Risk factors for high risk Human Papillomavirus (HR-HPV) infection among unscreened African women aged thirty-five to sixty-five years.

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MMed THESIS TITLE:

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DECLARATION

I, Michelle Ann De Souza, hereby declare that the work on this dissertation is based on my original research (except where acknowledgements indicate otherwise) and has not, in whole or in part, been submitted towards another degree, at this university or elsewhere. The university is empowered to reproduce either the whole or any portion of the contents for the purposes of research.

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List of abbreviations

AIC:	Aikaike's information criterion
AIDS:	Acquired immunodeficiency disease syndrome
ASCUS:	Atypical squamous cells of unknown significance
ASIR:	Age standardized incidence rates
BV:	Bacterial Vaginosis
CHF:	Community health forum
CHW:	Community health worker
CI:	Confidence interval
COC:	Combined oral contraceptive
DMPA:	Depot medroxyprogesterone acetate
DNA:	Deoxyribonucleic acid
DVI:	Direct visual inspection
ELISA:	Enzyme-linked immunosorbent assay
EU:	European Union
FDA:	Food and drug administration
HC-I:	Hybrid Capture I HPV test
HC-II:	Hybrid Capture II HPV test
HIV:	Human immunodeficiency virus
HSIL:	High grade squamous intra-epithelial lesion
HPV:	Human Papilloma virus
HR-HPV:	High risk genital Human Papilloma virus
HSV-1	Herpes simplex virus-1
HSV-2:	Herpes simplex virus-2
IARC:	International Agency for Research on Cancer
IV:	Intravenous
KOH:	Potassium hydroxide
LAIP:	Long-acting injectable progesterone
LSIL:	Low grade squamous intra-epithelial lesion
MM:	Michael Mapongwana Day hospital

NCI:	National Cancer Institute
NCR:	National Cancer Registry
Net-EN:	Norethindrone enanthate
OR:	Odds ratio
Pap:	Papanicoloau test
PCR:	Polymerase chain reaction
POR:	Prevalence odds ratio
PR:	Prevalence ratio
RNA:	Ribonucleic acid
SADHS:	South African Demographic Health Survey
SAT:	Screen and treat study
STI:	Sexually transmitted infection
USA:	United States of America
VIA:	Visual inspection with acetic acid

Dedication

This thesis is dedicated to the three most important people in the world to me; my husband Ross, my daughter Catherine and my daughter Josie. Without their constant love and support, this thesis would not have been possible and I thank them for this.

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Abstract

Title: Risk factors for high risk Human Papillomavirus (HR-HPV) infection among unscreened African women aged thirty-five to sixty-five years.

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Introduction: Persistent infection with high risk types of Human Papillomavirus (HR-HPV) is a known necessary cause of cervical cancer which is the second most common cancer in women around the world. Genital HPV infection is one of the commonest sexually transmitted infections in the world. This study was designed to evaluate the prevalence of HR-HPV in previously unscreened African women aged thirty-five to sixty-five years and to determine the socio-demographic, behavioural, contraceptive use and biological risk factors for HR-HPV infection among these women.

Methods: This was a cross-sectional analytic study design using data derived from a randomized control trial (SAT study) evaluating screen and treat modalities, which was located in an area called Khayelitsha in the Western Cape. At enrolment, all women underwent a clinical examination, completed a questionnaire on demographic characteristics and sexual behaviors, and provided blood samples for HIV testing. Samples for *Neisseria gonhorreae* and *Chlamydia trachomatis* were collected using endocervical cone-brushes and tested using the Hybrid Capture GC/CT DNA Assay Endocervical cone-brush samples were tested for Human Papilloma Virus (HPV) DNA using the Hybrid Capture II HPV DNA Assay. Wet mount exams were performed on-site during the clinical examination by trained study nurses to identify *Trichomonas vaginalis* and Bacterial vaginosis was assessed during the clinical examination using Amsel criteria. Data from the enrollment visit was analyzed for 6645 participants and a multiple logistic regression analysis was performed to evaluate the risk factors for HR-HPV infection.

