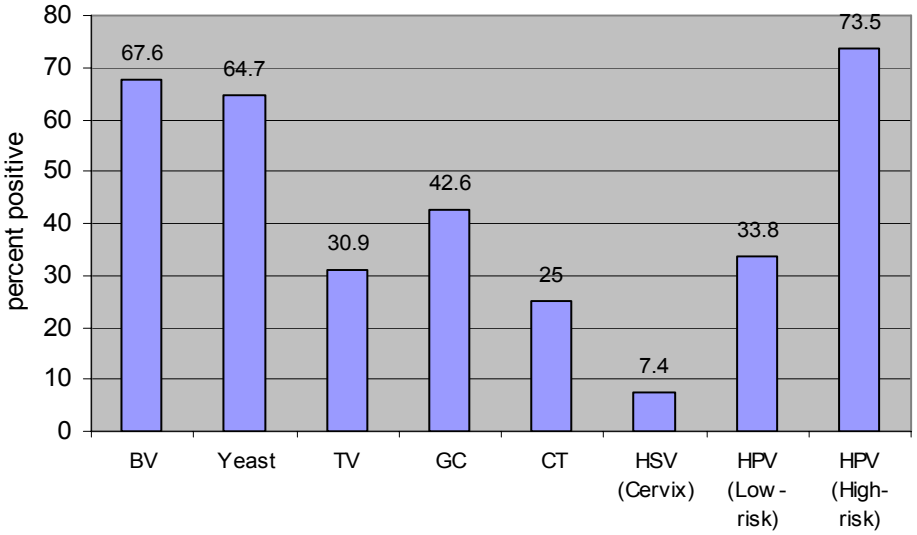

Figure 7.3 Aetiogical distribution of vaginal and cervical infections detected at the enrolment in 68 HIV-seropositive women

The manufacturer's information on the sensitivity of viral load estimations for the equipment used (cut-off - 400 copies/ml) was applied to define "detectable HIV" in cervicovaginal lavage (CVL) specimens as well as in plasma (PVL). At enrolment, HIV RNA was detected in CVL collected from 26 patients (38.2%). The median HIV RNA detected in the CVL of 26 HIV 'shedders' was 3068 copies/ml of CVL (± SE 2582, range 576 – 67640 copies/ml). The median log copies detected was 3.49 (± SE 0.1, range 2.8 – 4.8). The results of univariate analyses are shown in Table 7.3. Although higher rates of HIV shedding were detected among several groups of patients including those using injectable contraceptives (48.4% vs 34.4%, p 0.25); having a history of post-coital bleeding (45.5% vs. 29.3%, p 0.19); genital ulcerations (55.6% vs. 35.1%, p 0.23); vaginal discharge (38.7% vs. 25.0%, p 0.58) and cervical erosion (50.0% vs. 33.3%, p 0.18), these associations were not statistically significant. HIV shedding rates were not significantly different between patients with and without concomitant vaginal infections, namely, bacterial vaginosis, trichomoniasis or candidiasis (Table 7.3). Similar findings were observed in patients with gonococcal and/or chlamydial cervical infections as well as high-risk and low-risk HPV infections. Higher HIV shedding rates were observed in patients with HSV infection involving the cervix although the

difference did not reach statistical significance. (80.0% vs. 33.9%, Odds Ratio 7.8, 95% CI 0.8 – 74.5, Fisher's Exact Test, p 0.06)

Table 7.3 Assessment of potential risk determinants associated with HIV CVL shedding

Potential determinants	No/Total (%) with HIV shedding	Odds Ratio (95% CI)	p
Demographic & reproductive health			
Age			
15 - 19	4/11 (36.4%)	1	
20 - 29	20/49 (40.8%)	1.2 (0.3 - 4.7)	
30 & above	2/8 (25.0%)	0.58 (0.08 - 4.4)	
Current injectable contraceptive use			
No	11/32 (34.4%)	1.0	
Yes	15/21 (41.7%)	1.4 (0.5 - 3.7)	
History of post-coital bleeding			
No	16/46 (34.8%)	1	
Yes	10/22 (45.5%)	1.6 (0.6 - 4.4)	
STIs related signs and symptoms			
Presence of STI signs on external genitalia			
No	17/56 (30.4%)	1	
Yes	8/10 (80.0%)	9.2 (1.8 - 47.8)	0.008
Sores on external genitalia			
No	20/57 (35.1%)	1	
Yes	5/9 (55.6%)	2.3 (0.6 - 9.6)	
Vaginal discharge			
No	1/3 (25.0%)	1	
Yes	24/62 (38.7%)	1.9 (0.2 - 19.3)	
Cervical mucopus			
No	17/42 (40.5%)	1	
Yes	8/20 (40.0%)	1.0 (0.3 - 2.9)	
Cervical erosion			
No	14/42 (33.3%)	1	
Yes	12/24 (50.0%)	2.0 (0.7 - 5.6)	
STI pathogens			
Bacterial vaginosis			
No	10/22 (45.5%)	1	
Yes	16/46 (34.8%)	0.6 (0.2 - 1.8)	
Yeast infection			
No	7/24 (29.2%)	1	
Yes	19/44 (43.2%)	1.8 (0.6 - 5.3)	
Trichomonas vaginalis			
No	16/47 (34.0%)	1	
Yes	10/21 (47.6%)	1.8 (0.6 - 5.0)	
Neisseria gonorrhoeae			
No	15/39 (38.5%)	1	
Yes	11/29 (37.9%)	1.0 (0.4 - 2.6)	
Chlamydia trachomatis			
No	20/51 (39.2%)	1	
Yes	6/17 (35.3%)	0.9 (0.3 - 2.7)	
Any GC &/or CT infections			
No	15/32 (46.9%)	1	
Yes	11/36 (30.6%)	0.5 (0.2 - 1.3)	
Cervical Herpes simplex virus			
No	20/59 (33.9%)	1	
Yes	4/5 (80.0%)	7.8 (0.8 - 74.5)	
Cervical HPV (High-risk types)			
No	7/16 (43.8%)	1	
Yes	19/50 (38.0%)	0.8 (0.3 - 2.5)	
Cervical HPV (Low-risk types)			
No	14/43 (32.6%)	1	
Yes	12/23 (52.2%)	2.3 (0.8 - 6.4)	
Immunologic derterminants			
CD4 counts (/mm3)			
≥350	12/44 (27.3%)	1	0.009
200 - 350	9/13 (69.2%)	6.0 (1.6 - 2..2)	
<200	4/7 (57.1%)	3.6 (0.69 - 18.3)	
Plasma viral load			
≤20,000 copies	4/34 (11.8%)	1	<0.001
>20,000 copies	22/34 (64.7%)	13.8 (3.9 - 48.4)	

There was however, a significant direct association between HIV PVL and the level of HIV shedding in CVL (Pearson Correlation Coefficient = 0.542, p<0.001). A significantly higher proportion of patients whose PVL was higher than 20,000 copies/ml at enrolment shed HIV in their CVL than those with PVL ≤20,000 copies/ml (64.7% vs. 11.8%, Odds Ratio 13.8, 95% CI 3.9 – 48.4, p<0.001) (Table 7.4).

