FACTORS ASSOCIATED WITH ABNORMAL CERVICAL SMEARS IN HIV NEGATIVE WOMEN IN SOWETO

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Master of Public Health [Health measurement]

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DECLARATION

I, Adolphus Qedusizi Mntambo, declare that this research report is my own work. It is being submitted for the degree of Master of Public Health [Health measurement] in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any institution.

Signed:_____

On the:_____day of_____,_____

At: Johannesburg

DEDICATION

This research report is dedicated to patients who died, prematurely, from cervical cancer.

I also dedicate it to my wife who supported me.

PUBLICATIONS AND PRESENTATIONS

This work has not been published or presented before.

ABSTRACT

Introduction: Cervical cancer is caused by persistent infection with high-risk Human Papilloma Virus (HPV) and is a leading cause of cancer deaths in South African women aged 15-65years. We estimated prevalence of abnormal (Atypical squmous cells of unknown significance to invasive cervical cancer) cervical cytology and associated co-factors in 18-35-year old women who tested negative for Human Immuno deficiency Virus (HIV).

Method: This cervical lesion study was secondary analysis of data collected during a Microbicide Feasibility Study (MFS). MFS recruited 1100 women from public health care facilities. Women were interviewed and socio-demographic, sexual behaviour and clinical information was collected. If HIV negative, cervical and vaginal swabs were collected for Pap smear and laboratory testing for sexually-transmitted infections (STI). For the cervical lesion study, 808 women were eligible and 752 were enrolled in the study. Associations with abnormal cervical cytology were analysed using multiple logistic regression, and were reported as adjusted odds ratio (AOR) with a 95% confidence interval (CI).

Results: We analysed 570 cytology specimens. Prevalence of abnormal cervical cytology was 6.7% (95% CI 4.8-9.0). Women who had an abnormal cervical cytology result were more likely than those with normal cytology results to report abnormal vaginal discharge (OR 2.33; 95% CI 1.07-5.06; p=0.03). They were also more likely to have more than one child (OR 2.21; 95% CI 1.00-4.87; p=0.05).

Discussion and conclusion: Our study showed that LSIL is common in this younger age group. Because HPV infection and thus abnormal cervical cytology are high among the younger population, this result is not unexpected. Since most LSIL regress naturally, our data support the current South African screening protocol for cervical cancer.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

BV:	Bacterial vaginosis
CI:	Confidence interval
CIN:	Cervical intraepithelial neoplasia
CT:	*Chlamydia trachomatis*
HIV:	Human immunodefiency virus
HSV-2:	Herpes simplex virus (type 2)
HPV:	Human papillomavirus
HR-HPV:	High-risk Human papillomavirus
IARC:	International Agency for Research on Cancer
ICC:	Invasive cervical cancer
ISC:	*In-situ* cancer
IQR:	Inter-quartile range
NCR:	National Cancer Registry (South Africa)
NG:	*Neisseria gonorrhoeae*
OC:	Oral contraceptive
OR:	Odds ratio
AOR:	Adjusted odds ratio
Pap:	Papanicolaou
SIR:	Standardized incidence ratio
STI:	Sexually transmitted infection
TPHA:	*Treponema pallidum* haemagglutination test
TV:	*Trichomona vaginalis*

1.0 INTRODUCTION

Burden of cervical cancer

Cervical cancer is the second most common cancer in women throughout the world, and the most common cancer in women in low and middle income countries. About 371, 000 new cases of cervical cancer are diagnosed in the low and middle income countries each year (Parkin, 2005). Of the estimated 300 000 cervical cancer deaths annually, about 80% are from low and middle income countries (Parkin, 2005). According to the South African National Cancer Registry (NCR), black females (aged 0-74 years) had a lifetime probability of developing cervical cancer of 4.34 (1 in 23) in 1997 compared with American women (all races), who had a probability of 0.78 (1 in 129) (Mqoqi, 2003 and American Cancer Society, 2001). From figure 1, cervical cancer is leading cause of cancer deaths in women in South Africa.

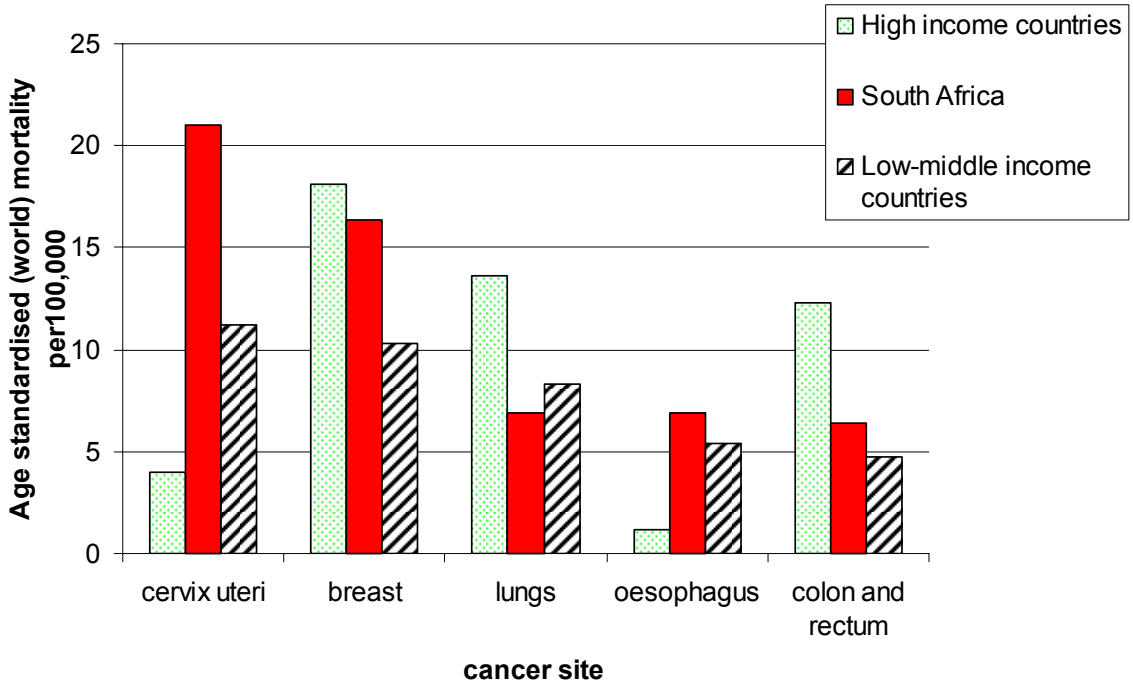


Figure 1: Top five cancer-related mortality rates in women, worldwide. (Ferlay, 2004)

Cervical cancer incidence increases sharply with age among South African women. In 2002, age-specific incidence rates (per 100 000 women per year) of cervical cancer was 19.6, 104 and 118 for women in the age groups: 15-44years, 45-54years and 55-64years. These rates were high compared with: 9, 44 and 50 for women in the world in the age groups: 15-44years, 45-54years and 55-64years. (Ferlay, 2004). The above cancer statistics highlight high levels of disparity in the burden of disease between high and low to middle income countries.

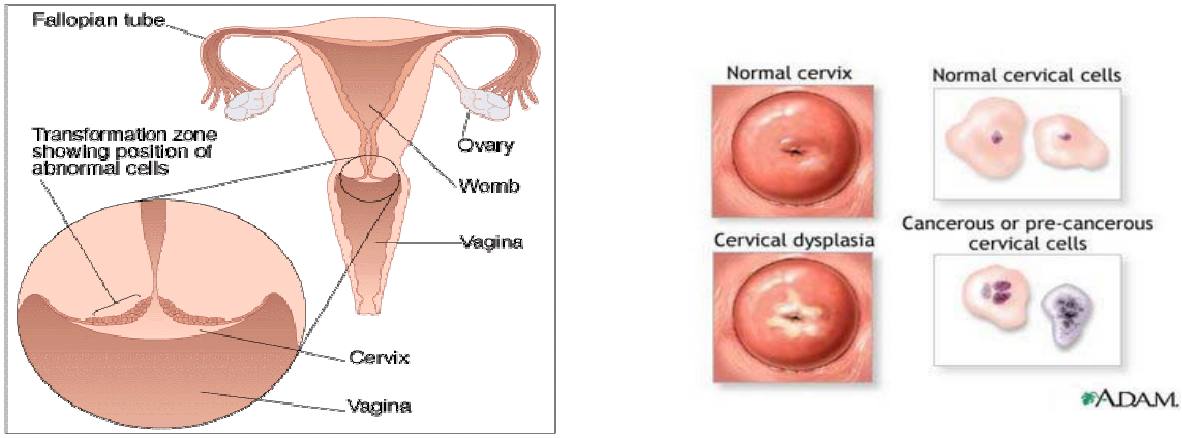
Squamous cell carcinoma

Squamous cell carcinoma accounts for approximately 80% of all cervical cancers. The second most common form of cervical cancer is adenocarcinoma (Dolinsky, 2002). Due to hormonal, physical and infectious influences, the cervical surface epithelia change throughout a woman's lifetime. This change may promote or interrupt cervical diseases development. Some of the changes include cervical ectopy, which exposes the transformation zone to infections. (Coombs, 2003). The transformation zone is a site of cell-structure change (from columnar to squamous cells) and lies just inside the cervix os, as shown in figure 2a. In cervical ectopy, the transformation zone becomes exposed.

Human papillomavirus (HPV) in the aetiology of cervical cancer

HPV is a double-stranded DNA-virus with a closed circular genome. It is a member of the Papovaviridae family. Over 100 HPV sub-types have been characterized molecularly and about 40 can infect the genital tract and can cause cancer or warts. (Bosch, 2005). HPV is a common sexually-transmitted infection (STI). Approximately 50% of sexually-

active men and women aged 15-49 years have been infected with HPV at some point in their lives (Schiffman, 2003 and Manhart, 2002).



a) Transformation zone (Cancerhelp, 2008) b) Cervix (Medlineplus, 2008)

Figure 2: Diagram showing (a) transformation zone close-up & (b) structure of the cervix

Almost all cases of cervical cancer are associated with high-risk HPV (HR-HPV). A matched case-control study found a 99.7% prevalence of HPV DNA in cervical cancer cases (Walboomers, 1999). HPV-16 and -18 cause almost half of all cervical cancers (Schiffman, 2003). Other high risk sub-types include HPV-45, -31, -33, -52, -58 and -35 (Munoz, 2006). Oncogenic HPV sub-types are thought to cause the production of two proteins, E6 and E7. These proteins turn off some host tumour-suppressor genes (American Cancer Society, 2005a). Turning off of these genes may allow uncontrolled growth of cells lining the cervix.

HPV-16 and HPV-18 have distinguishing biochemical and biological activities compared to low-risk genital HPV types. For example: HPV-16 and HPV-18 oncoprotiens E6/E7 bind p53/pRB protein, respectively, with a higher affinity than the corresponding LR-HPV proteins and an enhanced potential of HPV-16 and HPV-18 E2 proteins to activate transcription. (Pablo, 2005)

Natural history of HPV infection

HPV is a necessary, but not a sufficient cause of cervical cancer. As shown in figure 3 below, about half of HPV infections are transient and are cleared in less than two years (Claeys, 2002). Also, about 60% of low grade intraepithelial lesions may regress to normal. If HPV persists, cervical lesions form and may progress and develop into invasive cancer (Schiffman, 2003; Manhart, 2002; Munoz, 1997).

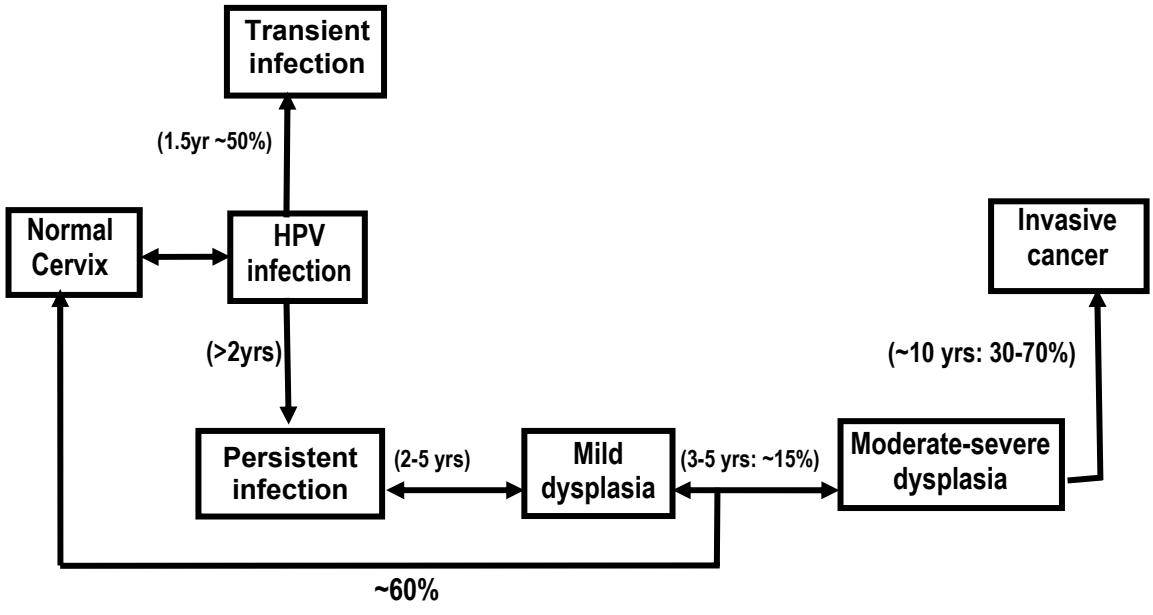


Figure 3: Natural history of cervical carcinogenesis (Schiffman, 2003 & Munoz, 1997).