Results: In total, 6645 participants were included in the analysis. Of these women, 1416 (21.3%, 95% confidence interval (CI); 20.3; 22.3) tested positive for HR-HPV infection. The multivariate logistic regression analysis showed that a positive Human immunodeficiency virus (HIV) status (odds ratio (OR); 4.08, 95% CI; 3.47; 4.80), previous sterilization (OR; 0.72, 95% CI; 0.61; 0.85), current use of condoms (OR; 2.15, 95% CI; 1.22; 3.80), current use of Depot medroxyprogesterone acetate (DMPA) (OR; 1.37, 95% CI; 1.13; 1.65), current use of Norethindrone enanthate (Net-EN) (OR; 1.39, 95% CI; 1.02; 1.88), currently married (OR; 0.71, 95% CI; 0.62; 0.81), mean number of live births (OR; 1.10, 95% CI; 1.06; 1.14), mean age in years (OR; 0.99, 95% CI; 0.98; 0.997) and currently employed (OR; 0.86, 95% CI; 0.74; 0.99) were significant in a model predicting the odds of infection with HR-HPV when adjusted for other socio-demographic, behavioural and biological variables and use of contraception.

Conclusions: In conclusion, this study shows that there is a very high prevalence of HR-HPV infection in African women aged thirty-five to sixty-five years living in Khayelitsha. The overwhelming association between HIV infection and HR-HPV infection in this study has very important clinical and policy implications in the communities where HIV infection, Acquired immunodeficiency disease syndrome (AIDS) and cervical cancer are major health problems. This study also adds onto the knowledge of risk factors for HR-HPV infection, but introduces the possibility of long-acting injectable progesterones (LAIP) having a significant effect on the prevalence of HR-HPV infection and highlights the need for further research into the risks of HR-HPV infection.

Chapter one
Introduction

1.1 The problem: Cervical cancer

Cervical cancer is a continuing major public health threat to many women around the world. It is the second commonest cancer in women worldwide (Sankaranarayanan, Ferlay. et al. 2006, Parkin, Bray. et al. 2005) with nearly 500 000 women diagnosed with cervical cancer every year and 273 200 deaths annually (Table 1.1) (Denny, Sankaranarayanan. et al. 2006). Around 80% of the cervical cancer deaths occur in developing countries such as South Africa, where it is the leading cause of cancer deaths in women (Bradshaw, Groenewald. et al. 2003). In developed countries the incidence rates are low, but before cervical screening programs were introduced in the 1960s and 1970s in countries like Europe and North America, the incidence rates were similar to the incidence rates seen in developing countries today (Parkin, Bray. et al. 2005).

Table 1.1: Cancers of the uterine cervix: incident cases and number of deaths in 2002. (Denny, Sankaranarayanan. et al. 2006)

	Cancer of the cervix	
Region	**No. of cases**	**No. of deaths**
Worldwide	492,800	273,200
More developed countries	83,400	39,500
Less developed countries	409,400	233,700
Southern Africa	7,600	4,400
Eastern Africa	33,900	27,100
Middle Africa	8,200	6,600
Northern Africa	8,100	6,500
Western Africa	20,900	16,700

In developing countries cervical cancer incidence rates derived from population-based registries are either outdated or incomplete and thus reliable data is difficult to obtain. In South Africa, the National Cancer Registry (NCR), a pathology-based cancer registry, was set up in 1986 and this registry relied upon histology reports from laboratories around the country. The NCR published the 1998-1999 cancer statistics in April 2005. The most current data on incidence of cervical cancer comes from this report (Mqoqi, Kellett. et al. 2005). The NCR reported that there were 6061 new cases of cervical cancer in 1998 and it was the leading cause of cancer in females in 1998. In 1999 it was the second leading cancer in South African women with 5203 new incident cases (Mqoqi, Kellett. et al. 2005). The lifetime risk of cervical cancer for all women was one in twenty-six in 1998 and one in thirty-one in 1999. The NCR also reported that the risk of developing cervical cancer increased with age, with women aged 65 to 69 years having the highest age standardized incidence rate (ASIR) of 136.4 per 100 000 women (Mqoqi, Kellett. et al. 2005). Table 1.2 shows the ASIR for different populations in South Africa taken from the NCR in 1998 and 1999. It shows the marked difference between the different population groups living in South Africa. The high ASIR (42.1 / 100 000) in African women was related to the lack of access to effective and well-managed cervical screening programs and although the ASIR (34.88 / 100 000) had decreased in 1999, cervical cancer was responsible for 33% of all cancers in African women in that year. It is acknowledged that the presented figures are probably underestimated for African women who may die in the rural areas without a histological diagnosis having been made at a laboratory and thus they have not been included in the register.