Table 7.4 Association between HIV PVL and shedding of HIV in CVL (initial visit)

HIV Plasma Viral Load (PVL)	n	Median HIV RNA Shedding (copies/ml) in Cervicovaginal Lavage Fluid (Range)	Number of Women Who Shed HIV in CVL (%)	Odds Ratio (95% CI)	p
High PVL (>20,000 copies/ml)	34	3,5421 (576 - 67,640)	22 (64.7)	13.8 (3.9 - 48.4)	<0.001
Low PVL (≤20,000 copies/ml)	34	7,992 (602 - 10237)	4 (11.8)		
Total	68	3,068 (576 - 67,640)	26 (38.2)		

1 vs 2, Mann-Whitney U, p<001

There was an increasing trend in the amount of HIV RNA detected in CVL in patients with higher PVL (R^2 Quadratic 0.264) (Figure 7.4).

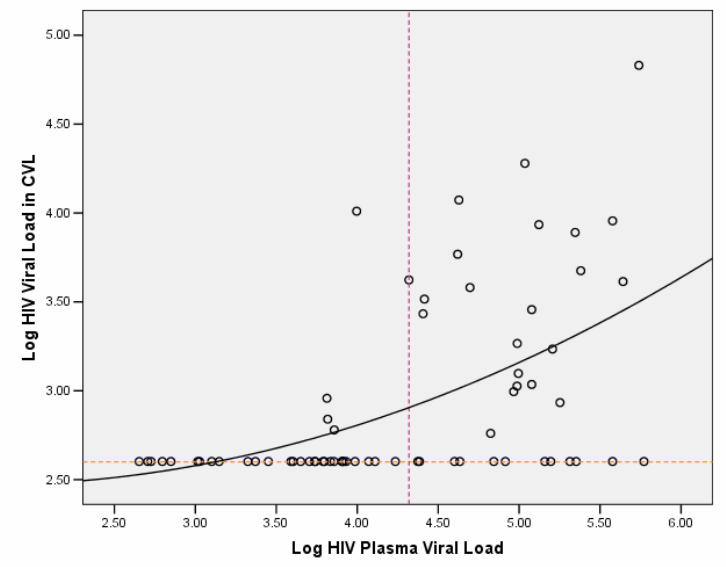

Figure 7.4 Association between HIV Plasma Viral Load (PVL) & HIV shedding in Cervicovaginal Lavage Fluid (CVL) *(Horizontal reference line approximates the PVL amount of 20,000 copies/ml and vertical reference line indicates approximates of undetectable limit of HIV copies in CVL. Quadratic curve fitting provides – R^2 Quadratic of 0.264)*

A stronger association between PVL and HIV shedding in CVL was found in the group of patients with $CD4^+$ counts 350 and above compared to those with $CD4^+$ counts lower than 350 cells/mm^3 (Table 7.5). Of 44 patients with $CD4^+$ counts 350 and above, 20 patients (45.5%) had high PVL (>20,000 copies/ml). Among these, 12 (60%) patients shed HIV RNA in their CVL. In contrast, none of 24 patients with low PVL (20,000 copies/ml & below) shed HIV (χ^2 19.8, p<0.001). The association between PVL and HIV shedding in CVL was not significantly associated in 20 patients with $CD4^+$ counts fewer than 350 cells/mm^3 (75% shed HIV in high PVL group vs. 50% in low PVL group, Fisher's Exact Test p 0.35).

Table 7.5 Association between CD4, HIV PVL and shedding of HIV in CVL

CD4 + Counts		HIV Shedding in CVL		Total
		HIV RNA Detected	No HIV RNA Detected	
≥350	High PVL (>20,000)	12 60.0%	8 40.0%	20
	Low PVL (≤20,000)	0 0%	24 100.0%	24
	Total	12 27.3%	32 72.7%	44
<350	High PVL (>20,000)	9 75.0%	3 25.0%	12
	Low PVL (≤20,000)	4 50.0%	4 50.0%	8
	Total	13 65.0%	7 35.0%	20

All patients were treated syndromically to cover all vaginal and cervical infections (i.e. ciprofloxacin 500 mg p.o. stat plus azithromycin 1 g p.o. stat plus metronidazole 400 mg p.o. bd for 7 days plus fluconazole 150 mg p.o. stat). Treatment for genital herpes (i.e. valaciclovir 500 mg p.o. bd for 5 days) was provided presumptively for those with suspected genital ulcerations or with significant cervical erosion and/or ulcerations.

The microbiological outcomes of patients at Day 7 are shown in Table 7.6. Most treatment failures or persistent infections were encountered in patients with bacterial vaginosis and/or yeast infections. Of 45 patients who were positive for bacterial vaginosis at enrolment, 16 (35.6%) were still positive at the follow-up visit at Day 7. Similarly, 21 out of 44 patients

(47.7%) with yeast infections continued to be positive at the follow-up visit. In the case of vaginal trichomoniasis and gonococcal cervicitis, all patients were cured by Day 7 while 4 patients (25%) with chlamydial cervicitis were still positive at Day 7.

Table 7.6 Treatment outcomes of patients with bacterial and fungal infections at Day-7

Outcome at Follow-up Visit	Bact. Vaginosis		Trichomo-niasis		Yeast Infection		Gonococcal Infection		Chlamydial Infection	
	n	%	n	%	n	%	n	%	n	%
Negative at Both Visit	18	26.5	45	66.2	14	20.6	36	52.9	49	72.1
Treatment Success	29	42.6	20	29.4	23	33.8	27	39.7	12	17.6
Treatment Failure**	16	23.5	-	-	21	30.9	-	-	4	5.9
Reinfection*	3	4.4	2	2.9	9	13.2	3	4.4	1	1.5
Unknown	2	2.9	1	1.5	1	1.5	2	2.9	2	2.9
Total	68	100	68	100	68	100	68	100	68	100

**remain +ve at follow-up visit; **continued +ve at follow-up visit*

All five women who were PCR positive for HSV from their cervical specimens were successfully treated with anti-herpes therapy. The majority of low- and high-risk HPV-positive patients were persistently positive on follow-up (68.2% for low-risk and 91.5% for high-risk HPV-positive patients) (Table 7.7).