Co-factors in the development of cervical cancer

While HPV is primary aetiological agent in cervical cancer, additional factors have been implicated in HPV persistence and progression and the development of cervical cancer. These factors include:

Age

Because cervical cancer takes many years to develop (see figure3), it is generally more common among older women. A multi-center prevalence survey was conducted in South Africa, to describe age-specific prevalence rates of cervical cancer in South African women presenting for screening. Results of this survey showed that cervical cancer is common among older women. Women with LSIL (2.42% of women screened) had an average age of 33.1 years, while women with HSIL (1.8%) had an average age of 37.97 years and women with invasive cancer (0.47%) had an average of 51.3 years. These age differences were statistically significant (Fonn, 2002).

In other settings, cervical cancer may develop at younger ages (<35 years) and dysplasia may progress more quickly to invasive disease (Jennings, 1992). Lancaster (1999) studied 10 000 Papanicolaou (Pap) smears from Ka-Ngwane (South Africa) and found that 78% of CIN III cases were 40 years old or younger. A descriptive case series in a population of sexually-active adolescents in New York found an increase in the prevalence of benign cellular changes from 8.7% in 1982-3 to 20.1% in 1992-3; prevalence OR 2.7; 95% CI 1.9-3.8) (Mangan, 1997).

There is evidence showing that regression or progression of pre-invasive cervical cancer is greatly influenced age of the patient. A modelling study was conducted, to assess regression of pre-invasive cervical cancer, using data from British Columbia Pap smear screening programme. This study showed that for women aged 18-34 years, 84% (95% CI 76-92%) of the new lesions will regress spontaneously. This regression rate was significantly reduced to 40% for women over the age of 34 years. (van Oortmarssen, 1991)

Genetic factors

The genetic background of the host may influence persistence of HPV infection. Among genetic factors thought to be involved in the susceptibility to squamous cell cervical cancer and disease outcome, two have been intensely investigated. These are the polymorphic genes of the major histocompatibility complex (MHC (viz. the human leukocyte antigen (HLA) complex)), as well as a particular polymorphism in the p53 gene (Maciag, 1999). MHC genes encode proteins involved in antigen presentation to T-cells (both $CD4^+$ and $CD8^+$ T-cells) and p53 is a tumor suppressor gene that regulates cell proliferation. In a case control study conducted in Montreal by Ades (2008), the HLA variant (B7-DRB1*1501-DQB1*0602 haplotype) was associated with a 83% reduction in risk of HSIL among HPV-16 and HPV-18 positive subjects (OR 0.17; 95% CI 0.05-0.54). Similar associations were also observed among Southern Chinese women (Chan, 2007).

Brown (2008) carried out series of experiments to study the interaction between HPV-16 DNA components and host p53. Their results suggested that p53 down-regulated HPV-16 DNA replication via the E2 protein. HPV-host genetic interaction depends, to a large extent, on the population and the HPV subtype being studied.

Gynaecological factors

High parity is thought to increase the risk of developing cervical cancer through the maintenance of the transformation zone on the exocervix for many years. This may facilitate exposure to HPV. Hormonal factors may also be involved in this process. (Munoz, 2006). A case-control study conducted in Mali, among 82 invasive cancer cases and 97 controls, showed that high parity and poor genital hygiene conditions were the main co-factors for cervical cancer in this population (Bayo, 2002). More recently, in a meta-analysis of 10 case-control studies, HPV-positive women with more than one child were at a higher risk of squamous-cell carcinoma of the cervix compared to those with no children, (OR 2.3; 95% CI 1.6-3.2). Women with more than six full term pregnancies were even much more likely, than those with no full-term pregnancies, to have squamous-cell carcinoma of the cervix (OR 3.8; 95% CI 2.7-5.5). Younger age at first full-term pregnancy was independently associated with an increased risk of ICC (risk ratio (RR) 1.07; 95% CI 1.06-1.09) for each decline in year. (Munoz, 2002).

Hormonal contraceptives

The use of hormonal contraceptives changes hormone levels which may disturb the normal environment and facilitate cervical infection. In a systematic review of 28 studies, together including 12,531 women with cervical cancer, oral contraceptive (OC) users had a greater risk of developing cervical cancer (Odds ratio (OR) 2.5; 95% CI 1.6-3.9) compared to non-users (OR 1.1; 95% CI 1.1-2.2) (Smith, 2003).

In 2002, the International Agency for Research on Cancer (IARC) conducted a meta-analysis of ten case control studies. This analysis was restricted only to women with a positive HPV DNA test. The risk of cervical cancer was higher in women who had used OC for ten or more years compared with never-users (RR 4.03; 95% CI 2.09-8.02) (Moreno, 2002). Because of HPV DNA assay inclusion, this analysis provided stronger evidence of an association between prolonged hormonal contraceptive use and risk of cervical cancer.

However, a case-control study conducted in South Africa, on 524 invasive cervical cancer cases and 1541 controls (including 254 HPV-positive controls) found no evidence suggesting that either injectable progestin-only or combined estrogen/progestogen OC increase the risk of clinically evident invasive cancer of the cervix. Progestin-only users were no more likely, than combined estrogen/progestogen OC-users, to have invasive cervical cancer, (adjusted (A) OR 1.0; 95% CI 0.8-1.3 versus AOR 0.8; 95% CI: 0.7-1.1) (Shapiro, 2003). Behavioural differences between OC users and other women may

confound the relationship and make it difficult to study the role of OC use in genital tract diseases.

Co-infection with HIV

Because HIV attacks the immune system, co-infection with HR-HPV and HIV undermines the ability of the immune system to fight the HPV. In this way, HIV may cause HPV persistence or speed up progression to invasive cervical cancer (ICC). This synergy is of concern especially in Southern Africa where HIV prevalence is high and anti-retroviral therapy (ART) is not widely accessible.

A case control study (138 cases and 138 controls) conducted in Tanzania found that HIV-1 positive women were more likely to have ICC compared with HIV-1 negative women (OR 2.9; 95% CI 1.4-5.9). Another case control study conducted in the Western Cape (South Africa) observed a strong association between squamous intraepithelial lesions (SIL) and co-infection with HIV and high risk HPV compared to women with neither infection (AOR 41.3; 95% CI 18.8-90.5). But there was no excess risk of invasive cervical cancer (ICC) (AOR 1.17; 95% CI 0.75-1.85). (Moodley, 2006). Sitas (2000) examined the relationship between HIV-1 and a number of cancer types that are common in South Africa. Their study focused in the Johannesburg area and used case control design. Women who were HIV positive were more likely, than those who were HIV negative, to have cervical cancer (OR 1.6; 95%CI 1.1-2.3).

Adam (2008) followed up a cohort of 575 women attending the colposcopy clinic at Chris Hani Baragwanath Hospital, Soweto, South Africa, to investigate clinical predictors of persistent cytological abnormalities in women who had had a large loop excision of the transformation zone (LLETZ). Half (49.4%) of the patients had persistent abnormal smears six months after LLETZ treatment. Of those patients who had persistent abnormality, 64% were HIV positive and 13% HIV negative, $p<0.001$. Of those patients who were HIV positive and had persistent cytological abnormality, 56% had CD4 count <200 versus 24% with CD4 count >499, $p<0.001$. Since highly active antiretroviral therapy (HAART) has recently been publicly-introduced in South Africa, it is unclear as to what effect this will have on excess mortality due cervical cancer.

Sexual behaviour and STI

Other factors which increase a woman's risk of getting HPV infection include having sex at an early age, having many sexual partners (lifetime or concurrent) and having sex with an uncircumcised male (Castellsague, 2001). These factors are all indicators of higher risk sexual behaviour and may be markers for infection with an STI.

A study of 1000 women seeking STI services in inner city Baltimore found evidence linking *Chlamydia trachomatis* (CT) to abnormal Pap smear (OR 2.04; 95% CI 1.0-4.15) (Kanno, 2005). In a longitudinal cohort study carried out to determine whether gynaecological infections other than HPV are related to the risk of cervical cancer or not found that both Trichomona vaginalis (TV) and positive *Herpes simplex virus*-2 (HSV-2) serology were predictors of cervical neoplasia, with standardized incidence ratio (SIR) of

6.4 (95% CI 3.7-10.0) and 12.0 (95% CI 2.4-34.0) respectively (Viikki, 2000). It is unclear if other agents operate independently from HPV or whether these associations are merely confounded by sexual behaviour. Other studies have found no evidence of association between cervical cancer and H*erpes simplex virus*-2 (RR 0.9; 95%CI 0.6-1.3) (Lehtinen, 2002), *Chlamydia trachomatis* (p=0.4) (Ghazal-Aswald, 2006) and again *Chlamydia trachomatis* (p>0.05) (Takac, 1999). These latter four studies should be interpreted with caution though since they have some methodological weaknesses which may diminish their power to detect association between these STI and cervical abnormality. These weaknesses included small sample sizes and use of *Chlamydia trachomatis* test kit with lower sensitivity in a low-prevalence population.

Cervical inflammation and inflammatory response

Evidence of an association between specific STI and cervical cancer is inconclusive. This has lead to continued search for other HPV cofactors in the development of cervical cancer. Tjiong (1999) studied cervicovaginal washings to establish whether inflammatory cytokines could be found in relation to cervical neoplasia. The concentrations of interleukin-6 (IL-6) and interleukin-8 (IL-8) increased with severity of cervical neoplasia.

Inflammation (and smoking) increase reactive oxygen species (oxygen-related free radicals), reduce antioxidant activity and adversely affect natural history of HPV infections. Free radicals cause genotoxic damage of host DNA (Castle, 2003) and HPV-infected cells can not effectively clear these cells. There is no evidence to suggest that

HPV alone induce inflammatory response (Castle, 2001). So, inflammatory response could be confidently linked to HPV cofactors rather than the HPV infection itself.

Smoking

Cigarette contains carcinogenic ingredients and cigarette smoking is linked to several cancers, notably as a cause of lung cancer. Cigarette smoking may reduce immunoresponse in the cervix, affect female hormone metabolism or cause direct genetic damage. A case-control study carried out in the USA among women exposed to oncogenic types of HPV found that current smoking was associated with a 2-fold increase in risk of cervical cancer (95% CI 1.2-2.8). In current smokers, there was a significant trend of increased risk with number of cigarettes smoked per day (p for trend = 0.003), but not with duration of smoking. (Shields, 2004).

Socio-economic status

Women from low socio-economic background have been shown to have an increased risk of developing cervical cancer. Poor access to screening is responsible for this disparity. Sub-standard nutritional status is another possible risk for women from impoverished backgrounds to develop cancer more than their affluent counterparts.

A case control study conducted in Bangkok found that a high intake of foods rich in vitamin A, especially high-retinol foods, was associated with a reduced risk of *in-situ* disease (OR 0.09; 95%CI 0.02-0.5) (Shannon, 2002). A literature review conducted by Garcia-Closas (2005) found no convincing evidence for an association between diet and

nutritional status and cervical carcinogenesis. But, this review found some biologically-credible association between fruits and vegetable intake and HPV clearance. There is some support for the hypothesis that antioxidant nutrients may play a protective role in cervical carcinogenesis. However, current available evidence for an association between diet, nutritional status and cervical HPV carcinogenesis is not yet convincing.