Table 1.2: Summary statistics for cervical cancer, 1998 and 1999 (Mqoqi, Kellett. et al. 2005)

Population group	Age standardized incidence rates (ASIR)	
	1998	**1999**
Asian	16.39	11.02
African	42.1	34.88
Coloured	29.04	26.35
White	14.5	12.04
Total	**34.43**	**29.72**

1.2 Aetiology of cervical cancer

Persistent infection with high risk genital types of Human Papillomavirus (HR-HPV) is a known necessary cause of cervical cancer and its precursors (Munoz, N. 2000, Walboomers, Jacobs. et al. 1999). Research has shown that the worldwide prevalence of HR-HPV in cervical cancer specimens is 99.7% and HR-HPV infection has the highest "worldwide attributable fraction" reported for a specific cause of a major cancer (Walboomers, Jacobs. et al. 1999). The findings mean that cervical cancer will not develop without the presence of a persistent infection with HR-HPV (Bosch, Lorinez. et al. 2002). This has important clinical relevance as women that are at high risk of cervical cancer are those women that have a persistent infection with known HR-HPV types.

In the cervix, the cervical transformation zone is the area that is uniquely susceptible to Human papillomavirus (HPV) carcinogenicity. This area is also easily accessible for clinicians to detect and monitor the early changes in the cervix which later lead to cervical cancer. This has allowed for much research to be carried out in determining the critical pathway from the normal cervix to cancer formation (Schiffman, Castle. 2003). There are three important steps in cervical carcinogenesis which include: HR-HPV transmission and acquisition, progression to precancer and finally cancer invasion of the cervix.

1.3 Justification/ rationale for this study

Cervical cancer is still a major public health burden in South Africa as shown by the burden of disease statistics for South Africa (Bradshaw, Groenewald. et al. 2003). Persistent infection with HR-HPV types is a known necessary cause of cervical cancer and its precursors (Walboomers, Jacobs. et al. 1999). Some studies have been done on the prevalence of HR-HPV infection in South Africa and the Western Cape and these have shown that there is a high prevalence of HR-HPV infection in these communities (Denny, Kuhn. et al. 2000, Allan, Marais. et al. 2006). Much research has been done on risk factors for HR-HPV infection in developed countries but there is limited published knowledge on the risks of HR-HPV infection in South African women. Infection with HIV has been associated with HR-HPV infection in South Africa but there is no data on the use of contraception and other behavioural risk factors and the association with HR-HPV infection in our communities.

The motivation for this research is to determine the prevalence of HR-HPV infection in previously unscreened African women and to analyze the risk factors for HR-HPV infection in African women aged thirty-five to sixty-five years living in the Western Cape, South Africa. It is important to understand the risk factors in these women so that appropriate preventative programs can be developed for South African women at risk of HPV infection specifically.

1.4 Overall aim of the study

This study was designed to determine the risk factors for HR-HPV infection for women aged thirty-five to sixty-five years living in Khayelitsha, South Africa.

1.5 Objectives of the study

1. To determine the prevalence of HR-HPV infection in women aged thirty-five to sixty-five years living in Khayelitsha, South Africa.

2. To ascertain which of the following factors were associated with HR-HPV infection in a previously unscreened population of women:

a. demographic factors – age, marital status, type of housing, education level, employment status.
b. behavioural – age at first intercourse, number of partners in lifetime, sexual activity in last month, age at first pregnancy, number of pregnancies, number of live births, smoking habits, use of alcohol, use of barrier contraception, use of hormonal contraception.
c. biological – infection with Human immunodeficiency virus (HIV), presence of sexually transmitted infections (STI) and previous treatment for sexually transmitted infections.