Table 7.7 Treatment outcomes of patients with viral STIs at Day 7

Outcome at Follow-up Visit	Herpes Cervicitis		HPV (Low-risk)		HPV (High-risk)	
	n	%	n	%	n	%
Negative at both visits	53	77.9	35	51.5	10	14.7
Treatment success*	5	7.4	7	10.3	4	5.9
Treatment failure**	-	-	15	22.1	43	63.2
Reinfection***	-	-	4	5.9	4	5.9
Unknown	10	14.7	7	10.3	7	10.3
Total	68	100	68	100	68	100

**viral clearance in HPV patients; ** viral persistence in HPV patients;*

**** viral appearance in HPV patients*

PVL was measured at follow-up in all cases, and two patients had undetectable PVL at Day 7. The median PVL at Day 7 was 16,970 copies/ml (range 505 – 1,060,813 copies/ml) and the median log PVL was 4.2 (±SE 0.12, range, 2.7 – 6.03). When compared to their PVL status at enrolment, 31 out of 34 patients with high initial PVL (i.e. >20,000 copies/ml) continued to be in the high PVL group at follow-up. Similarly 33 out of 34 patients with low PVL (i.e. ≤20,000 copies/ml) continued to be in the low PVL group on follow-up. Of 26

patients who shed HIV in their CVL at the enrolment visit, 10 (38.5%) stopped shedding HIV at follow-up, while seven of 42 patients who did not shed HIV at enrolment shed HIV in their CVL at follow-up.

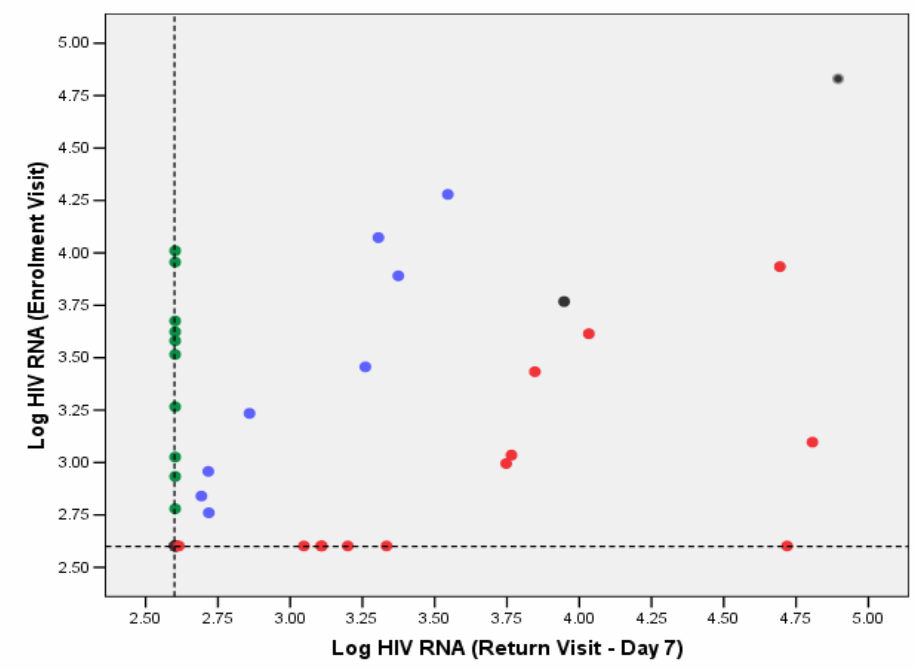

Figure 7.5 Scattergram of Log HIV RNA in CVL at enrolment and return visits
(Green – patients stopped shedding; Blue – patients declined in amount of shedding; Red – patients started shedding or increased amount of shedding; Horizontal & vertical reference lines represent detection cut-offs for HIV viral load in CVL, i.e. 400 copies/ml)

Of 16 patients who continued to shed HIV in their CVL, eight (50%) showed some decline in the amount of shedding with a median log reduction of 0.305 (range – 0.04 – 0.76) (Figure 7.5). The other eight cases showed increases in the amount of shedding with a median log increase of 0.58 (range – 0.07 – 1.71). In seven patients who started shedding at follow-up, the median HIV RNA shed in the CVL was 1,287 copies/ml (range 412 – 52,378 copies/ml). The median log HIV copies in the CVL was 3.1 (range 2.61 – 4.72).

Similar to the findings at the enrolment visit, there was a significant association between the high PVL and HIV shedding in CVL (Table 7.8).

Table 7.8 Association between HIV PVL and shedding of HIV in CVL (return visit)

HIV Plasma Viral Load (PVL)	n	Median HIV RNA Shedding (copies/ml) in Cervicovaginal Lavage Fluid (Range)	Number of Women Who Shed HIV in CVL (%)	Odds Ratio (95% CI)	p
High PVL (>20,000 copies/ml)	32	35,111 (412- 78,604)	19 (59.4)	11.7 (3.3 - 41.1)	<0.001
Low PVL (≤20,000 copies/ml)	36	8,192 (493 - 1,582)	4 (11.1)		
Total	68	2,154 (412 - 78,604)	23 (33.8)		

1 vs 2, Mann-Whitney U, p<001

A similar association was observed between PVL level and amount of HIV viral shedding in CVL at the follow-up visit (Figure 7.6).

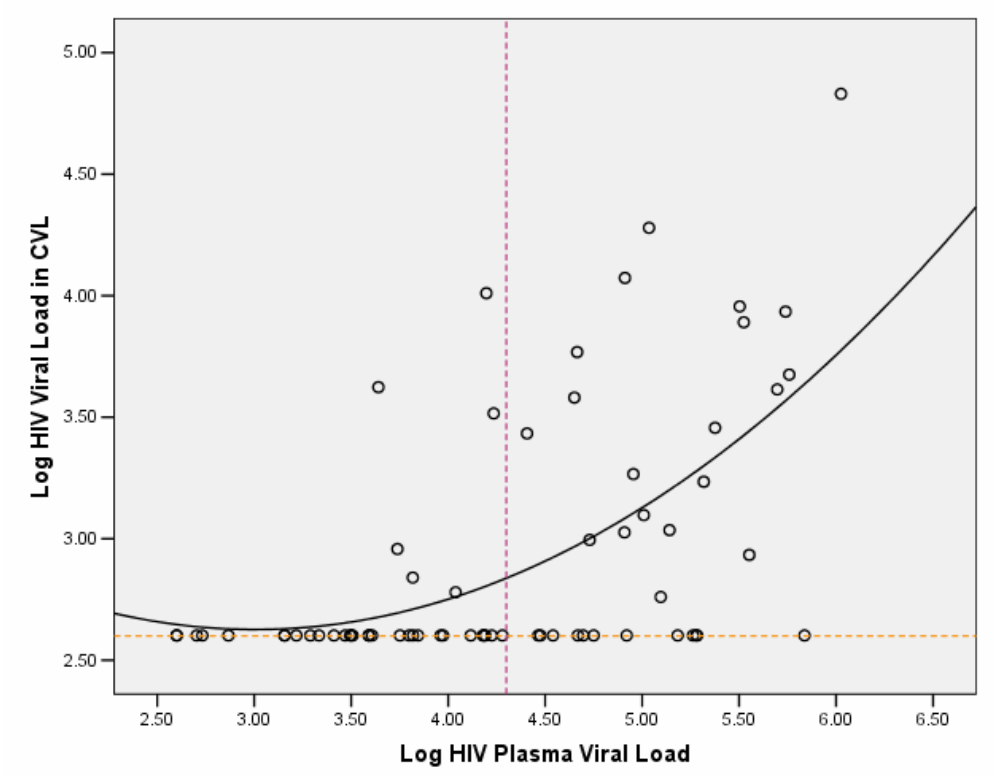

Figure 7.6 Association between HIV Plasma Viral Load (PVL) & HIV shedding in Cervicovaginal Lavage Fluid (CVL) at the follow-up visit
(Horizontal reference line approximates the PVL amount of 20000 copies/ml and vertical reference line indicates approximates of undetectable limit of HIV copies in CVL. Quadratic curve fitting provides – R^2 Quadratic of 0.326)