Cervical cancer is a preventable disease:

Primary prevention of cervical cancer

Condoms are effective in preventing HIV and other STI, but not HPV. Condoms' failure to prevent HPV is linked to the fact that HPV is highly transmissible and can be passed by skin-to-skin contact with any HPV-infected area not covered by the condom. Condoms do reduce the risk of developing cervical cancer. (American Cancer Society, 2005b). Condom use was shown to be associated with higher rates of regression of cervical intraepithelial neoplasia and clearance of cervical HPV infection in women and with HPV-associated penile lesions in men (Holmes, 2004).

A meta-analysis of twenty studies was conducted to determine whether condom use prevents genital HPV infection and HPV-related conditions or not. This analysis found inconsistent evidence that condom use reduces the risk of becoming HPV DNA-positive (Manhart, 2002). It is therefore likely that the relationship between condoms and reduced cervical cancer prevalence is linked to condom's ability to prevent the other STI, which are cofactors in the development of cervical cancer once HPV infection has occurred.

Other promising primary prevention strategies are vaccines, microbicides that are effective against HPV and male circumcision (MC). A review paper on HPV vaccines reported on a bivalent vaccine which incorporates HPV-16 and -18 and a quadrivalent vaccine formulated to protect against genital wart-causing HPV-6 and -11 and HPV-16 and -18. Both these vaccines were shown to be safe and over 90% efficacious in preventing HPV infections and pre-cancerous lesions (Ault, 2006). Microbicides are novel products that prevent STI *in vitro*. They are currently in phase 3 clinical trials. Since first generation microbicides will be non-specific, they may be more likely, than subsequent generations, to be active against HPV. In a review by MC has been shown to be about 48-60% efficacious in preventing HIV acquisition in men. Its role in preventing HPV has yet to be confirmed.

Secondary prevention of cervical cancer

Secondary prevention using Pap smear screening is currently the cornerstone of current cervical cancer prevention programmes. It relies on detecting sub-clinical changes in populations at risk of developing cervical cancer, and then treating these abnormalities to prevent progression to an advanced stage. The Bethesda System (TBS) is the most widely used system for describing cervical smear cytology results. This system was developed in 1988 and was last revised in 2001. The three main categories in TBS are shown in box 1 below (Apgar, 2003).

Box 1: Three main categories in the TBS:

1. Negative for intraepithelial lesion or malignancy: this category means that no signs of cancer or precancerous changes or other significant abnormalities were found.

2. Epithelial cell abnormalities: the cells of the lining layer of the cervix show changes that might be cancer or precancerous condition. This category is divided into several groups for squamous cells and glandular cells. The epithelial cell abnormalities for squamous cells are called:

- Atypical squamous cells (ASC). ASCs are further divided into ASCUS (atypical squamous cells of unknown significance) and ASCH (atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions)
- Low-grade squamous intraepithelial lesions (LSILs)
- High-grade (H) SILs: these are more likely than LSILs to develop into cancer if they are untreated.
- Squamous cell carcinoma: this shows that the woman is likely to have an invasive squamous cell cancer.

3. Other malignant neoplasms: these can be uncommon forms of cancer and affect cervix very rarely (Fonn, 2002).

Cervical cancer screening procedures using cytology, especially follow-ups and costs associated with cytology, limit access to screening. Laboratory processing, sample transport and patient time account for a significant proportion of total cervical cancer screening costs in developing countries (including South Africa) Goldhaber (2006). Alternative methods of cervical cancer screening have been implemented, especially in low-resourced countries. These methods include: naked eye visual inspection of the cervix uteri after applying diluted acetic acid (VIA), or Lugol's iodine (VILI) or with a

magnifying device (VIAM). Other settings also test for human papillomavirus DNA using high risk probe of the Hybrid Capture-2 assay (HC2).

In a study of 2944 women aged 35-65 years in Cape Town, direct visual inspection with acetic acid and HPV DNA testing identified similar numbers of high grade squamous intra-epithelial lesions and invasive carcinoma cases as Pap smear did, but with lower costs (Denny, 2000). Goldie (2005) showed that very cost-effective strategies in South Africa are those that require the fewest visits (such as HPV DNA testing).

A meta-analysis of 11 studies, conducted in Africa and India, was undertaken to assess the accuracy of five cervical cancer screening tests (VIA, VILI, VIAM, Pap smear and HC2). All participants underwent colposcopy examination and abnormal colposcopy patients had punch biopsies taken. Visual tests and colposcopy were highly correlated. Sensitivity and specificity for the visual tests were over 79% and 84%, respectively. Sensitivity and specificity for Pap smear was 57% and 93%, respectively and HC-2 test showed a sensitivity of 62% and a specificity of 94%.

South African screening guidelines recommend that each woman receives three Pap smear screenings in her lifetime, with a ten-year interval between smears, commencing at not earlier than age 30 years (National Department of Health-SA, 1997). This screening programme was introduced in year 1997 and uses age as a risk factor for cervical cancer, because mild dysplasia normally regresses spontaneously in younger women and cervical cancer takes in excess of ten years to develop.

Cervical cancer incidence has declined in high income countries where populations at risk have access to high quality cervical screening programmes (Kitchener, 2006). South Africa, like many other low to middle income countries, has not succeeded in controlling cervical cancer mainly because the screening programme has not been implemented successfully and programme coverage remains low in these countries, as depicted by figure 4 below. However, within South Africa, mortality has declined in populations that have had better access to services historically (white females), compared to South African coloured women (Nel, 1994 and Bailie, 1996). This further highlights disparity between under- and adequately-resourced settings within the country.

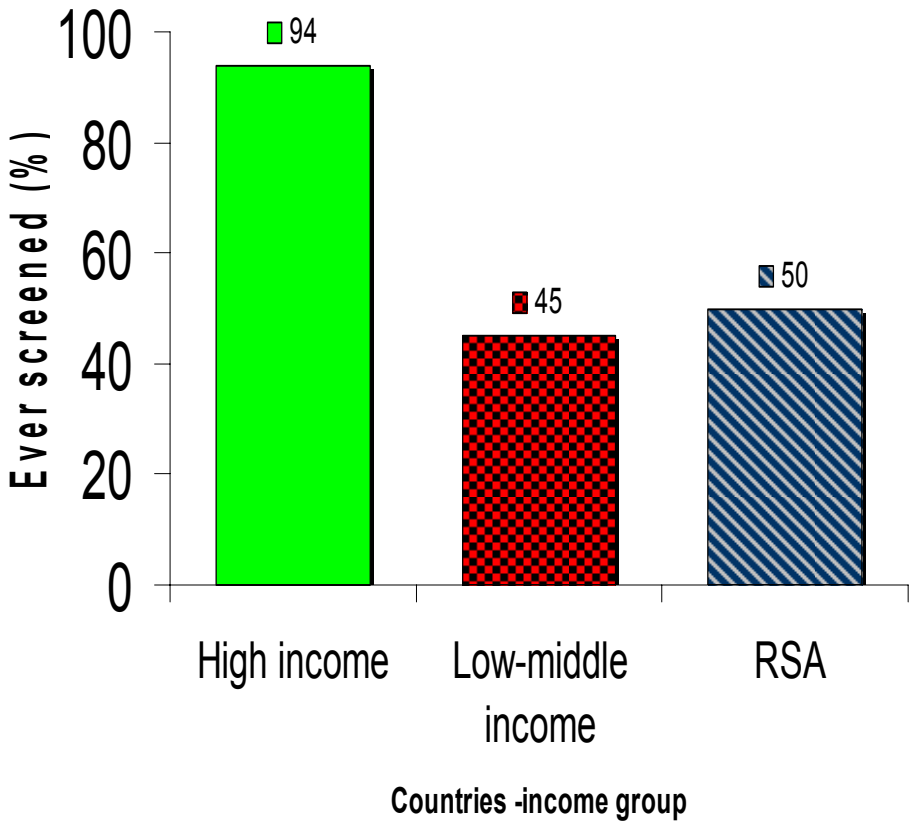


Figure 4: Cervical cancer screening coverage, women aged 25-64years (Gakidou, 2008)

Statement of the problem

Cervical cancer is the most common cancer in South African female population. It is caused by persistent HR-HPV infection interacting with certain factors. Currently South Africa relies on the screening programme to detect and manage pre-cancerous lesions, and this programme recommends screening based on the woman's age as a risk indicator. There is little data on prevalence of abnormal smears in South Africa population, especially in the younger age group, outside of Western Cape.

Rationale for this study

Evidence from previous studies is unclear and inconsistent about risk factors associated with abnormal cervical cytology and development of cervical cancer. The present study provides analysis of association between selected risk factors and abnormal cervical cytology, in a population of young women (18-35 years old) at risk for HIV and other STI.

Since most women with HPV do not progress to develop invasive cervical cancer, understanding which other risk factors, in addition to age, are associated with abnormal cervical cytology may help improve cervical cancer screening programme by extending screening to women with these additional risk factors and ultimately improve existing cervical cancer control programme in South Africa.

Research question

How common is abnormal cervical smear cytology in sexually active women from Soweto, aged 18-35 years, and what are the factors associated with abnormal cervical smear cytology results?

1.1 Study Objectives

Main objective

To determine factors associated with abnormal cervical smear results in women participating in the Microbicide Feasibility Study.

Specific objectives

1) To estimate prevalence of abnormal cervical smears in this population.
2) To determine socio-demographical, gynaecological and sexual behaviour factors associated with abnormal cervical smear cytology, in this population.

2.0 METHODS

2.1 Setting

This current protocol is a secondary analysis of data collected at an enrolment visit of a Microbicide Feasibility Study (MFS). MFS was a prospective cohort study conducted in preparation for an efficacy trial of a vaginal microbicide. The main objectives of this study were to estimate: HIV incidence, rate of condom use and rate of cohort retention over twelve months, prior to starting a phase 3 trial. Women were screened for HIV and pregnancy and enrolled into the study if negative for both tests. At the enrolment visit, participants were examined for cervical cytological abnormalities –because women with abnormal cervical cytology were ineligible to participate in a vaginal microbicides trial, swabs collected for STI, and blood drawn for *Herpes simplex virus*-2 and syphilis serology. Enrolment into the MFS study occurred from 13 January 2002 to 27 January 2004.

This study was conducted in Soweto, which is a largest peri-urban township in South Africa. Soweto has an estimated 305 000 households and a population of about 1,3 million people. This township lies about 15 kilometres south west of Johannesburg City, and thus its name: South Western Townships. The population of Soweto is predominantly Black and all 11 of South Africa's official languages are spoken in Soweto. Unemployed household heads make up 40% of the Soweto population, and 28% of households earn below R800 a month. (Loots, 2008)

2.2 Study design and participants

Women were invited, from the community and from primary health care facilities, to come to the study site for eligibility screening and participation. Consenting participants were included in this analysis if they were: HIV negative aged 18-35 years, sexually active, using a recognised method of contraception, enrolled in the MFS and had a cervical cytology result. The Microbicide Feasibility Study recruited women aged 18-35 years old, because they were deemed to be at increased risk of acquiring HIV. Participants also had to be on a reliable contraception, because microbicide trials aimed at minimising the risk of women becoming pregnant and possibly exposing foetus to a trial product.

Participants received transport reimbursement of R50.00 at the end of the visit. All participants were HIV negative because one of the objectives of the MFS study was to estimate HIV incidence. This is an analytical cross-sectional study of data collected during the enrolment visit of the MFS.

2.3 Clinical procedures

All participants gave written and signed informed consent. Socio-demographic factors, gynaecological factors and sexual behaviour data were collected using a structured questionnaire. Interviews were conducted by trained staff members in a private setting. Two licensed rapid tests (Abbot Determine and Unigold) were used to test for HIV antibodies. Medical history, general and pelvic examinations were performed at the enrolment visit.

Cervical examination included:

1. Visual inspection of the cervix to assess for:

- Size and shape of cervix.
- masses noted
- sores/ulcers
- redness
- oedema
- non – menstrual bleeding, and if present , source of non menstrual bleeding e.g. – cervical polyp; through the cervical os; cervical ectopy.
- cervical mucous and friability.

2. Bimanual Examination included:

- Palpating the cervix and noting any tenderness on moving the cervix from side to side.
- Palpating both adnexae for masses and tenderness.
- Palpating the uterus to establish its size, consistency and presence of any masses.

When the study started, Ayers spatula (wooden spatula) were used and procedure was changed half-way through the study to using the cervical brush.

Syndromic management of STI was done and all clinical assessments were carried out by a qualified nurse.

2.4 Laboratory procedures

Swabs collected from the endocervix were assessed for Neisseria *gonorrhoeae* (NG) and *Chlamydia trachomatis* using a qualitative commercial PCR (Roche Amplicor ®). Swabs taken from the vaginal wall were cultured in Diamonds media for TV, and used to prepare slides for Gram stain and diagnosis of Bacterial vaginosis (BV) using Nugent's criteria. Blood was collected for syphilis serology. RPR reactive specimens were confirmed with Treponema pallidum haemagglutination test (TPHA). *Herpes simplex virus*-2 serology was performed using the Focus HerpeSelect type-specific ELISA. Laboratory tests were performed by Contract Laboratory Services (CLS), in Johannesburg General Hospital.