3. To determine the effect of age on the above mentioned risk factors by stratifying the dataset by three age categories namely age 35-39 years, 40-49 years and 50-65 years.

4. To make recommendations regarding cervical cancer prevention programmes and targeting women who may be more at risk for education and prevention programs.

1.6 Literature review on Human Papillomaviruses and risk factors

The literature review will focus on the Human Papillomavirus and the risk factors for HR-HPV infection.

1.6.1 Human Papillomaviruses

Human papilloma virus (HPV) is a small, nonenveloped, double-stranded deoxyribonucleic acid (DNA) virus that infects epithelial cells through tissue disruptions. HPV's are classified into different types and over 120 types of the HPV have been identified. There are at least 40 types which infect the epithelial lining of the anogenital tract and 15 types have been classified as high risk or cancer inducing (Trottier, Franco.

2006, Wiley, Masongsong. 2006, Munoz, Bosch. et al. 2003). The high risk types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. HPV 16 and 18 are the most common high risk types that are found and are together associated with about 70% of all cervical cancers worldwide (Wiley, Masongsong. 2006). A further three types namely HPV 26, 53, and 66 are probable high risk types. The low risk or benign HPV types cause genital warts and many low-grade intraepithelial lesions of the cervix and the most important low risk types are 6 and 11 (Trottier, Franco. 2006, Munoz, Bosch. et al. 2003).

HPV infection is spread via skin to skin contact. They infect basal cells in the stratified epithelium via small disruptions in the tissues. HPV infection disrupts the normal functioning of the cell but normally there is very little inflammation associated with the infection which would attract a large immune response. Previous research has shown that the body's immune response to the presence of HPV's can be delayed and some women may never develop antibodies (Wiley, Masongsong. 2006). The HPVs may become integrated into the human DNA in the cell and this occurs often in some high grade precancer lesions and cancer lesions (Wiley, Masongsong. 2006).

Genital HPV infection is one of the commonest sexually transmitted infections in the world (Trottier. Franco. 2006, Wiley, Masongsong. 2006) and in the United States of America (USA) approximately 6.4 million new infections occur annually (Ault, K.A. 2006). The prevalence of HPV infection ranges from 30.5% in Mozambique, to 9% in Canada and 13% in West Africa (Wall, Scherf. et al. 2005, Bosch and de Sanjose. 2003). In Asia, the prevalence rate was shown to be 38.8% in University students that were sexually active (Shin, Franceschi. et al. 2004). In Brazil, the HR-HPV prevalence has been shown to be between 22.1% and 31.8% and it is one of Brazil's most important sexually transmitted infections (STIs) (de Lima Soares, de Mesquita. et al. 2003). The HR-HPV prevalence in the Western Cape was found to be 17% in a study which ended in December 2001 (Allan, Marais. et al. 2006). This study also showed that the age-specific prevalence of HR-HPV was 37.1% in women aged less than 30 years, 21.1% in those women aged 30-39 years, 12.9% in women aged 40-49 years and 14.7% in women aged

50-59 years. Similarly a previous study done in Khayelitsha in women aged 35-65 years showed the prevalence of HR-HPV infection to be 16.2% (Denny, Kuhn. et al. 2000).

The prevalence of HR-HPV infection is highest in young sexually active women (Trottier, Franco. 2006) and in most populations there is a decreasing prevalence of HR-HPV infection with increasing age despite ongoing sexual activity (Trottier, Franco. 2006, Wiley, Masongsong. 2006, Kjaer, Svare. et al. 2000). It has been postulated that this effect of age on the prevalence of HR-HPV infection, is due to an infected women developing an immune response that prevents infections in the future (Trottier, Franco. 2006, Kjaer, Svare. et al. 2000). This effect of age on the prevalence of HR-HPV infection is important when comparing prevalences from different studies. Some researchers have found that there is a decreasing prevalence of HR-HPV infection with age after thirty years but then prevalence seems to increase slightly again in women older than 50-55 years (Allan, Marais. et al. 2006, Sellors, Karwalajtys. et al. 2002). This age related prevalence pattern of a high prevalence in women aged 30 years or less and then a decreasing prevalence with increasing age until age 50, whereby the prevalence increases again slightly will need to be evaluated in this study setting to determine if this pattern occurs in our sample of women.