The characteristics of 10 patients who ceased shedding HIV at follow-up are shown in Table 7.9. HIV shedding ceased in four patients who were cervical HSV PCR positive and had received presumptive anti-herpes treatment at enrolment. Among them, three had high PVLs ranging from 81,110 to 573,791 copies/ml. Of six HSV-negative patients, three had a low PVL (i.e.≤ 20,000) and the remaining had high PVLs (i.e. >20,000 copies/ml) at Day 7.

Table 7.9 Characteristics of 10 patients who ceased vaginal shedding of HIV at follow-up visit

	age	CD4*	PVL at Day 1#	HIV in CVL at Day 1#	PVL at Day 7#	HSV at Day 1	Reproductive Tract Infections at Enrolment	Positive Rx Outcomes	Negative Rx Outcomes
1	27	668	377,979	9,029	318,459	Neg	Yeast, TV, HPV (both types)	Clearance of Yeast, TV & HPV (both types)	-
2	29	12	240,388	4,734	573,791	Pos	HSV, HPV (both types)	Clearance of HSV, HPV (Low-risk)	Positive for BY & Yeast
3	29	49	178,798	858	356,916	Pos	HSV, Yeast, TV, HPV (both types)	Clearance of HSV, TV	Persistent Yeast, HPV (Both types)
4	22	294	97,065	1,062	81,110	Pos	HSV, BV, Yeast, TV, GC & HPV (both types)	Clearance of all pathogens except HPV (both types)	Persistent HPV (both types)
5	18	412	26,005	3,277	17,175	Pos	HSV, Yeast, TV, GC, CT & HPV (high-risk)	Clearance of all pathogens except HPV (high-risk types)	Persistent HPV (high-risk types)
6	26	294	97,366	1,845	90,136	Neg	TV and RPR +ve	Clerance of TV	Positive for HPV (high-risk)
7	32	-	49,737	3,807	44,734	Neg	Only Yeast	Clearance Yeast	-
8	26	435	20,855	4,202	4,367	Neg	BV, TV, GC, HPV (high risk types) & RPR +ve	Clearance BV, TV & GC	Persistent HPV (high-risk types)
9	18	262	9,918	10,237	15,732	Neg	BV only	Clearance of BV	Positive Yeast
10	21	326	7,211	602	10,924	Neg	BV & Yeast	Clearance of BV & Yeast	-

** cells/mm^3; # copies/ml*

Among seven patients who started shedding HIV in CVL at follow-up, five (71.4%) had PVLs of >20,000 copies/ml at both visits. The median PVL for all patients was 69,758 copies/ml (range: 1,062 – 377,967 copies/ml) at enrolment and 46,636 copies/ml (range: 1649 – 687,718 copies/ml) at follow-up. Treatment failure for BV was observed in three patients and one became BV-positive at follow-up. Treatment failure occurred in three patients with yeast infection. None of these patients was positive for HSV.

Of 16 patients who continued to shed HIV in CVL at the follow-up visit, eight (66.7%) were successfully treated for BV, six for infection caused by yeast (46.2%), four for TV (80%), eight for GC (100%) and five for CT infection (100%). The median PVL of patients on their return visit was 116,672 copies/ml (range: 5,472 – 1,060,813 copies/ml) and 87.5% of patients had high PVLs (>20,000 copies/ml).

Of 35 patients who did not shed HIV in CVL at all visits, nine (36%) were persistently positive for BV, ten (45.5%) for yeast infection, three (33.3%) for CT infection and none for GC. Two patients with HSV (one from a cervical swab and one from an ulcer) were successfully treated. The majority (80%) of patients had low PVL (<20,000) and the median PVL at enrolment and follow-up were 6,267 copies/ml (range 450 – 590,203 copies/ml) and 6,587 copies/ml (range 505 – 192,204 copies/ml) respectively. Seven patients had high PVLs at enrolment with a median PVL of 156,764 copies/ml (range 23,671 – 590,203 copies/ml). Six of the seven patients continued to have high PVLs at follow-up visit with a median PVL of 104,355 copies/ml (range 29,905 – 192,204 copies/ml).

7.5 Discussion

In sub-Saharan Africa, the majority of HIV transmission that has occurred during the past decade was through heterosexual transmission with the number of women infected with HIV infection outnumbering the men in 2003[11]. African women appear to be infected at an earlier age than African men, and the difference in HIV prevalence between the sexes continues to grow. UNAIDS has reported that, in sub-Saharan Africa, 57% of adults infected are women, while 75% of young people infected are women and girls. Also, the findings of the

Demographic and Health Survey (1998) and other behavioral studies conducted in South Africa indicate that young African women in the region tend to have male partners who are older than themselves and this has resulted young women engaging in sexual relationships with those that were more likely to have a higher STI rate and more likely to be HIV-positive than younger men[12]. It is inevitable that the gender inequalities prevailing in the region make it much more difficult for African women to negotiate condom use. Sexual violence targeting women is reportedly very common and a higher risk of HIV transmission is anticipated in the presence of tissue trauma which occurs more frequently in women subjected to violent sexual abuse. In South Africa, a survey of 1366 women attending antenatal clinics in Soweto found significantly higher rates of HIV infection in women who were physically abused, sexually assaulted or dominated by their male partners as measured by the South African adaptation of the Sexual Relationship Power Scale (SRPS). The study also produced evidence that abusive men are more likely than non-abusers to be HIV-seropositive[13]. The HIV sentinel surveillance data collected from the Esselen Street STI Clinic in Johannesburg have shown that more than half of women attending the clinic with STI symptoms were infected with HIV in 1998[14]. It is possible that, with an increasing number of sexually active women becoming HIV-seropositive, any changes in patterns of sexual networking or sexual practices (e.g. appearance of sexual networks between young boys and women older than themselves or young girls who had previous sexual relationships with older men etc.), may rapidly propagate HIV transmission into other population groups.