2.5 Data management

All questionnaires were checked for quality before entered into the database. Data were double-entered using Epi-info 6 (CDC, Atlanta, 2002). Discrepancies between first and second data entry were resolved. The current analysis uses data collected during the Microbicide Feasibility Study. Selection of risk factors and of demographics for the current analysis was informed by the literature review on factors which have been shown to be associated with cervical cancer and on possible confounders. Data was analysed using STATA 8.2 (StataCorp, Texas, 2005)

2.6 Statistical analysis

Outcome variable:

The main outcome of interest was abnormal cervical cytology result defined according to the Bethesda classification system as "Epithelial cell abnormalities". The results of cervical cytology were categorized into two groups, as follows:

1) Positive: if any of the cervical abnormalities (ASCUS, ASCH, LSIL, HSIL or ICC) were detected in Pap smears. This category grouped together all the above results because of limited sample size.
2) Negative: if no cervical abnormalities were detected in Pap smears.

Main exposure variables

Main exposures that were explored were age, socio-economic status, cohabiting status, parity, contraceptive use, condom use, number of sexual partners, genital symptoms, current STI infection (CT, GC, TV, BV, *Herpes simplex virus*-2, syphilis) and history of STI infection.

Unadjusted analysis

Categorical variables were analysed using the number and percentages in each category and using the chi-squared or fishers exact test to test for association. Strength of association was shown using OR.

Controlling for confounding

Multiple logistic regression was used to adjust for confounding variables. Backward stepwise regression was used during exploratory analyses of model building. This

analysis began with a full or saturated model, which included all variables whose p-values were less than 0.30, during the unadjusted analysis, or which were stated a priori. As a rule of thumb, a less restrictive p-value is used to include more factors initially, and eliminate non-significant variables from this bigger model in an iterative process (Hosmer, 2000). The fit of the model was tested after the elimination of each variable to ensure that significant variables were not excluded from the model.

Likelihood ratio test was used to assess the model fit after each iterative step. The model building had been completed when no more variables could be eliminated from the model, without making the model weaker. The likelihood-ratio test was used to test the significance of each coefficient in the model. Likelihood-ratio test was used because it is more robust, compared to the Wald test, when coefficients are large or when the sample size is small.

2.7 Ethical issues

The main study received ethical clearance from The Wits Human Research Ethics Committee (HREC), with ethics reference number: M02-01-09 (appendix B). The study information was explained to volunteers before they were invited to participate. Information discussed included: confidentiality, reimbursement and that participation was voluntary. All volunteers gave signed informed consent to indicate their willingness to participate in the study. This secondary analysis protocol was also approved by HREC on 30 January 2007, with ethics reference number: M070113 (appendix B).

3.0 RESULTS

3.1 Participation in the study

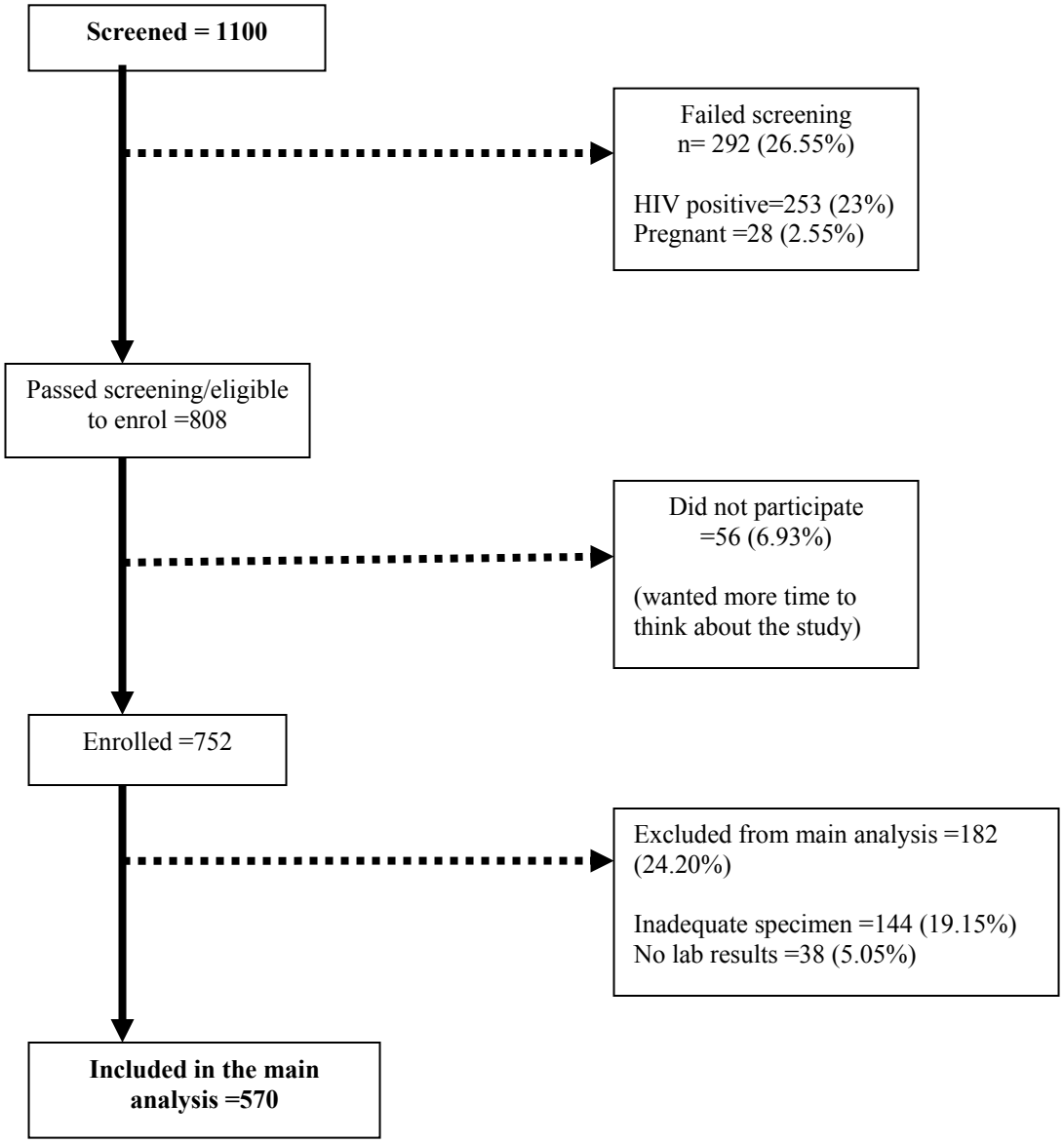


Figure 5: Diagram showing selection of study participants

Participation rate

Figure 5 above shows flow diagram of study participants. Most participants, 253/1100 (23%), were screened out because they tested HIV positive. Participation was high amongst those who were eligible to participate, 752/808 (93.1%).

Pap smear adequacy rate

Of the 752 women who participated in the study, 182 (24.2%) were excluded from final analysis, because their repeat cytology specimens were either unsuitable or inadequate for laboratory analysis (n=144) or laboratory results were missing/lost (n=38). The adequacy (satisfactory) rate of the slides submitted to the laboratory for cytology was thus 80.0% (570/714). So, the main analysis included 570 participants.

3.2 Characteristics of study participants

The majority of participants (55.6%) were aged between18 and 24 years. The median age of women with abnormal cervical cytology was not different from that of women with no cervical cytology abnormality [22.5 years (IQR 21-27) compared to 24 years (IQR 20-28), respectively]. As shown on table 1 below, the vast majority of participants did not stay with their partners (82.6%), had no income (68.8%) and had one or no child (79.2%). Few (8.2%) participants reported having had more than one sexual partner in the last three months.

Table 1: Characteristics of study participants.

Characteristic	Participants (n)	% (95 CI)
Age group (yrs): 18 to 24	317	55.6 (51.4 - 59.7)
25 to 35	253	44.4 (40.3 - 48.6)
Highest education attained -grade: >=12	330	57.9 (53.7 - 62.0)
<12	240	42.1 (38.0 - 46.3)
Socioeconomic status: Has income	392	31.2 (64.8 - 72.6)
Has no income	178	68.8 (27.4 - 35.2)
Lives with partner: Yes	99	17.4 (14.3 - 20.7)
No	471	82.6 (79.3 - 85.7)
Sexual partners (concurrent): >1	47	8.3 (6.1 - 10.8)
<=1	523	91.7 (89.2 - 93.9)
Current contraceptive: Hormonal	325	57.0 (52.8 - 61.1)
Non-hormonal	245	43.0 (38.9 - 47.2)
Parity: >=1	240	42.3 (38.2 - 46.5)
Nil	327	57.7 (53.5 - 61.8)
Ever pregnant: Yes	357	62.6 (58.5 - 66.6)
No	213	37.4 (33.4 - 41.5)
Use condom for contraception: Yes	194	34.0 (30.1 - 38.1)
No	376	66.0 (61.9 - 69.9)
Lower abdominal pain*: Yes	91	16.0 (13.1 - 19.3)
No	478	84.0 (80.7 - 86.9)
Vaginal discharge*: Yes	85	14.9 (12.1 - 18.1)
No	484	85.1 (81.9 - 87.9)
Genital sores*: Yes	37	6.5 (4.6 - 8.9)
No	531	93.5 (91.1 - 95.4)
HSV-2 seropositive: Yes	301	54.2 (50.0 - 58.4)
No	254	45.8 (41.6 - 50.0)
RPR positive: Yes	15	2.7 (1.5 - 4.4)
No	540	97.3 (95.6 - 98.5)
GC positive: Yes	8	1.5 (0.7 - 3.0)
No	518	98.5 (97.0 - 99.0)
CT positive: Yes	60	11.4 (8.8 - 14.4)
No	467	88.6 (85.6 - 91.2)

Characteristic	Participants (n)	% (95 CI)
BV positive: Yes	298	53.8 (49.5 - 58.0)
No	256	46.2 (42.0 - 50.5)
TV positive: Yes	28	5.0 (3.3 - 7.1)
No	533	95.0 (92.9 - 96.7)
Yeast positive: Yes	52	9.5 (7.2 - 12.3)
No	496	90.5 (87.7 - 92.8)

(Table 1 continued). *In the past 4 weeks.

3.3 Prevalence of abnormal (ASCUS-ICC) cervical cytology

From table 2 below, the prevalence of any abnormal cervical cytology was 6.7% (95% CI 4.8-9.0). Pap smear results in the group LSIL to HSIL were 5.3% (95% CI 3.6-7.4).

Table 2: Main outcome: prevalence of abnormal cervical cytology

Cytology results	Frequency	% (95% CI)
Normal	532/570	93.3 (91.0 - 95.2)
Abnormal	38/570	6.7 (4.8 - 9.0)
ASCUS	8/570	1.4 (0.6 - 2.7)
LSIL	23/570	4.0 (2.6 - 6.0)
HSIL	7/570	1.2 (0.5 - 2.5)
Total	570	100.0

3.4 Factors associated with abnormal cervical cytology

3.4.1 Unadjusted analysis

Table 3 below summarises the results of univariate analysis. Access to income was associated with a lower risk for cervical abnormality (OR 0.68; 95% CI 0.33-1.44). Also, cohabiting was associated with a lower risk for cervical abnormality (OR 0.54; 95% CI 0.14-1.57). However, in both these instances, this protection was not statistically significant. None of the other demographic factors were associated with abnormal cervical cytology.

Table 3: Factors associated with abnormal cytology results, unadjusted analysis.