Although HPV infections are very common, in most cases the infections are cleared spontaneously and only about 7% of infections persist after five years (Molano, Van den Brule. et al. 2003). The evidence is clear that the risk of cervical precancerous lesions or cervical intraepithelial neoplasia (CIN) increases proportionately to the number of cervical specimens taken over time that test positive for HR-HPV types (Trottier, Masongsong. 2006). The persistence of HR-HPV infection as an important step in the development of cervical carcinogenesis is accepted internationally, but the exact definition of what constitutes a transient or persistent infection has not been agreed upon and further population studies need to still be completed. Along with the HR-HPV persistent infection, there are certain co-factors which when present lead to the development of precancer and possibly invasive cervical cancer in women who are HR-HPV positive. Co-factors such as smoking and contraceptive use have been extensively

studied (Castle, Walker. et al 2005, Mcintyre-Selman, Castle. et al. 2005, Plummer, Herrero. et al 2003, Herrero, Brinton. et al. 1990). Both men and women can be infected with many different types simultaneously and acquiring immunity to one type doesn't seem to protect one from the other types (Wiley. Masongsong. 2006).

1.6.2 Testing for HR-HPV infections

There are two molecular tests to test for HPV infection that have been approved by the US Food and Drug Administration (FDA) and that are commercially available. There is the Hybrid Capture I HPV test (HC-I) and now the more sensitive Hybrid Capture-II HPV test (HC-II) (Digene Corporation, Gaithersburg, MD) which tests for 13 different high-risk HPV types and five low-risk types. The HC-II test has an additional 4 probes for the detection of HPV types and more samples can be processed at any one time. The HC-II test kit uses a liquid hybridization kit which uses ribonucleic (RNA) probes against HPV DNA genomic targets followed by signal amplification. The HC-II tests for the following HR-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 and five low risk HPV types namely 6, 11, 42, 43 and 44. One RNA probe detects the low risk types (A) and the B RNA probe detects the high risk types. One disadvantage of this method is the lack of HPV genotype information from this system. However this test is easy to do in clinical settings and does not need a special laboratory to avoid the cross-contamination of specimens or reagents.

The gold standard test for HPV detection is the polymerase chain reaction (PCR) technique which use consensus primers such as MY09/11 and GP5+/6+ and others to identify specific types of HPV (Denny, Wright. 2005). These processes however need to be done in specialized laboratories which have specific designs to prevent contamination. There are no commercially available PCR tests yet but this should change in the near future. The systems like non-amplification Southern and dot blot hybridizations with type-specific polymerase chain reaction (PCR) and general-primer PCR can detect HPV genotype (Huang, Chao. et al. 2006) and this gives these methods an advantage over the HC-II method. The most commonly used tests for research trials are the HC-II tests and

the PCR detection methods and the sensitivities and specificities of these tests are high and very similar (Soderlund-Strand, Rymark, et al. 2005, Venturoli, Cricca. et al. 2002).

1.6.3 HPV testing in primary prevention of cervical cancer

The Papanicoloau (Pap) smear test has been the standard cytology screening method for detecting pre-invasive lesions of the cervix. However there are limitations to this test such as its high false negative rate and the medical and legal consequences of this. Besides being used for studies on aetiology of cervical cancer, testing for HR-HPV infection as an additional primary screening test has become a very popular concept. HR-HPV testing has been tested previously in primary screening in asymptomatic women along with the Pap smear (Franco, E.L. 2003. It has also been used on women with abnormal Pap smear results as a complementary test to the Pap smear as well as in women that have had precancerous lesions treated and are undergoing follow-up for recurrence of the disease (Franco, E.L. 2003). HR-HPV testing has a higher sensitivity for detecting high grade squamous intra-epithelial lesions (HSIL) compared to the Pap smear, but it has a lower specificity. However when it is used in conjunction with the Pap smear test, the screening efficacy of the Pap smear test improves, which may allow for increased screening intervals (Ratnam, Franco. et al. 2000). The decision to use HR-HPV testing on its own as a primary screening test would depend on the setting and the available resources for each country. However HR-HPV tests can show a high false positive rate for cervical disease which could see many more women being referred for colposcopy to exclude disease. In low-resource settings, such as South Africa, this would put an extra burden on health services which are already oversubscribed. At present HR-HPV testing is not available in the public sector in South Africa unless in a research setting, but it is available in the private sector.