Studies have also reported that HIV is commonly detected in the genital fluids of women and STI co-infection could potentially increase the infectiousness of HIV[15]. However, the findings on the role of RTI/STIs in increased infectiousness of HIV are sketchy and inconsistent. In a randomised controlled study conducted in Rakai, Uganda, no association was found between the increase in transmission probability per coital act involving discordant couples and the presence of concurrent gonorrhoea, chlamydial infection, bacterial vaginosis, or trichomoniasis[15]. Unlike findings in Europe and the USA, the RCTs conducted in Africa indicate a higher transmission probability per act from women to men than from men to women[16-19] . Therefore, the presence of a large number of sexually active HIV-infected

women may also lead to an accelerated HIV epidemic in the coming years, unless intervention activities targeting HIV-seropositive women are employed to lower the transmission efficiency including reduction of infectiousness of HIV (Figure 7.7).

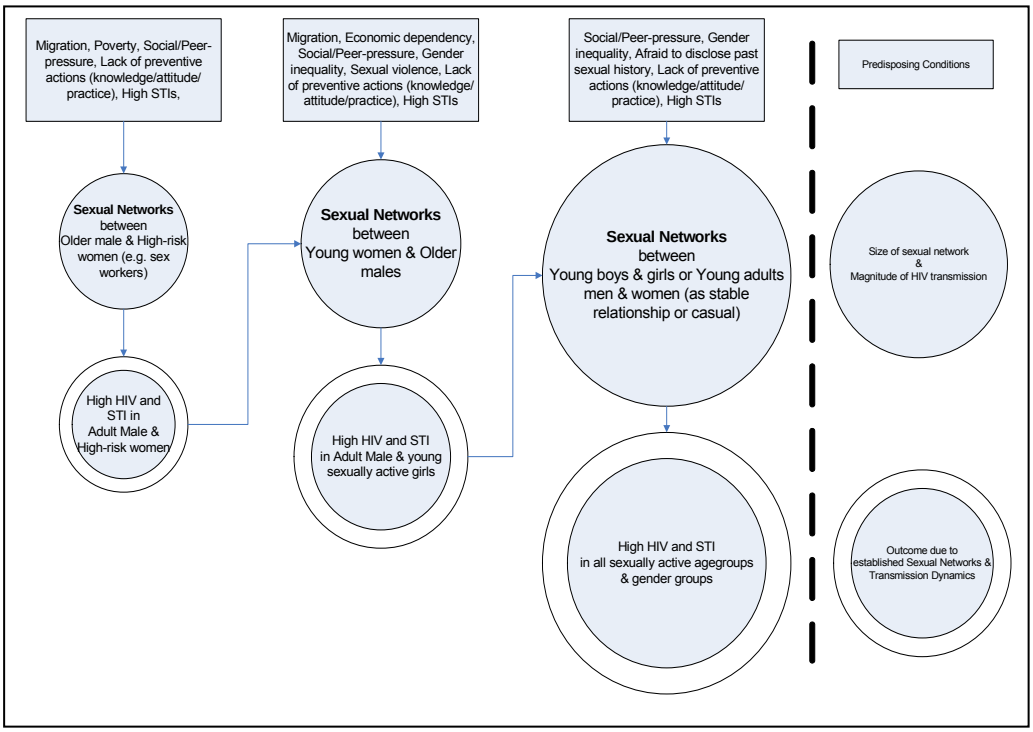

Figure 7.7 Simplified hypothetical framework on possible transmission dynamics & impact of HIV infection in southern Africa.

In the studies reported here, we explored the level of HIV shedding in cervicovaginal lavage of HIV-seropositive women with or without co-infection with RTI/STI. The study population was relatively young with over 60% of subjects recruited being under 25 years of ages with a mean age of 24 years. The majority had no clinical and laboratory evidence of immunodeficiency and 68.8% had $CD4^+$ counts of 350 cells/mm^3 and above. There was no significant association between individual RTIs/STIs and HIV shedding in our study. Four of five patients with positive cervical HSV infection however, shed HIV in their CVL at enrolment and stopped shedding at the follow-up visit after receiving anti-herpes therapy with valacyclovir. Our study found that HIV plasma viral load was the main determinant for vaginal shedding of HIV in terms of frequency as well as quantity of shedding. When we used a proxy cut-off of 20,000 copies/ml of PVL, the frequency and quantity of shedding were significantly higher in patients with PVL >20,000 copies/ml (Odds ratio 13.8; 95% CI 3.9 – 48.4). In

addition, a positive association between log PVL and log HIV copies in CVL was also observed (R^2 = 0.264). This association was stronger in those with CD4 counts ≥350 as none of the 44 patients with PVL <20,000 had detectable HIV RNA in their CVL, despite having other potential risk determinants such as RTI/STIs, cervical ectopy and lack of contraceptive use. This finding has an important implication as sexual activity may be much higher among those without AIDS-related signs or symptoms than in AIDS cases. Therefore, interventions to reduce HIV infectiousness should target immunocompetent women. Our results also support the findings of larger community-based RCT studies conducted elsewhere. Quinn *et al* reported in their study on heterosexual transmission of HIV-1 among serodiscordant couples in Rakai, Uganda that the mean serum HIV-1 RNA level was significantly higher among HIV-1-seropositive participants whose partners seroconverted, than among those whose partners did not seroconvert (90,254 copies/ml vs. 38,029 copies/ml, P=0.001) [20]. Apart from a younger age and circumcision status of male partners, they found no other potential determinants such as STIs were associated with HIV transmission. Many other studies have also reported the association between high PVLs (e.g. primary or early HIV infection) and infectiousness of HIV [5, 21-25].

Theoretically, if co-infection with RTI/STI is making a significant contribution to HIV infectiousness through increased shedding of HIV in genital secretions, any successful treatment of RTI/STI should reduce or stop genital shedding of HIV. Ghys et al reported that successful treatment of STIs reduced shedding of HIV in genital secretions of female sex workers in Cote d'Ivoire. In their study, the association between viral load and HIV shedding was not significant within the same CD4 levels and therefore it was not included in their multivariate analysis. The conclusion was made that increased infectiousness of HIV is associated with immunosupression and STIs[8]. In our studies, the prevalence of multiple vaginal and cervical infections was extremely high and treatment failure at follow-up was high for BV and yeast infection. All gonococcal and trichomonas infections were successfully treated although some women had new infections detected at follow-up. We did not find a significant association between successful treatment of RTI/STI and reduction in HIV shedding among our patients. Similar to the finding during the enrolment visit, high PVLs at

the return visit were significantly associated with a higher frequency of HIV shedding and the amount of HIV shed in the CVL at the return visit (Odds ratio 11.7, 95% CI 3.3 – 41.1).

Of the 10 patients who stopped shedding HIV at follow-up, four had cervical HSV infection at enrolment which was treated successfully using anti-herpes treatment presumptively. Shedding was reduced to undetectable levels in four of these HSV-positive patients, despite three of them having high PVLs ranging from 81,110 to 573,791 copies/ml at the return visit. This association between HSV and HIV infection in women needs to be explored further with large scale studies. In contrast, successful treatment of RTI/STI such as BV (6 patients), yeast infection (6 patients), trichomoniasis (4 patients), gonorrhoea (8 patients) and chlamydial infection (5 patients), did not stop HIV shedding. The median PVL in these patients was high (116,672 copies/ml) and 87.5% had PVL of more than 20,000 copies/ml at follow-up.