Risk factor	Women with abnormal cells No (%)	Women with normal cells No (%)	Crude OR (95% CI)
Age group (years): 18 to 24	24 (63.2)	293 (55.1)	1.0
25 to 35	14 (36.8)	239 (44.9)	0.72 (0.33-1.48)
Education (years): Les than 12	18 (47.4)	222 (41.7)	1.0
12 or more	20 (52.6)	310 (58.3)	0.80 (0.39-1.64)
Has no income	15 (39.5)	163 (30.6)	1.0
Has income	23 (60.5)	369 (69.4)	0.68 (0.33-1.44)
Not co-habiting	34 (89.5)	437 (82.1)	1.0
Co-habiting	4 (10.5)	95 (17.9)	0.54 (0.14-1.57)
Sexual partners: 1 or nil	35 (92.1)	488 (91.7)	1.0
More than 1	3 (7.9)	44 (8.3)	0.95 (0.18-3.21)
Use condom for contraception:			
No	26 (68.4)	350 (65.8)	1.0
Yes	12 (31.6)	182 (34.2)	0.89 (0.40- 1.87)

Risk factor	Women with abnormal cells No (%)	Women with normal cells No (%)	Crude OR (95% CI)
One or no child	7 (18.4)	111 (21.0)	1.0
More than one children	31 (81.6)	418 (79.0)	0.85 (0.31-2.04)
Ever been pregnant: No	11 (28.9)	202 (38.0)	1.0
Ever been pregnant: Yes	27 (71.1)	330 (62.0)	1.5 (0.70-3.43)
[1]Non-hormonal contraceptive	14 (36.8)	231 (43.4)	1.0
Hormonal contraceptive use	24 (63.2)	301 (56.6)	1.32 (0.64-2.81)
Abnormal vaginal discharge*:			
No	28 (73.7)	456 (85.9)	1.0
Yes	10 (26.3)	75 (14.1)	2.17 (0.90-4.83)
Lower abdominal pain*: No	30 (78.9)	448 (84.4)	1.0
Yes	8 (21.1)	83 (15.6)	1.44 (0.55-3.36)
Genital sores*: No	34 (89.5)	497 (93.8)	1.0
Yes	4 (10.5)	33 (6.2)	1.77 (0.43-5.41)
HSV-2 : Negative	12 (33.3)	242 (46.6)	1.0
Positive	24 (66.7)	277 (53.4)	1.75 (0.82-3.92)
Syphilis: Negative	35 (97.2)	505 (97.3)	1.0
Positive	1 (2.8)	14 (2.7)	1.03 (0.02-7.16)
[2]Cervical infections: Negative	30 (90.9)	435 (87.9)	1.0
Positive	3 (9.1)	60 (12.1)	0.73 (0.14-2.44)
[3]Vaginal infections: Negative	15 (39.5)	222 (41.9)	1.0
Positive	23 (60.5)	308 (58.1)	1.11 (0.54-2.33)

(Table 3 continued). *In the past 4 weeks.

[1]Non-hormonal contraception includes: no contraception, condom, IUD and diaphragm.

[2]Past infections (NG and CT).

[3]Current infections (BV, Yeast and TV)

Of the gynaecological factors analysed, the odds of having abnormal cervical cytology result were slightly higher for those women who reported ever being pregnant (OR 1.5; 95% CI 0.70-3.43; p=0.267) and also for those who used hormonal contraceptives (OR 1.32; 95% CI 0.64-2.81; p=0.429). But these associations were not statistically significant; p-values were 0.267 and 0.429 respectively.

None of the behavioural factors were associated with abnormal cervical cytology result. The odds ratio of having abnormal cervical result was 0.95, 95% CI 0.18-3.21, p-value=0.935 for participants who reported having had multiple sexual partners in the last three months; and was 0.89, 95% CI 0.40-1.8, p-value=0.741 for participants who reported using condoms as their method of contraception.

Self-reported genital symptoms, in the past four weeks, were associated with having abnormal cervical cytology result. Participants who reported having abnormal vaginal discharge in the last 4 weeks were more likely to have an abnormal Pap smear result (OR 2.17; 0.90-4.83). This association was statistically significant with a p-value of 0.042. Whilst reporting abdominal pain, and genital sores was associated with having abnormal cervical cytology result, these relationships were not statistically significant (OR 1.44; 95 % CI 0.55-3.36; p=0.378 and OR 1.77; 95 % CI 0.43-5.41; p=0.300 respectively). Participants who tested positive for HSV-2 were more likely to have abnormal Pap smear result, But this association was not statistically significant (OR 1.75; 95% CI 0.82-3.92; p=0.166). None of the other laboratory-diagnosed STI were associated with abnormal cervical cytology result.

3.4.2 Adjusted analysis: multiple logistic regression

In the saturated model, the odds of having abnormal cervical cytology result were lower for participants who were in the older age group (25-35 years) (adjusted OR (AOR) 0.50; 95% CI 0.22-1.11; p=0.09). The odds of having abnormal cervical cytology result were higher for participants with more than one child (AOR 2.16; 95% CI 0.96-4.85; p=0.06), with abnormal vaginal discharge (AOR 2.09; 95% CI 0.95-4.61; p=0.07), and for those who tested positive for HSV-2 serology (AOR 2.13; 95% CI 0.88-4.17; p=0.10). However, evidence to support associations for age and HSV-2 serology was weak.

Main effects model

Table 4 below shows the final model (main effects model), after a few iterative steps which began with selected factors from table three above. Abnormal vaginal discharge and multi-parity were associated with abnormal cervical cytology result. Participants with abnormal cervical cytology were more likely to have an abnormal vaginal discharge compared to those without an abnormal discharge (AOR 2.33; 95% CI 1.07-5.06; p=0.03); and were also more likely to have more than one child (OR 2.21; 95% CI 1.00-4.87; p=0.05).

Table 4: Factors associated with abnormal cytology results, adjusted analysis

Variable		Women with abnormal cells No (%)	Women with normal cells No (%)	Adjusted OR (95% CI)
Age group (yrs):	18 to 24	24 (63.2)	293 (45.1)	1.0
	25 to 35	14 (36.8)	239 (44.9)	0.51 (0.24-1.10)
[1]More than one child		7 (18.4)	111 (21.0)	2.21 (1.00-4.87)
One or no children		31 (81.6)	418 (79.0)	
Abnormal vaginal discharge:	Yes	10 (26.3)	75 (14.1)	2.33 (1.07-5.06)
	No	28 (73.7)	456 (85.9)	

[1]From univariate analysis, parity was not a significant risk factor, but was included in multiple regression because it was stated a priori.

4.0 DISCUSSION

Worldwide, cervical cancer is second only to breast cancer when it comes to incidence of cancer in women. Available, but out-dated, statistics indicate that South African women have a high risk of developing cervical cancer. (Mqoqi, 2003) The main objective of this study was to determine the prevalence and factors associated with abnormal cervical cytology results in HIV negative women from Soweto.

Prevalence of abnormal cervical smears:

Our data show a high prevalence of abnormal cervical cytology [6.7% (95%CI 4.8-9.0), given the younger age of the participants. The median age of women with abnormal cervical cytology was not different from that of women with no cervical cytology

abnormality [22.5 years (IQR 21-27) compared to 24 years (IQR 20-28), respectively]. This prevalence rate is lower than that observed in a South African survey (Fonn, 2002) and it compares to an observation made in an STI clinic in Baltimore (Kanno, 2005) but the Baltimore population included patients over the age of 35 years. Our

Factors associated with cervical cancer:

Abnormal vaginal discharge was associated with increased risk of having abnormal cervical cytology result (OR 2.33, 95% CI 1.07-5.06, p=0.003). Cervical glands normally produce mucus-containing discharge. When the cervix has an infection or a lesion, inflammation occurs. Inflammation is a non-specific physiological response to tissue damage caused by foreign material, like infectious micro-organism and douching. During inflammatory response, immune response cells (viz. phagocytes and natural killer cells) are recruited to the site of inflammation. These immune response cells release inflammatory mediators (viz. cytokines IL-6 and IL-8) (Tjiong, 1999). Also, non-specific protective antimicrobial oxidants are produced. But, these oxidants can also cause oxidative damage to host DNA, thus forming precursors for cancer. Abnormal discharge is then produced, which changes in either colour or texture or amount or smell. So, abnormal vaginal discharge is a non specific indication of a genital infection and inflammatory response. Other authors have also observed association between abnormal vaginal discharge and abnormal cervical cytology (Greenberg, 1999) and have argued that this non-specific immune response to infection or inflammation better explains association between cervical cancer and cervical infection or inflammation.

Our study found an association, albeit weak, between abnormal cervical cytology and having more than one child (OR 2.21; 95%CI 1.00-4.87; p=0.05). Since our study population is relatively young, having more than one child may be an indicator of higher-risk sexual activity, which is unprotected sex. Other studies have shown similar relationship between abnormal cervical cytology and multi-parity. Parity is thought to increase the risk of cervical cancer development through the maintenance of the transformation zone on the exocervix for many years, which may facilitate exposure to HPV.

Factors not associated with cervical cancer:

Use of hormonal contraceptives was not associated with abnormal cervical cytology result (OR 1.32; 95% CI 0.64-2.81; p-value=0.429). The use of hormonal contraceptive can cause alterations in hormone levels thus disturbing normal vaginal flora and making it easier for STI, including HPV, to infect the cervical lining. However, we could not find evidence linking hormonal contraception use and abnormal cervical cytology result. Other authors, including IARC studies, noted increased risk after ten years of OC use (Moreno, 2002). But, our results agree with what a Western Cape (South Africa) study observed. In this case-controlled study, authors compared risk of invasive cervical cancer between injectable progesterone-only users and combined estrogen/progesterone oral contraceptive users and found no evidence of an increased risk, regardless of duration or recency of use (Shapiro 2003).

It is common in our population for nulliparous young women to delay initiation of contraception. Given the relatively younger age of our study participants, exposure to hormonal contraception would not have been long enough to result in the development of abnormal cervical cells. Also, our data reported on hormonal contraceptive, whereas the IARC studies and the Western Cape study looked at oral contraceptive and injectable progesterone, specifically.

Age was also not associated with abnormal cervical cytology result. Women with abnormal cervical cytology result were slightly younger than those with no abnormality, but this difference was not statistically significant (p=0.09). Most cancers take many years to develop. So, how possible was it to observe a high prevalence of abnormal cervical cytology result in this young cohort? Increased frequency of SIL at younger age has been attributed to early sexual initiation and differences in the biological maturity of the immune system and cervix.

We did not measure age of participants at sexual debut, but a national youth survey conducted in South Africa showed that South African youth start experimenting with sex at younger age. This survey found that 48% (95% CI 45-52) of youth aged 15-19 years old had ever had sex (Pettifor, 2004). This early age at sexual debut could account for some of the cervical abnormality observed in our study at a relatively younger age. However, observing mainly low-grade abnormalities is consistent with what would be expected in young women, since HPV infections peak soon after initiating sexual activity with normally transient infections.

Condom use was not associated with cervical cytology result (OR 0.89; 95% CI 0.40-1.87; p=0.741). Condom use reporting may be unreliable or condoms do not offer reliable protection against HPV. The latter reason is highly-plausible because there is evidence to suggest that HPV can be transmitted through condom-unprotected pubic areas. The significance, in cervical cancer pathogenesis, of HPV acquired in this way is unclear.

Limitations of the study

Study design-related limitations:

Since this is a cross sectional study, temporal relationship between exposures and outcome could not be ascertained since they were both measured at the same time. Therefore, this study can not show if exposure variables caused the outcome, for those risk factors that are not fixed. Also, the outcome of the lesions could not be assessed because women were not followed up.

Behavioural data was self-reported. Researchers have questioned the validity of self-reported data, because respondents often provide socially-acceptable responses (Weinhardt, 1998). Attempts were made to address this potential problem by matching interviewer and participant on age and gender; conducting interviews in private rooms and stressing confidentiality during the informed consent process and throughout the interviews; and ensuring that interviewers were trained to be non-judgmental with regard to the various sexual behaviours, attitudes and norms reported by the respondents.

Recall period may have introduced recall bias. Sexual behaviour history depended on the recall ability of the participants. Recall was restricted to a period of no greater than three months in order to minimize this bias.

Limitations specific to this study:

Since this study is secondary analysis of data collected for another purpose –the Microbicide Feasibility Study, data was limited to that which was collected for the main study. Data on smoking was not available and controlling for HPV was not possible, because HPV was not measured. Variables such as contraceptive use lacked detail on length of use. The study was also not sufficiently powered to detect differences in more than one level of exposures. Because the Microbicide Feasibility Study recruited women aged 18-35-years old, our study population was also limited to this age group.

Because of sample size limitation, cytology results ranging from ASCUS up to ICC were lumped together into one category (abnormal cervical cytology). An attempt was made during the analysis to separate the HSIL results, but this did not alter the outcome of the analysis. Classification bias was possible with a Pap diagnosis. Pap smear cytology has low sensitivity, ranging from 30-87% (Nanda, 2000), and thus gives a moderate rate of false negative results. Given our population prevalence of abnormal cervical cytology (about 5%), low sensitivity could lead to an under-estimation of prevalence through false negatives. Despite this limitation, Pap smear examinations are approved by the FDA (FDA, 2004) and the test has been used extensively (and successfully despite lower prevalence) in high income countries. This method is also recommended by WHO

(WHO, 2008) for screening for cervical pre-cancerous lesions. We therefore found it reasonable to use Pap smear to assess for the outcome in our study.