1.6.4 Risk factors for HR-HPV infection

There have been numerous studies on the risk factors for HR-HPV in women living around the world. There have been differing results due to the fact that different

populations have been studied and different risk profiles have been found depending on whether the study concentrated on high or low risk HPV types or what age group of women was studied (Trottier, Franco. 2006). Many of the risk factors are sexual activity indicators but other risk determinants have been researched and they are often categorized as demographic risk factors, behavioral factors and biological risk factors.

1.6.4.1 Demographic risk factors

Demographic risk factors such as marital status and age have been investigated and those that are linked to possible behavioral factors have been found to be important. Women that are single, separated or divorced were found to be at risk of HR-HPV infection in a study performed in 2000 in Canada (Sellors, Karwalajtys. et al. 2002). This could be attributable to transmission of infections from new sexual partners. In the USA, a study looking at risk factors for HR-HPV infection in Mexican women born in the USA, showed that single women were more at risk of HR-HPV infection and this may be due to single women or their partners not being monogamous (Giuliano, Papenfuss. et al. 1999). Munoz, Kato. et al (1996) argue that a geographical variation in HR-HPV prevalence which they found in their study, is related to an earlier first sexual debut.

Besides the sexual behaviour risk determinates, the most consistent risk factor for HR-HPV infection is age (Trottier, Franco. 2006, Nyari, Kalmar. et al. 2004, Kjaer, Srave. et al. 2000, Kjaer, Van den Brule. et al. 1997, Munoz, Kato. et al. 1996). The relative odds for detecting HR-HPV infection decreases with increasing age (Van den Brule. et al. 1997). Most studies have found a marked decrease in risk once the women reaches age 30 or more (Trottier, Franco. 2006, Sellors, Karwalajtys. et al. 2003). Generally most HR-HPV infections in younger women are transient, and only a minority of women develop persistent infection. In older women, the HR-HPV infection is more likely to be a persistent infection and more relevant as a risk factor for the development of cervical cancer. There is also a decreased HR-HPV prevalence in older women which has previously been explained by changing sexual activity in older women. However a study in Danish sex workers, where sexual activity was similar in all ages, still found a

decreased HR-HPV prevalence in older women (Kjaer, Srave. et al. 2000). It is postulated that there is an acquired immune response which is responsible for the decreased prevalence in older women and is important for the natural history of HR-HPV infection (Del Amo, Gonzalez. et al. 2005, Kjaer, Svare. et al. 2000). Age has also been found to confound the associations between certain risk factors and HR-HPV infection (Del amo, Gonzalez. et al. 2005). Thus age will be an important risk factor to analyze in any risk factor analysis.

1.6.4.2 Behavioural risk factors

Certain behavioral factors such as sexual activity, smoking and alcohol use have been found to be important risk factors for HR-HPV infection. It has been consistently shown that sexual activity is the most important risk factor for incident HR-HPV infection (Wiley, Masongsong. 2006) and the most established risk factor is an increased number of lifetime sexual partners (Ahmed, Madkan. et al. 2006). The age of onset of sexual intercourse especially below 20 years of age is significantly associated with the presence of genital HR-HPV infection (Sellors, Karwalajtys. et al. 2002). The age of onset of sexual intercourse may be a marker for other sexual activity risks as for example the younger the age of sexual debut, the greater the number of lifetime partners there may be and this may be a determinant on its own. However it has been postulated that the presence of cervical ectopy during adolescence maybe the reason for the increased risk of age of sexual debut. However a study done by the International Agency for Research on Cancer (IARC) in 2005, which studied over 11 000 women, found that early age at sexual debut was not significantly related to HR-HPV positivity (Vaccarelle, Franceschi. et al. 2006: 326). This study did find that lifetime number of sexual partners was associated with HR-HPV positivity in their study participants. The partner's sexual behaviour was also significant after adjusting for age and number of women's sexual partners (OR; 1.45, 95% CI; 1.24; 1.70) (Vaccarelle, Franceschi. et al. 2006: 326). Similarly a population based study in Costa Rico, showed that age at first intercourse was not an independent risk factor (Herrero, Castle. et al. 2005). The number of sexual partners was an important predictor of HR-HPV infection in some studies (Wiley, Masongsong. 2006, Herrero,