Major limitations of our study include: small sample size (67 women), a high prevalence of multiple RTIs/STIs in the same subject and no long-term follow-up after the Day-7 visit. Our findings however, support the current concept that phase-specific interventions are needed to maximise the impact of STI control on HIV transmission at the community level[26-27]**.** Several debates and critical analyses followed after conflicting outcomes were apparently obtained following two major community-based RCTs conducted in Mwanza, Tanzania and Rakai, Uganda[28-29]. In Mwanza, the improved clinical management of symptomatic STD treatment, was associated with a reduction in HIV incidence by 38% [95% confidence interval 15 - 55%] over a period of 2 years[30]. Periodic mass treatment of bacterial STI in Rakai, Uganda however, did not reduce HIV incidence over the same period, despite achieving similar reductions in STIs[31].

Some authors conclude that the impact of STI treatment programmes on HIV transmission may depend more on behavioural risk reduction than on the stage of the epidemic[32-33]**.** Others argue that STIs may play a less important role in HIV spread in mature HIV epidemics as was

the case in Uganda. Non-bacterial STIs, notably genital herpes, were more common in Rakai; and rounds of mass treatment in Rakai may have been followed by rapid reinfection[34] .

Based on our findings, one of the important and generally recognised interactions between STIs and HIV, namely that STIs increase the infectiousness of HIV may not be universal and should probably be more realistically interpreted as conditional. It is possible that other high-risk factors in the study population may have obscured the importance of HIV transmission and acquisition in the present study, e.g. high transmissibility in recently acquired HIV infection when PVLs are high may have been such a confounding factor.

The high rates of STIs that were detected in our study population at an early stage of the HIV epidemic provide an early warning for the potential for rapid spread of HIV. This early warning sign is based on the role of acute STIs as a proxy marker for the presence of high-risk sexual behaviours (i.e. unprotected sex with high-risk partners) as well as increased susceptibility to HIV if he/she is exposed. The lack of effective intervention, targeting core transmitters or high-risk sub-population groups during the early phase of the HIV epidemic has lead to dire consequences for southern Africa which will become apparent in the years ahead. Any intervention should include enhanced STI treatment and control, risk-reduction counselling of high-risk individuals, education for behavioural change and partner notification and treatment. This should theoretically reduce two components involved in the reproductive equation, namely, the probability of transmission and the frequency of sexual partner change.

In the mid-1990s, an accelerated spread of HIV occurred in southern Africa with almost one third of the sexually active population in South Africa were infected by the early 2000s. During this accelerated phase of spread leading to the high incidence era, the probability of transmission might have been largely dependent of the prevalence of STIs in HIV-seronegative individuals, and the high-level HIV viraemia associated with primary infection during the early phase of HIV acquisition[35-36]**.** As the HIV epidemic became more mature, the number of individuals in the infectious pool steadily increased with time. Some proportion of people living with HIV will have reached a stable but low plateau or set point in their HIV viral

load, while others will have higher viral load as HIV infection progresses to AIDS. Those patients with high PVLs will exhibit ~~with~~ high-level genital HIV shedding and an increased probability of per-coital transmission, with or without the co-factor effect of concurrent STIs. Therefore, the role of concurrent STIs may be negligible in increasing the HIV transmission probability from HIV-seropositive people, particularly in those who already have high PVLs. Meanwhile, concurrent STIs may contribute to an increased transmission probability of asymptomatic HIV patients with a low viral set-point (therefore low PVL) who may not shed HIV in their genital fluid during the STI-free period.

In conclusion, it is clear that STI treatment is not a “magic bullet” in controlling the spread of HIV. However, the role of treatment in reducing the transmission probability of HIV transmission in a population is dynamic and varies according to different phases of the HIV epidemic. In addition, the role of some viral STIs such as genital herpes should not be under-estimated as their hyper-endemic status in a community could weaken the potential effect of STI prevention and treatment activities which are traditionally focused on curable bacterial STIs. Many RCTs are being conducted or planned to estimate the effect of episodic and suppressive treatment of HSV on HIV acquisition and shedding. In addition, the availability of HAART in many developing countries may influence the transmission probability of HIV through lowering the viral load in previously treated naïve patients and thereby, reduce the infectiousness of HIV. Current ART treatment guidelines recommend initiating therapy when HIV-seropositive individuals become symptomatic and qualify as AIDS patients. Therefore, present programmes may not have any significant impact on the sexual transmission of HIV which is probably driven largely by asymptomatic individual with high PVL.

Finally, the findings of our studies and those of other researchers have reached a common conclusion – there is a strong and dynamic bidirectional interaction between STIs and HIV. This important knowledge must be optimally utilised and adapted in order to control HIV at various phases of the epidemic globally. However, scientific knowledge should be applied with caution in places with diverse social, cultural and epidemiological backgrounds, and the “one size fits all” principle is not appropriate for our attempts at controlling STI and HIV.

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CHAPTER 8

General discussion and conclusions

8.1 Strengthening STI surveillance in resource-poor countries

As described in previous chapters, the effects of STIs on HIV transmission have been documented in many studies, including those conducted in South Africa. It is obvious that the population-attributable fraction (PAF) of STI co-factor effects on new HIV infections in a population is highly dependent on the phase of HIV epidemic. It is estimated that PAF of STI co-factor effects could be significantly greater in populations during the growth phase of the HIV epidemic than in those populations experiencing a mature HIV epidemic[1].

In all the study populations, we observed that high level STI epidemics preceded the explosive spread of HIV infection among high-risk individuals. Following this early phase of the epidemic, HIV infection spread into family units or ill-defined sexual networks, finally resulting in wide-spread transmission within the general population[2-3].

Several groups have explored the potential use of the incidence and prevalence of conventional STIs as important measures of risk behaviour for HIV[4-7]. Based on these experiences, it has been proposed to use the incidence of selective STIs as biological markers for the potential risk of spread of HIV infection in a population[8.] In 2002, WHO conducted an expert consultation meeting in Treviso, Italy to devise methods for the estimation of the global incidence of STIs and their association with HIV infection. The meeting recommended the selection of proxy STI indicators to monitor the potential risk of HIV infection in a population[9]. The complexity of natural history and clinical presentation of STIs, however, imposed a major barrier to the implementation of comprehensive STI surveillance systems to generate interpretable data on what the WHO recommendations are based, particularly in resource-poor countries. As such, the quality of STI surveillance data may vary between countries and the interpretation of STI data could often be difficult.

WHO and UNAIDS have recommended strengthening STI surveillance as an essential component of second-generation surveillance of HIV infection. In order to improve STI surveillance activities, WHO and UNAIDS issued guidelines for sexually transmitted infection surveillance in 1999[10]. The studies reported here demonstrate the importance of triangulating data collected from different recommended STI surveillance components, using a tiered surveillance approach. This approach provides useful information on both temporal changes in incidence of STI syndromes as well as changes in aetiological patterns of specific STI syndromes due to emerging or re-emerging STIs, for example, the increasing role of genital herpes in the aetiology of GUD in recent years[11-16].