Non-participation bias was possible because not all recruits participated. Non-participants were requested three times to enrol into the study, after which researchers felt that further persuasion would exert undue pressure on the women and would thus be unethical. Enrolled participants were more likely to be slightly older than non participating women. Younger women may have felt at a greater risk of testing HIV-positive and decided not to participate. Thus generalization of results should be done with caution. However, participation rate in our study was good (93.1%), thus mitigating any bias that may have resulted from this age difference. Age was also adjusted for in the main analysis.

Women who screen regularly for cervical cancer have a lower risk of developing cervical cancer (Brinton, 1992 and Shields, 2004). We did not collect data on participants' screening history. It was therefore not possible to adjust for this potential confounder in our analysis. But, study participants were relatively younger than the Nationally-recommended age for cervical screening. We therefore have no reason to think that screening history is an important confounder in our study.

Male-partner circumcision (MC) status was not measured in our study. There is evidence showing that MC reduces the likelihood of HPV infection and thus of cervical cancer (Munoz, 1997 and Schiffman, 2003). At a population level, high prevalence of MC results in low prevalence of HPV and thus low prevalence of ICC. High levels of MC

also results in low levels of HIV and other STI, thus resulting in lower risk of transmitting HPV. So MC could potentially reduce prevalence of HPV and ICC. Strong evidence linking MC with a high (up to 60% in the Orange Farm circumcision study (Auvert, 2005)) reduction in risk of contracting HIV for men recently came from randomised controlled trials in RSA, Uganda and Kenya. In the light of this evidence, it would be important to study the effect of MC in the development of cervical cancer.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Our study showed that LSIL abnormality is common in this population of younger women. An abnormal cervical cytology result was associated with abnormal vaginal discharge and with having more than one child.

Recommendations:

- Similar studies looking at co-factors for cervical cancer should preferably measure: HPV, detailed contraceptive data –such as length of use.
- Findings from this study support current screening practice, because many LSIL would either regress naturally or take many years to progress into cancer.

6.0 APPENDIX A: Summary of relevant studies of risk factors for cervical cancer development

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Adam, et al.	2008	Cohort - prospective. Median time to follow-up: 122 days	women attending colposcopy clinic at CHBH, Soweto, South Africa	persistent cytologic abnormality	increased risk associated with: HIV (self reported), CD4 count<200cells/cubic mm	loss to follow-up was high (overall)=41.3%
Ades, et al.	2008	Case-control	Montreal, Canada. 381 cases: histologically-confirmed CIN 2 or worse, referred to colposcopy clinic. 884 controls: women from outpatient clinics with normal cytology	>=CIN 2	certain HLA alleles and haplotypes were protective	
Ault	2006	Review: recent RCT	Women aged 15-25 yrs. No history of prior abnormal cervical cytology, <=6 male sexual partners, seronegative for specific HPV	persistent HPV (specific types) infection, clinical disease	Intervention: HPV vaccines (16 alone; 16 & 18; 16, 18, 6 & 11). Results: All 3 vaccines were highly efficacious (71-100%).	Participants' follow-ups were: 18-48 months (short). So, duration of vaccine-induced immunity is unknown. It is also not known if vaccines are HPV type specific or they confer cross immunity.
Auvert, et al.	2005	RCT	Orange Farm, South Africa, men, 18-24 yr old.	HIV incidence rate	Intervention: male circumcision (MC). Result: MC offered 60% (32-76) protection against HIV acquisition.	Unblinded intervention.
Bayo, et al.	2002	Case-control	Mali. Hospital-based. 82 ICC cases. 97 age-matched controls.	ICC	HPV DNA status, parity>10: OR=4.8 (1.5-14.7), never douched: OR=17.6(4.2-74.7), re-using home-made feminine napkins: OR=45.9(8.8-238.7) and polygamous marriage: OR=5.3(1.3-21.3)	Parity was associated with cervical cancer, only when it was very high.

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Beral, et al.	1988	Cohort - prospective	UK women. OC users=23000. Non-users=24000.	Incidence & mortality rates from genital tract cancers.	OC ever-users had excess ICC of 8 per 100 000 woman-years compared with never-users.	Good follow-ups (woman-yrs): ever-users=257 028 & never-users=182 866
Buga	1998	Cross-sectional	260 University of Transkei students, South Africa	cervical cancer awareness & risk factors	Ever heard of cxca: 74.3%. Knew HPV causes cxca: 68%. Unsure if cxca could be prevented: 62.8%. Reported history of STI: 42.2%. Absence of barrier contraceptive: 94.6%	Satisfactory response rate: 74.3%
Castle, et al.	2001	Case-control	Women from Guanacaste, Costa Rica.<50yrs. Cases: 95 with abnormal cytology, 220 with HPV DNA+. Controls: 130 without HPV DNA.	High-grade lesions in women who are HPV DNA+	severe cervicitis: OR=1.9 (0.9-4.1)	Selection of controls unclear. Controls fewer than cases.
Chan, et al.	2007	Cross-sectional	Nicaragua. All women attending women's health programme. N=1185	prevalence and risk factors of STI and cervical neoplasia.	77% reported STI symptoms. 7.7% had abnormal cytology result. Risk factors: age <30: OR=5.2(2.2-12.0); being employed: OR=2.4(1.1-5.0); STI symptom: OR=3.3(1.0-10.9)	
Fonn, et al.	2002	Cross-sectional. Multicentre study	Women presenting for screening (N=19 686).	age-specific cervical cytology abnormality	Risk of cxca associated with: age (every increase of 1yr): OR=1.013(p=0.0090); parity (each additional birth): OR=1.076 (p=0.0085); clinically noticeable cervical discharge: OR(p=0.0150)	Diverse study population. Big sample size.

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Garcia-Closas, et al.	2005	Systematic review of epidemiologic evidence: 10 RCT, 5 prospective studies, 3 nested case-control prospective studies, 15 case-control studies	Observational studies controlling for HPV infection published between: Mar1995-Nov2003 and all RCT published: Jan1991-Nov2003. All studies had to have a control group. All but 2 studies were done in developed countries -mainly U.S.	HPV persistence or risk of cervical squamous neoplasia (ISC and ICC)	HPV persistence: possible protective effect (fruits, vegetables, vitamin C & E, beta- & alpha-carotene, lycopene). Protective effect of cervical neoplasia: probable (folate, retinol, vitamin E and possible vegetables, vitamin C & B12, alpha- & beta-carotene, lycopene). High blood homocysteine was probable associated with increased risk of cervical neoplasia.	
Ghazal-Aswald, et al.	2006	Cross-sectional	United Arab Emirates, Al-Ain district. 728 women attending primary and secondary care.	cervical smear abnormality	No association with chlamydia: chi2=0.6, p=0.4	Cervical abnormality: Low prevalence population (1.51% (0.66-2.4))
Jennings, et al.	1992	Cohort - retrospective, 5-yr period.	Women diagnosed with ICC at Groote Schuur Hospital, South Africa. 82 women <35yr old & 82 women >=35yrs old.	Incidence, grades of cxca & survival following treatment (surgery or chemotherapy).	No difference in incidence of abnormal cervical cytology: 11.7% (women <35yrs). <35yr presented with earlier stages of the disease: p=0.0031. no difference in survival times: p=0.8500	Incidence for women >=35yrs not shown. Cxca was prevalent in women <35yrs
Kanno, et al.	2005	Cross-sectional: record-review.	Baltimore: inner-city public STD clinic (2001-2002)	Prevalence and risk factors of abnormal cervical cytology.	prevalence of abnormal cytology: 5.7%, independently associated with: age (p=0.010) OR=0.64 (0.45-0.90), genital warts (p<0.001) OR=7.03 (2.82-17.53), chlamydia(p<0.049) OR=2.04 (1.00-4.15)	

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Lancaster, et al.	1999	Cross-sectional	Mpumalanga, South Africa. 10000 cytology smears, from previously unscreened population, were analysed. Results were compared with similar studies from other previously unscreened populations Pretoria (urban) & Transkei (rural)	Prevalence and risk factors of abnormal cervical cytology.	Prevalence of abnormal cytology: 3% (Mpumalanga), 5% (Pretoria) & (Transkei). ~75% of cases were younger than 41yrs. 79% of cases with >=6 children had CIN3-ICC compared with 16% with only 1 child.	Analysis mainly descriptive. No adjusted analysis was done.
Lehtinen, et al.	2002	Nested case-control study	Cases: 178 cxca, controls: 527. Selected from a 5-yr cohort study of 550,000 women from: Finland, Norway & Sweden. .	Cervical carcinoma	HSV-2: no association found.	Authors also did meta analysis of 6 longitudinal studies, which also showed no association between HSV-2 and cxca.
Mangan, et al.	1997	Descriptive case series.	Sexually-active adolescents with Pap smear result between: Jan1982-Dec1983 (N=557) and Jan1992-Dec1993 (N=871).	Prevalence (and 10-yr change) of abnormal cytology results.	Prevalence of abnormal cytology: 2.8% (1983) vs 11.7% (1993), with OR=4.7(2.7-8.3). Prevalence of benign cellular changes also increased from 8.7%(1983) to 20.1%(1993), OR=2.7(1.9-3.8)	
Manhart, et al.	2002	Meta-analysis	Included 20 studies published in English between: 1980-2000, identified in MEDLINE.	Endpoints: HPV infection, HPV subtypes, warts, SIL, CIN	Condom use: no consistent evidence of reduction in risk of becoming HPV-DNA positive.	Conclusions not definitive due to inadequate measures of condom use.

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Moreno, et al.	2002	Pooled data from 10 case-control studies	Women from: Asia (Thailand, the Philippines), Africa (Morocco), South America (Brazil, Peru, Paraguay, Columbia) & Spain. Cases (ICC, ISC) =1853, controls (normal cytology) =1916.	cervical cancer risk	Oral contraceptive use. Never-users vs.: <=5yrs of use OR=0.73(0.52-1.03), 5-9yrs of use OR=2.82(1.46-5.42), >10yrs of use OR=4.03(2.09-8.02)	Diverse study population. Big sample size.
Munoz, et al.	2002	Pooled data from 10 case-control studies	Women testing positive for HPV DNA, infection from: Asia (Thailand, the Philippines), Africa (Morocco), South America (Brazil, Peru, Paraguay, Columbia) & Spain. Cases (ICC, ISC) =1800, controls (normal cytology) =255.	cervical cancer risk	Association between number of full-term pregnancies and cervical squamous-cell cancer: Parity: >=7 versus nulliparous: OR=3.8(2.7-5.5), parity=1 or 2: OR=2.3(1.6-3.2).	Diverse study population. Big sample size.
Munoz, et al.	2006	Review of epidemiologic evidence: mixed designs	Studies from all over the world (developed and developing) contributed data.	HPV DNA, abnormal cervical cytology/histology	In one study, HPV DNA was detected in 99.7% of the cervical tumours leading to conclusion that HPV is a necessary cause of cxca. HPV-16, -18, -45, -31, -33, -52, -58, and -35 (descending order) were responsible for about 90% of cxca worldwide. Conclusion: Established risk factors: smoking, long-term OC-use. HIV coinfection and high parity. Probable cofactors: HSV-2 coinfection, Chlamydia trachomatis coinfection, diet and nutrition.	Detailed review of studies done by IARC across different countries using common study protocol and well-validated PCR assays for detection of HPV that were carried out in a central laboratory.

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Nel, et al.	1994	Record-review	Cytological, histological and oncological records (of 1990) from academic hospitals of University of Orange Free State, South Africa. N=684 (533=black, 151=white).	CIN and ICC in black vs white patients	Black women presented with a more advanced disease: p<0.001. The disease peaked at an earlier age in black women.	The study is purely descriptive.
Shannon, et al.	2002	Case-control	Siriraj Hospital, Bangkok, Thailand. Women. For the ISC analysis: cases=50, controls=125. For the ICC analysis: cases=134, controls=384. Controls were identified from family planning or gynecologic clinics associated with the Siriraj Hospital.	ISC, ICC	ISC vs clinic controls: protective effect of: high intake of vitamin-A (trend OR=0.25(p<0.001) and retinol (trend OR=0.40(p<0.001)	

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Shapiro, et al.	2003	Case-control	Coloured and black women in Western Cape, South Africa (Jan1998-Dec2001). Cases=524 (first occurrences of histologically-confirmed ICC), recruited from public gynecology oncology clinics at tertiary hospitals. Controls=1541 with a sub-group of 254 HPV-positive controls, recruited from same hospitals or local hospitals or the community health centers. Controls were matched for decade of age, ethnicity and residence.	cxca risk	Injectable progestogen contraceptives: OR=1.0(0.8-1.3), combined estrogen/progestogen oral contraceptives: OR=0.8(0.7-1.1).	Authors adequately matched cases and also looked at long-term use.
Shields, et al.	2004	Case-control: retrospective review.	235 cases of squamous carcinoma. 486 controls	HPV DNA seropositivity, cxca	Increased risk of HPV DNA+ among controls was associated with: number of sexual partners, black race & OC use. Condom was protective. Reduced risk of cxca among HPV-exposed women was associated with: screening, black race & yeast infection. Increased risk in the same group was associated with: current smoking: 2-fold increase, low education and income, and history of non-specific genital infection.	