Castle. et al 2005, Kataja, Syrjanen. et al. 1993) but in younger women recent sexual behavior seems to be more important than lifetime sexual partners (Sellors, Karwalajtys. et al. 2003, Munoz, Kato. et al. 1996,). However due to the high prevalence of HPV, even one previous sexual partner can be a risk factor of acquiring HR-HPV infection (Wiley, Masongsong. 2006).

The number of pregnancies a woman has ever had, has been postulated to be a risk factor for HR-HPV infection. A recent report done on the IARC HPV prevalence surveys showed that nulliparous women had a 40% increased odds of HR-HPV infection compared to parous women (OR; 1.40, 95% CI; 1.16; 1.69). However women who had five or more full-term pregnancies had similar odds of HR-HPV infection compared to women who had only one full-term pregnancy (Vaccarella, Herrero. et al. 2006). A study done by Kjaer. et al in 1997, showed that a higher number of live births was associated with a lower relative odds of HR-HPV infection after adjusting for confounding factors (OR; 0.40, 95% CI; 0.20; 0.70) in women with one or more live births compared to women who had never been pregnant (Kjaer, Van den Brule. et al. 1997). Other studies have found that the number of live births increases the odds of HR-HPV infection (Hildesheim, Gravitt. et al. 1993) while other studies have found no association with parity and prevalence of HR-HPV infection (Aggarwal, Gupta. et al. 2006).
Research has shown that smoking cigarettes is associated with an increased risk of HR-HPV infection, pre-invasive cervical lesions and cervical cancer. Smoking is linked to metaplasia and cell proliferation and the by-products of tobacco effect the local immunity of the cells and disturb certain cell death pathways which may induce instability in the cells (Wiley, Masongsong. 2006). In the early 1980s, researchers in Europe found that current smoking increased the risk for HR-HPV infection (Kataja, Syrjanen. et al. 1993) and Wang et al (2003) found this to be true in their population-based population of 10 000 women in Costa Rica. Recently studies have shown that smoking increases the risk of cervical cancer among HR-HPV positive women and thus acts as one of the co-factors in the pathway from HR-HPV infection to cancer (Plummer, Herrero. et al. 2003). A randomized trial conducted by the National Cancer Institute (NCI) to evaluate different management strategies for certain categories of cervical disease, found that current

smoking was only weakly positively associated with the risk of HR-HPV infection but found that HR-HPV-positive smokers were more likely to have cervical disease than HR-HPV-positive non-smokers (McIntyre-Seltman, Castle. et al. 2005). However some studies show that there is not a consistent association between smoking and the risk of HR-HPV infection (Baseman, Koutsky. et al. 2005, Burk. Ho. et al 1996,) or no association between the number of cigarettes smoked per day and presence of HR-HPV infection (Harris, Kulasingam. et al. 2004).