8.2 Strength of interactions between HIV and different STIs

We observed that conventional STIs vary in the magnitudes of interaction with HIV infection, as has been documented in several studies[17-18]. We found particularly strong interactions between genital herpes and HIV. At the individual level, HIV-seropositive patients with genital herpes were more frequently found to have atypical clinical presentations, delays in spontaneous healing, a longer duration of HSV shedding and increased association with HIV shedding from ulcer and genital exudates. These findings were similar to those found in studies conducted elsewhere[19-21].

As was the case with HSV infection, there was a strong association between HIV and HPV infection in both men and women. A higher prevalence of HPV infection was found among HIV-seropositive patients in our study population and this may reflect the higher frequency of recurrences and/or longer duration of infection (i.e. persistency). The interaction between HIV and HPV types associated with a higher risk for cervical cancer could influence future trends of cervical cancer in southern Africa. Cervical cancer causes high morbidity and mortality in women in southern Africa and demands innovative strategies for its containment. The role of targeted and enhanced cervical cancer screening in women who are positive for high-risk HPV types should be further explored to reduce premature mortality due to cervical cancer in the region.

Some interactions between syphilis and HIV infection were difficult to assess in our outpatient setting, as specialised investigations such as CSF and ophthalmological examinations are not performed routinely. However, we found that the biological false positive reactions in syphilis serology (i.e. RPR) are not a common occurrence in our HIV-seropositive study population. On the other hand, syphilis serology could be falsely negative in patients with PCR-confirmed primary syphilis who are co-infected with HIV and other aetiological agents causing GUD. We confirmed that the recommended treatment of syphilis provided effective clinical and microbiological cure in HIV-seropositive patients with primary syphilis.

The clinical presentation and treatment responses of cases of chancroid were similar in HIV-seropositive and HIV-seronegative patients. Also, mixed infections involving chancroid and genital herpes were found to be common, particularly in HIV-seropositive patients (Section 3). The effectiveness of syndromic treatment targeting only bacterial causes of genital ulceration was significantly reduced due to the persistent ulcerations as a result of co-infection with genital herpes. The need for corrective action to include genital herpes therapy in the syndromic management protocol has been discussed at WHO[22]. Further research studies to explore the effect of episodic and suppressive herpes therapy on HIV transmission are currently underway[23].

8.3 Impact of co-existing HIV epidemic on control of STIs

Efforts for the control and elimination of STIs in southern Africa have been affected positively as well as negatively by the co-existing HIV epidemic. After a decade of neglect in controlling the hyper-endemic STI situation in the region, measures to prevent and control of STI have been incorporated into a core intervention program to contain the HIV epidemic in many affected southern African countries. More resources were made available for STI control programmes during the 1990s and many outreach and innovative STI intervention activities were implemented. Among these activities, the introduction of syndromic management of STIs played a crucial role in controlling curable STIs and in the process achieved a significant reduction in the duration of the infectiousness of bacterial STIs[24-25]. The implementation of region-wide surveillance programmes including detection of anti-microbial resistance among

bacterial STIs provided valuable information for selecting the best treatment algorithms, particularly for gonorrhoea and chancroid[26-27]. Population-based cross-sectional prevalence studies were conducted among low- and high-risk populations and the results of these studies provided a better understanding of the burden of infection due to asymptomatic or inapparent infectious individuals[28]. As a result, the focus of intervention activities has been extended to involve asymptomatic infectious individuals in the community through various outreach intervention activities. Important intervention activities include periodic presumptive therapy for high-risk women in mining areas (e.g. Lesedi and Mothusimpilo Projects) and enhanced partner notification and treatment at municipal clinics. Our studies have clearly shown the impact of these intervention activities on the incidence of curable STIs among the mine workers who were their main clients. Enhanced screening and treatment of syphilis in high-risk population groups (e.g. all patients with new STI episodes) has led to a rapid decline in syphilis seropositivity in these groups. In some areas, the prevalence of RPR positivity among STI patients has reached a lower level than that of pregnant women. Overall, the improvement in community awareness on safer sex practices and STI services has led to a rapid decline in incidence and prevalence of STIs in the region.

Meanwhile, co-infections involving conventional STIs and HIV were highly prevalent among high-risk population groups in the region during the study period. This situation has contributed to more frequent occurrences of atypical presentations, altered natural history of STIs, and reduction in effectiveness of some syndromic STI treatment algorithms – especially as a result of herpes co-infections.

In our studies, the relative prevalence as well as absolute number of cases of genital herpes have increased significantly and a stronger association was found between herpes and HIV-seropositivity than other STIs. The atypical presentations, lack of spontaneous healing and long duration of HIV shedding from herpetic ulcerations are issues of major concern for the containment of spread of HSV and HIV in the region. In our cross-sectional study on GUD aetiology, we reported that an increasing proportion of HIV-seronegative patients also presented with genital herpes. In addition, a high seroprevalence of HSV-2 was found in HIV-

seronegative individuals. The impact of increasing prevalence of a viral ulcerative STI with known potential of causing recurrent clinical or sub-clinical ulcerations among sexually active population could be an additional facilitating factor for continued spread of HIV in the region. Based on our study findings, a firm recommendation should be made to include anti-herpes therapy in the syndromic management algorithms for GUD in areas where high proportion of GUDs is attributed to genital herpes. Some countries such as Botswana have already established a national plan to provide episodic treatment of genital herpes at public clinics[29-30].

We also found high levels of association between HPV and HIV infection in our study population. We found that the most widely known determinants of spread of HPV infection such as the number of life-time sexual partners, age at sexual debut and a high prevalence of hormonal contraceptive use are very common in high-risk women. Together with high a prevalence of HIV, there is potential for continued increase in the number of HPV infections among women in southern African. Although it was not statistically significant, our study reported a high prevalence of HPV types associated with higher cervical cancer risk (high-risk HPV) among older women and the prevalence was higher in HIV-seropositive older women. Estimating the potential impact of such a high prevalence of HIV and HPV co-infection on the incidence of cervical cancer is complex. The causal relationship between high-risk HPV infections and cervical cancer has been well documented and the probability of progression could be higher in patient with persistent or chronic infections[31-33]. Cervical cancer has been included as a AIDS-defining illness in developed countries as the incidence of this condition is higher among HIV-seropositive women[34]. The excess morbidity and mortality of cervical cancer as a result of the co-existing HIV epidemic in developing countries has, however, not been documented in the literature. It is possible that the HIV epidemic may increase the burden of HPV infection and cervical cancer in developing countries and this effect may become more visible with longer life-expectancy in HIV-seropositive women due to the anticipated increase in accessibility to HAART.