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Sitas, et al.	1997	Case-control	Greater Johannesburg & Soweto, SA, between: mid-1990-Dec2005. Black patients aged 15-50yrs. Cases: presenting (for the first time) with cancer believed to have infective aetiology, N=588. Controls: 325 patients with cancers other than those suspected to have infectious cause.	cancers linked to an infective agent (includes cxca)	HIV infection: no association with cxca OR=0.6(0.2-1.9), p=0.9. HIV was associated with Kaposi sarcoma OR=61.8(19.7-194.2), p<0.001 and non-Hodgkin's lymphoma OR=4.8(1.5-14.8), p=0.007	Men included in sample size. These results are inconsistent with numerous studies linking HIV and cxca. Small number of cxca in this study may have contributed in this null finding.
Sitas, et al.	2000	Case-control	Greater Johannesburg & Soweto, SA, between: 1995-Jan1999. Black patients aged >=18yrs. Cases: presenting (for the first time) with cancer believed to have infective aetiology and with cardiovascular disease, N=4883(females=3404). Controls: patients with cancers other than those suspected to have infectious cause and vascular disease, N= 844(females=556).	cancers linked to an infective agent (includes cxca)	HIV infection associated with: cxca OR=1.6(1.1-2.3), Kaposi sarcoma OR=21.9(12.5-38.6), p<0.001 and non-Hodgkin's lymphoma OR=5.0(2.7-9.5), vulval cancer OR=4.8(1.9-12.2)	

Author/s	Year	Study design	Study population	Main outcome	Risk factors	Comments
Smith, et al.	2003	Systematic review of epidemiologic evidence: 28 studies (24 case-control studies, 4 cohort).	Total N=12 531 women with ICC or ISC. 16 studies done in high income countries: 8 in USA, 8 in Europe.	ICC, ICS	Hormonal contraceptives were associated with ICC for all women: <5yrs: RR= 1.1(1.1-1.2), 5-9yrs: RR=1.6(1.4-1.7) and >=10yrs: RR=2.2(1.9-2.4). Similar pattern was observed with HPV positive women and with ICS.	Marginal effect, but consistent data. Large sample size, narrow confidence intervals.
Takac, et al.	1999	Case-control	Cases: 423 patients undergoing conisation of uterine cervix at the Gynaecology and Perinatology clinic of Maribor Teaching Hospital, Slovenia. Controls: 108 women undergoing regular gynaecological investigation.	CIN	Chlamydia trachomatis: chi2=0.29, p>0.05. Cases were older (p<0.01) and were mostly multiparous (p<0.01)	No confounders were considered in the analysis.
Viikki, et al.	2000	Longitudinal cohort study	19114 women attending mass screening in Finland in 1985-1990. Women were followed up until 1994.	CIN	Factors associated with cxca were: Trachomonas vaginalis: SIR=6.4(3.7-10), HPV: SIR=5.5(4.2-7.2) and HSV-2: SIR=12(2.4-34).	Strong evidence reported.

CXCA= cervical cancer. ICC=invasive cancer. ISC: in situ cancer. CIN=cervical intraepithelial neoplasia. RCT=randomised controlled trial. OC=oral contraceptive. (95%CI). SIR=standardized incidence ratios

7.0 APPENDIX B: ETHICS CLEARANCE CERTIFICATES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mntambo

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M070113**
PROJECT	Factors Associated with Abnormal Cervical Smears in HIV Negative Women in Soweto
INVESTIGATORS	Mr AQ Mntambo
DEPARTMENT	School of Public Health
DATE CONSIDERED	07.01.26
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.01.30 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr SD Moretlwe

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-01-09

PROJECT A Feasibility Study In Preparation For A Phase III Microbicide Trial In Women Attending Family Planning And Post Natal Services In Soweto, South Africa

INVESTIGATORS Dr/Ms S/B Delany/Nkala

DEPARTMENT School of Clinical Medicine, CH Baragwanath Hospital

DATE CONSIDERED 02-01-25

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 02-04-05 **CHAIRMAN**.....(Professor P E Cleaton-Jones)

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

c c Supervisor: Dr S Delany
Dept of School of Clinical Medicine, CH Baragwanath Hospital

Works2\lain0015\HumEth97.wdb\M 02-01-09

DECLARATION OF INVESTIGATOR(S)

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

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

8.0 APPENDIX C: STUDY QUESTIONNAIRES

SCREENING FOR ELIGIBILITY (V-1A)
These questions will be asked at the point of recruitment.

A	**Office use only**					
	Date of interview		Interviewer's initials		Form used 1=English 2=Zulu 3=Sotho	
	Time interview started:					

Interviewer: *Provide participant with a Participant Information Sheet, and confirm that she gives consent to be screened for eligibility to participate in the study after reading the following:*

Hello, my name is _____, and I work for the Reproductive Health Research Unit (RHRU) at Chris Hani Baragwanath Hospital in the New Nurses' Home Building.

The RHRU is conducting research on the health of women in Soweto. We would like to know more about the rate of HIV infection in women in Soweto, the extent to which women in this community are able to persuade their partners to use condoms, and to determine the factors that might prevent the women from participating in the research for a longer period. We would like you to answer a few questions of a personal nature about yourself, where you live, your partners, and your attitude to HIV testing. Your name will not be used on this form, and all your answers will be kept confidential. Your participation in this interview is voluntary, and you are free to withdraw at any time without jeopardising the care that you usually receive at this clinic or any clinic in the future.

I'd like you to confirm that you understand the purpose and procedures of this study, and that you give consent to continue with this interview.

Verbal consent given?		*Circle response*	
	Yes	1	
	No	2	**End interview**

B	PARTICIPANT INFORMATION	Response	Instruction
4.	What is your date of birth?		
	dd/mm/yyyyy		**Skip to 4b**
	Don't know	98	
			Note: Eligible if born between 1967 and 1984
4a	If not known, what is your age? years		
	Don't know		
4b	**Screening ID (Date of birth, initials)**		
	dd/mm/yy/initials		
	Fill this information in the space at the bottom of every page		
4c	Can we contact you with further information on this study?		
	Yes	**1**	
	No	**2**	**Skip to 5**
	No response	**99**	**Skip to 5**
4d			
	If yes, please give contact details		**Go to Locator form**
Interviewer: *I will start by asking you some questions about yourself, your schooling, your home, and where you go for health care services.*			
5	What is your home language?	**Circle one**	
	isiZulu	1	
	isiXhosa	2	
	Sesotho	3	
	North Sotho (Sepedi)	4	
	Isindebele	5	
	IsiSwati	6	
	Setswana	7	
	Xitsonga	8	
	TshiVenda	9	

	English	10	
	Afrikaans	11	
	Other	12	
	(Specify)		
	No response	99	
6	What is your religion?	**Circle that which best applies**	
	Christian	1	
	Traditional African	2	
	Islam	3	
	None	4	
	Other	5	
	Specify		
	No response	99	
7	What is the highest level of education that you have completed?	**Choose Grade or Standard**	
	Grade		
	OR		
	Standard		
	Tertiary	93	
	Did not attend school	94	
	Don't know	98	
	No response	99	
8	Do you stay in Soweto?		
	Yes	1	
	No	2	**Skip to 8a**

	If yes, specify area	**Choose one**	
	Braam Fischer	1	
	Chiawelo	2	
	Central Western Jabavu	3	
	Diepkloof	4	
	Dlamini	5	
	Dobsonville	6	
	Doornkop	7	
	Dube village/ext	8	
	Jabavu	9	
	Jabulane	10	
	Klipspruit west	11	
	Kliptown	12	
	Khwezi	13	
	Mapetla	14	
	Meadowlands	15	
	Merafe	16	
	Mlamlankunzi	17	
	Mofolo	18	
	Molapo	19	
	Moletsane	20	
	Moroka	21	
	Mzimhlophe	22	
	Naledi	23	
	Nancefield	24	
	Noordgesig	25	
	Orlando east/west	26	
	Phefeni	27	
	Phiri	28	
	Phomolong	29	
	Pimville	30	
	Protea	31	
	Sgodiphola	32	
	Snake Park	33	
	Tladi	34	
	White city Jabavu	35	
	Zola	36	
	Zondi	37	
	Other	38	
	Specify area		
			Note: Eligible if living in Soweto

8a	If you **do not stay in Soweto**, where do you stay?		
	(Specify area)		
	No response	99	
8b	If you stay **outside Soweto**, are you likely to move to Soweto in the next 12 months?		
	Yes	1	
	No	2	
	Don't know	98	
	No response	99	
8c	How long have you stayed where you are currently living?		
	years		
	Don't know	98	
	No response	99	
8d	How many nights have you stayed away from home in the last month?		
	nights		
	Don't know	98	
	No response	99	
9	Where do you usually go for health care services?	**Circle all that apply**	
	Private clinic	1	**Skip to 10**
	Private doctor	2	**Skip to 10**
	Hospital	3	**Skip to 10**
	Chemist	4	**Skip to 10**
	Traditional healer	5	**Skip to 10**
	Public sector clinic	6	
	Other		
	Specify		**Skip to 10**
	No response	99	**Skip to 10**

9a	If you go to a public clinic where do you usually go to?	**Circle all that apply**	
	Bophelong/Snake Park	1	
	Chiawelo	2	
	Deipkloof	3	
	Green Village	4	
	Itereleng/Dobsonville	5	
	Jabavu	6	
	Klipspruit West	7	
	Lenasia	8	
	Lilian Ngoyi	9	
	Mandela Sisulu /Phomolong	10	
	Meadowlands	11	
	Mofolo	12	
	Mofolo South	13	
	Moroka	14	
	Nokuphila /Doornkop	15	
	Orlando	16	
	Pimville	17	
	Senaone	18	
	Siphumlile/Snake Park	19	
	Tladi	20	
	Tsepisong/Doornkop	21	
	Zola	22	
	Other	23	
	Specify		
	No response	99	
	Note: Eligible if using Soweto primary health care clinics		
10	Describe the type of housing in which you stay	**Circle that which best applies (only one answer)**	
	House	1	
	Room inside/outside the house	2	
	Shack	3	
	Flat	4	
	Other	5	
	Specify		
	No response	99	

10a	What is your housing made of?	**Circle that which best applies (only one answer)**	
	Bricks	1	
	Corrugated iron	2	
	Wood	3	
	Mud	4	
	Grass	5	
	Prefabricated	6	
	Other	7	
	Specify		
	No response	99	
10b	How many rooms are there in your house?		
	Number		
	No response	99	
10c	How many adults (persons over the age of 18) live there with you?		
	Number		
	No response	99	
10d	How many children (persons under the age of 18) live there with you?		
	Number		
	No response	99	
11	Do you have a source of income?		
	Yes	1	
	No	2	**Skip to 12**
	No response	99	**Skip to 12**

11a	If YES, where or who is your income from?	**Circle that which best applies**	
	Own salary or earnings (working)	1	
	Parent(s) (father/mother)	2	**Skip to 12**
	Partner (husband/boyfriend)	3	**Skip to 12**
	Sibling (brother or sister)	4	**Skip to 12**
	Government grant	5	**Skip to 12**
	Donations	6	**Skip to 12**
	Relative(s)	7	**Skip to 12**
	Other	8	**Skip to 12**
	Specify		
	No response	99	
11b	What job(s) do you do?	**Circle that which best applies**	
	Employed full time	1	
	Employed part time	2	
	Self employed	3	
	Unemployed	4	
	Student/scholar	5	
	Other	6	
	Specify		
	No response	99	

Interviewer: Now I'd like to ask you some questions about you and your sexual partner or partners. I'd like to remind you that this information is confidential.