8.4 Role of HIV viral load in transmission of HIV

Our studies demonstrated unique patterns of HIV shedding from genital fluid in patients co-infected with STI and HIV. The majority of patients with genital ulcerations shed HIV in their ulcer exudates; therefore, their infectiousness could be increased in settings where continued, unprotected sexual activity in the presence of active ulceration is common. HIV shedding in ulcer exudates also continued when there were treatment failures as a result of failed chancroid therapy or untreated genital herpes. The successful treatment of herpes in men and women was found to be associated with a decline or cessation in HIV shedding into ulcer exudates or genital fluid. Some HIV-seropositive patients with large ulcerations did not shed HIV in the ulcer exudates. We found that HIV-shedding in genital ulcer exudates was strongly associated with HIV PVL. A similar finding was also demonstrated in women presenting with vaginal discharge. Our studies have shown that HIV viral load is the main determinant for HIV shedding in both men and women presenting with STIs. This finding has been supported by other studies conducted elsewhere[35-36]. It is possible that the role of the STI co-factor effect in HIV shedding might be much reduced or negligible in patients with a high HIV plasma viral load (e.g. primary HIV infection, immunocompromised patients etc.). It is also important that the potential effect of increased accessibility of highly active anti-retroviral therapy (HAART) on HIV shedding in patients with conventional STIs should be investigated[37].

8.5 STI co-factor effect on HIV transmission

The determinants of sexual transmission of HIV in developing countries are many and complex and their dynamics should be assessed in a wider social, economic and behavioural context. It would appear that the interrelationships between behavioural, biological and immunological determinants vary depending on different phases of HIV epidemic. It is however, difficult but not impossible to quantify the STI co-factor effect on HIV transmission. Several attempts have been made to measure STI co-factor effects through community-based randomised controlled intervention trials, as well as by utilizing various mathematical modelling methods[38].

An improved understanding of co-factor effects would contribute to the better preparedness and implementation of more effective interventions, and quality monitoring and evaluation activities for the containment of both the STI and HIV epidemics. In fact, morbidity and mortality due to conventional STIs are still very high in many developing countries and effective STI intervention activities are required to reduce the burden of these diseases. Such activities could have a considerable impact on the course of HIV infection in specific population groups or countries. Caution should however be exercised as the overall magnitude of the impact may vary according to prevailing local determinants including broader issues such as social structure, behavioural patterns, STI prevalence and the stage of the HIV epidemic at a specific location or country.

8.6 Limitations of studies reported in this thesis

Major limitations of our studies include those associated with the cross-sectional study design of some of our studies, and the inherent poor follow-up rate associated with highly mobile population groups in selective short-term follow-up studies.

Our studies measured the associations rather that causal relationships since most information was collected from cross-sectional facility-based studies. A community-based randomised control study design could not be implemented due to the constraints in resources. We provided however strong support to monitoring and evaluation studies of community-based and outreach intervention activities implemented in the study areas. The findings of these studies have been published elsewhere[39-41].

Although the size of the mine workforce in Goldfields of South Africa mines is relatively stable, the total mine population served by our research clinic declined slightly during the study period owing to the restructuring of smaller mines. No significant differences in demographic characteristics and STI incidence were however observed between miners employed in the restructured smaller mines and those in the remaining mines.

The changing trends of STI syndromes and their aetiological patterns among goldminers might have been affected by other confounding factors such as temporal changes in health seeking behaviour for specific STIs, changes in clinical diagnosis criteria, and changes in laboratory methods. Although we did not conduct health-seeking behavioural studies in the mines during the study period, the utilization of STI services was relatively stable throughout the years. Provision of STI services in the mines are incorporated in the primary health care services of the Goldfield Mines and therefore all STI patients receive services free-of-charge. We are confident that the majority of STI episodes occurring in the serving mines were treated at our reference clinic although some patients may have been treated at a mine hospital outpatient facilities. The trends in prevalence of individual STI syndromes and their aetiologies were monitored solely by our study team during late 1980s till 2001, using standard clinical surveillance and laboratory criteria. It therefore provided good surrogate information relating to the STI situation among migrant miners during the study period.

The changing performance of laboratory tests also influenced temporal changes in aetiological patterns of STI syndromes. In our study, the majority of laboratory tests were conducted in the Reference Centre for STIs (RCSTI) at the South African Institute for Medical Research (SAIMR) and other collaborating laboratories such as National Institute for Virology (NIV), South Africa and the Centers for Disease Control and Prevention (CDC), USA. Comparable laboratory methods were included in all studies although newly developed and more sensitive tests were employed where available. Although the prevalence of STIs was assessed using the most sensitive and specific laboratory techniques available, temporal trends were compared by using both original and new laboratory methods.

HIV shedding in genital fluid was studied as a surrogate measure for increased infectiousness of HIV-seropositive patients with STIs. Our study has shown some important findings about the determinants of HIV shedding in genital ulcerations and cervico-vaginal lavages. We could not however measure the level of HIV shedding in seminal fluid as all male patients enrolled in the study had active ulcerations and collection of semen was considered problematic in our study population. During informal discussion with the investigator, the

majority of patients consulted expressed reservation on donating semen even after their STIs had resolved.

Caution should be exercised in the interpretation of HIV shedding which we used as a surrogate marker for infectiousness of HIV-seropositive patients. Follow-up studies using discordant couples are the best way to measure determinants of HIV transmission[42-44]. These types of studies are not feasible in study populations comprising migrant miners or highly mobile groups (such as inner city populations). Lack of commitment of female patients to return for repeated follow-up visits was the most important limitation for the studies conducted at the Esselen Street STI clinic. To improve the quality of Pap smears, we made an attempt to collect Pap smear specimens after completion of antibiotic therapy. The majority of patients however could not commit themselves to return for follow-up appointments. For this reason, Pap smear specimens were collected at enrollment only. This problem also negated our plans to conduct a long-term follow-up programme for HPV-positive patients.

We found that multiple vaginal and cervical infections were very common in our study population, and that syndromic treatment of vaginal discharge often failed to achieve microbiological cure on completion of treatment, particularly for bacterial vaginosis and vaginal candidiasis. Limitations of the study on vaginal shedding of HIV included the small sample size and multiple potential determinants associated with HIV shedding in genital fluid. Although we collected information on all possible determinants, the small sample size limited the analysis of our finding. HIV PVL was however consistently associated with HIV shedding in our study. Further studies should be conducted using larger sample sizes in easy-to-follow up population groups such as women attending family planning clinics.

Overall, our studies explored the interactions between HIV and STIs in southern Africa through research studies integrated with the routine patient care and the surveillance activities of RCSTI. The results of these studies provided valuable inputs to improve STI surveillance, prevention and management in the region. Despite the aforementioned inherent limitations associated with facility-based studies, several research questions have been

addressed and answers provided based on our findings. Randomised controlled trials are underway in South Africa to explore further the relative importance of HSV-2 infection in acquisition of HIV infection.

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