12	Are you ***currently*** married (traditional or legal) or living with a man/woman with whom you have a sexual relationship?	**Circle that which best applies**	
	Currently married, living with spouse	1	***Skip to 12b***
	Currently married, living with other sexual partner	2	**Skip to 12b**
	Not married, living with sexual partner	3	**Skip to 12c**
	Currently married, not living with spouse or any other sexual partner	4	**Skip to 12d**
	Not married, not living with sexual partner	5	**Skip to 12d**
	No current partner (single)	6	
	No response	99	

12a	If no current partner (single), do you expect to be sexually active in the next year (12 months)		
	Yes	1	**Skip to 14**
	No	2	**Skip to 14**
	Don't know/unsure	98	**Skip to 14**
	No response	99	**Skip to 14**
	Note: Eligible is sexually active or planning to be sexually active in the next 12 months.		
12b	Does your spouse have any other wives that you know of?		
	Yes	1	
	No	2	
	Don't know/unsure	98	
	No response	99	
12c	How many nights did your partner spend **away from home** in the last month (30 days)?		
	Nights		**Skip to 13**
12d	How many days/nights in the **last month (30 days)** did you spend with your partner?		
	Nights		
13	Do you have any other sexual partner/s ***currently*** (apart from your spouse and/or the sexual partner you live with)?		
	Yes	1	
	No	2	**Skip to 14**
	No response	99	**Skip to 14**

13a	If **YES**, how many?		
	Number of other partners		
	No response	99	
13b	How many days or nights a month **in total** do you spend with this other sexual partner?		
	Use additional space if more than one other sexual partner currently		
	days/nights		
	days/nights		
	days/nights		
	No response	99	
Interviewer: **I'm now going to ask you some questions about your pregnancies, children and family planning history.**			
14	How many pregnancies have you had?		
	Number		**If zero (0), Skip to 16**
	No response	99	
15	How many living children do you have?		
	Number		
	No response	99	
16	Do you wish to have children/more children?		
	Yes	1	
	No	2	**Skip to 17**
	Don't know/unsure	98	**Skip to 17**
	No response	99	**Skip to 17**
16a	If **YES**, in the next 2 years?		
	Yes	1	
	No	2	**Skip to 17**
	No response	99	**Skip to 17**
16b	If **YES**, in the next year?	Yes 1	

	No	2	
	No response	99	
17	What contraceptive method are you using currently?	**Circle all that apply**	
	Injectable	1	**Skip to 17a**
	Pill	2	
	Loop/intrauterine device	3	
	Condom	4	
	Natural methods	5	
	None	6	
	Other	6	
	Specify		
	No response	99	
17a	If you are using an injectable contraceptive, what is the name of that injectable contraceptive?		
	Nur-Isterate	1	
	Depo-Provera	2	
	Don't know	98	
	No response	99	
Interviewer: **I'd like to ask you some questions about your feelings regarding HIV testing.**			
18	Have you ever been tested for HIV? Yes	1	
	No	2	
	No response	99	
19	Are you willing to be tested for HIV? Yes	1	
	No	2	**Skip to 19 b**
	Don't know/unsure	98	**Skip to 20**
	No response	99	**Skip to 20**
			Note: Eligible if agrees to be tested for HIV
19a	If **YES**, why?	**Circle all that apply**	
	Don't always use a condom	1	
	Don't trust sexual partner	2	

	Have a new sexual partner	3	
	Sexual partner has or had an STI	4	
	Want to know my HIV status	5	
	Having a baby	6	
	Do not expect a positive result	7	
	No reason	8	
	Other	9	
	Specify		
	No response	99	
19b	If **NO**, why not?	**Circle all that apply**	
	Only one partner	1	
	Trust my partner	2	
	Use condoms all the time	3	
	Afraid	4	
	Don't have an STI	5	
	No reason	6	
	Other	7	
	Specify		
	No response	99	
20	Do you think that you are at risk of being infected with HIV?		
	Yes	1	
	No	2	**Skip to 20b**
	Don't know/unsure	98	
	No response	99	

20a	If **YES**, why?	**Circle all that apply**	
	Don't trust my partner	1	
	Have more than one partner	2	
	Don't use condoms all the time	3	

	Could be raped at any time	4	
	Have had an STI	5	
	I am not in good health	6	
	Other	7	
	Specify		
	No response	99	
20b	If **NO**, why?	**Circle all that apply**	
	Abstaining from sex	1	
	Always use condoms	2	
	Only have one partner	3	
	Trust partner	4	
	Have not had an STI	5	
	I am in good health	6	
	Other	7	
	Specify		
	No response	99	
21	Are you currently enrolled in any other research studies?		
	Yes	1	
	No	2	
	No response	99	
	Note: Not eligible if currently enrolled in any other research studies		

C	**INTERVIEWER CHECK FOR ELIGIBILITY**		
21a	Age 18-35 (Q4)		
	Yes	1	
	No	2	
21b	Lives in Soweto (Q8)		
	Yes	1	

		No	2	
21c	Planning to be sexually active in the next 12 months (Q12)			
		Yes	1	
		No	2	
21d	Using a reliable method of contraception (Q17)			
		Yes	1	
		No	2	
21e	Willing to be tested for HIV (Q19)			
		Yes	1	
		No	2	
21f	Not currently enrolled in any other research studies			
		Yes	1	
		No	2	

	If the answer to all the above questions is YES, then the participant if ELIGIBLE.		**If any of the answers is NO, then the participant is INELIGIBLE.**	
	IF ELIGIBLE, **"Thank you for answering these questions. We would like to invite you to come to our clinic on the 1st Floor, New Nurses Home, Chris Hani Baragwanath Hospital for a follow up visit some time in the next two weeks when it is convenient for you. Please take a copy of this information sheet home with you to read, and discuss with your family, if you wish. We look forward to seeing you at the clinic."**		**IF NOT ELIGIBLE,** **"Thank for taking the time to answer these questions. If you have any further questions about the study, please contact us on the numbers provided on the information sheet."**	
	Time interview completed:		Initials	

	ENROLMENT INTERVIEW FORM (V0A)		
	These questions will be asked within 30 days of screening visits V-1A and V-1B being completed.		
A	**Office use only**		
1.	Date of interview [ ] [ ]/[ ] [ ]/[ ] [ ] [ ] [ ]		
	d d / m m / y y y y		
2.	Interviewer's initials [ ] [ ] [ ]		
3.	Enrolment ID [ ] [ ] /[ ] [ ]/ [ ] [ ] [ ] [ ]/[ ] [ ] [ ]/[ ] [ ] [ ] [ ]		
	d d / m m / y y y y / initials /unique number		
	Interviewer:		
	Hello, my name is _____, and I am from the Reproductive Health Research Unit. We would like to ask you some questions about your relationships, sex life, condom use & contraceptive use. We would like to ask these questions so that we can understand how people's relationships work. Please remember that any information you give me will be handled with strict confidentiality, which means no one else will know about your answers. If you don't want to answer any of these questions, please say so and I will go on to the next question.		
B	**Relationships** *Please can you confirm that your relationship status has not changed since your last interview.*		
4.	Are you ***currently married*** (traditional or legal) or living with a man/woman with whom you have a sexual relationship?	**Circle that which best applies**	
	Currently married, living with spouse	1	
	Currently married, living with other sexual partner	2	
	Not married, living with sexual partner	3	
	Currently married, not living with spouse or any other sexual partner	4	Skip to 5
	Not married, not living with sexual partner	5	Skip to 5
	No current partner (single)	6	Skip to 5
	No response	99	Skip to 5

4a	Does your spouse or partner have any other wives or partners that you know of?		
	Yes	1	
	No	2	
	No response	3	
5.	Have you had sex in the **last three months**?		
	Yes	1	Skip to 6
	No	2	
	No response	99	Skip to 6
5a.	If you have not had sex in the **last three months**, can you tell me the reason why?		
	Abstinence following delivery	1	Skip to 9
	Chose abstinence	2	Skip to 9
	Sick	3	Skip to 9
	No partner	4	Skip to 9
	Other	5	Skip to 9
	Specify	6	Skip to 9
	No response	99	Skip to 9
6.	How many sexual partners in total have you had in the last **3 months**, including casual and once off encounters?		
	Total number of sexual partners		
	No response	99	Skip to 9

7. Please can you define your relationship with each of these people, according to the following categories:

Interviewer: **please list each partner mentioned in** question 6 **above, and asked the participant to categorise according to the four categories listed below. Please note the number of partners per category in the table below.**

		Main partner (Married or living together as spouse)	**Regular** (Not married, not living together)	**Casual**	**Other**
7a.	Total number of partners in each category in the last 3 months?				

7b.	Total number of partners in each category in the past 4 weeks?				
8.	**Interviewer**: *With reference to the partner you have mentioned, could you please tell me…*				
		Main partner	**Regular**	**Casual**	**Other**
8a.	How many times did you have vaginal sex with each partner in the ***last*** week (7 days)? (Number of times)				
8b.	How many times did you have vaginal sex with each partner in the last ***four*** weeks (Number of times)				
8c.	Did you engage in anal sex with your partner in the last ***four*** weeks?				
	Yes	1	1	1	1
	No	2	2	2	2 Skip to 9
	No response	99	99	99	99 Skip to 9
8d.	If YES, how many times in the last four weeks did you engage in anal sex with your partner? (Number of times)				
C	**Condom use**				
	Have you ever used condom when having sex?				
9.	Yes	1			
	No	2			Skip to 9b
	No response	99			Skip to 11
9a.	If YES, what were the reasons for using a condom?		**Circle all that apply**		
	No reason	1			Skip to 10
	Don't trust partner	2			Skip to 10
	To avoid pregnancy	3			Skip to 10
	To avoid infection (STIs/HIV	4			Skip to 10
	Other	5			
	Specify				Skip to 10
9b.	If NO, what was the reason for not using a condom?		**Circle all that apply**		
	No reason	1			Skip to 11
	No need	2			Skip to 11
	Did not have one	3			Skip to 11
	Do not like them	4			Skip to 11

	Raped	5	Skip to 11
	Do not know about them	6	Skip to 11
	Do not know how to use them	7	Skip to 11
	Could not afford them	8	Skip to 11
	Other	9	Skip to 11
	Specify		Skip to 11

10.		**Main partner**	**Regular**	**Casual**	**Other**
a.	How many times in the last week did you use a condom with each partner? (Always, sometimes, never)				
b.	How often did you use condom with each partner in last four weeks? (Always, sometimes, never)				
c.	Did you use a condom the last time you had sex with each?				
	Yes	1	1	1	1
	No	2	2	2	2
	No response	99	99	99	99

	Do you always have condoms when you need them?		
11.	Yes	1	Skip to 13
	No	2	
	No response	3	Skip to 13
	What keeps you from having condoms when you need them?	**Circle only one**	
12.	Ran out	1	
	Don't know where to get them	2	
	Can't afford to buy them	3	
	Clinic ran out of condoms	4	
	Don't use condoms	5	
	Other	6	
	Specify		
D	**Sexual hygiene practices**		
13.	Do you ever douche? By douching I mean, do you ever use liquids or solutions to cleanse the inside of your vagina?		
	Yes		
	No		Skip to 17
	No response		Skip to 17

14.	What liquids or solutions do you usually use for douching?	**Circle all that apply**	
	Traditional Herbs	1	
	Vinegar	2	
	Water	3	
	Coca-cola	4	
	Detergent or washing powder	5	
	Disinfectant (Savlon, Dettol, etc)	6	
	Betadine douche bought over the counter	7	
	Soap and water (including Sunlight)	8	
	Other	9	
	Specify		
15.	When do you usually douche?	**Circle only one**	
	Before sex	1	
	After sex	2	
	Before and after sex	3	
	In the morning	4	
	In the morning and evening	5	
	Whenever I feel like	6	
	Other	7	
	Specify		
16.	In the last 4 weeks how often did you douche?	**Circle only one**	
	Every day	1	
	Every time I had sex	2	
	Sometimes (at least once in the last month but not everyday)		
	Never		
	Other		
	Specify		
17.	Do you ever insert any other products or substances into your vagina?		
	Yes	1	
	No	2	End
	No response	99	End

18.	In the last 4 weeks did you insert any other substances into your vagina?		
	Yes	1	
	No		End
	No response		End
	What products or substances did you insert into your vagina?	**Circle only one**	
19.	Towels, sponges, clothes	1	
	Toilet paper, tissues, cotton wool	2	
	Disinfectant	3	
	Snuff	4	
	Traditional herbs	5	
	Vinegar	6	
	Tampon	7	
	Other	8	
	Specify		
20	How often in the last 4 weeks did you insert the products or substances you have mentioned into your vagina?	**Circle only one**	
	Every day	1	
	During menses	2	
	After menses	3	
	Before sex	4	
	After sex	5	
	Before and after sex	6	
	Whenever I feel like it	7	
	Other	8	
	Specify		

Thank you very much for your time and for sharing this information with us. All this information will be kept confidential.

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