1.6.4.3 Use of contraception

Although the use of condoms has been suggested to prevent HPV infection (Winer, Hughes. et al. 2006, Herrero, Castle. et al. 2005, de Sanjose, Almirall. et al. 2003), it is known that because the condoms do not cover all infected surfaces and because condoms are often only used after some intimate contact has already taken place, there will not be 100% protection (Wiley, Masongsong. 2006, Winer, Lee. et al. 2003, Kjaer, Svare. et al. 2000). Some studies have shown that HR-HPV associated disease may be less common among those women whose partners consistently use condoms (Manhart, Koutsky. et al. 2002). A recent meta-analysis by Manhart and Koutsky, of over 20 trials which investigated the role of condoms in HPV transmission, concluded that the use of condoms does not protect against getting the HR-HPV infection, but the use of condoms may give some protection against HR-HPV associated disease like genital warts, precancerous lesions and cervical cancer (Manhart, Koutsky. et al. 2002). Some studies have also showed that the use of condoms could aid in the clearance of the HR-HPV infection (Hogewoning, Bleeker. et al. 2003).

Current combined oral contraceptive (COC) use has been associated with HR-HPV infection in some studies (Ahmed, Madkan. et al. 2006, Castle, Walker. et al. 2005, Herrero, Castle. et al. 2005, Green, Berrington De Gonzalez. et al. 2003, Winer, Lee. et al. 2003, Molano, Posso. et al. 2002, Munoz, Kato. et al. 1996).

There are not many studies in the literature that have looked at the risk of long-acting injectable contraceptives on the odds of HR-HPV infection. A cross-sectional study done at the United States-Mexico Border in 2001 showed that in a multivariate analysis, the current use of injectable contraceptives was significantly associated with HR-HPV infection (Giuliano, Papenfuss. et al. 2001). The authors concluded that because sexual history was accounted for in the multivariate model, the injectable contraceptives would have had an effect on the persistence of the HPV virus and not on new infections. The injectable contraceptives are progesterone-based and some studies have shown that progesterone has an effect on HPV persistence and also progression to pre-invasive cervical cancer (Sonnex, C. 1998). The exact mechanism has not been found but in the laboratory it appears that progesterone increases HPV cell transformation (Pater, Bayatpour. et al. 1990) and oncogene transcription (Yuan, Auborn. et al. 1999). The current use of long-acting injectable contraceptives has been shown to increase the risk of high grade pre-invasive cancer in HR-HPV positive women (OR; 1.6, 95% CI; 1.2; 2.1) (Castle, Walker. et al. 2005). A case control study in Latin America in the early 1990s concluded that prolonged use of long-acting injectable contraceptives increased the risk of cervical cancer especially for women who had stopped the method more than five years before the trial interview (Relative Risk (RR); 2.4, 95% CI; 1.0; 5.7) (Herrero, Brinton. et al. 1990). Further studies need to be done to evaluate the effect of progesterones on HR-HPV infection.

1.6.4.4 Biological risk factors

Biological risk factors such as infection with other sexually transmitted infections have been found to be important risk factors for HR-HPV infection (Ferenczy, Coutlee. et al. 2003, Jay, Moscicki. et al. 2000). Studies have shown that Human immunodeficiency virus (HIV) infection is a risk factor of genital HPV infection and that HPV infection is detected more frequently in HIV-positive women and is more persistent in HIV-positive women (Branca, Garbuglia. et al. 2003, Ferneczy, Coutlee. et al. 2003, Jay, Moscicki. et al. 2000). Studies done in Africa have also shown HIV-positive women are more likely to have HR-HPV infection compared to HIV-negative women (OR; 4.6, 95% CI; 2.8; 7.5)

(Moodley, Hoffman. et al. 2006). Women infected with HIV are at a higher risk of pre-invasive lesions of the cervix and this has been shown in Africa (Moodley, Hoffman. et al. 2006, La Ruche, You. et al. 1998, Laga, Icenogle. et al. 1992) and in developed countries (Branca, Garbunglia. et al. 2003).

It is postulated that this association is due to the HIV-associated CD4 T cell immunosuppression, but there may be mechanisms other than CD4 suppression that enhance HPV proliferation (Moscicki, Ellenberg. et al. 2000, Palefsky, Minkoff. et al. 1999). Research trials have shown that CD4 count and HIV viral load play a role in activation of HPV replication and HPV detection (Palefsky, Minkoff. et al. 1999). HIV-positive women with CD4 counts less than 200/mm^3 were at the highest risk of HR-HPV infection, while HIV-positive women with CD4 counts of more than 200/mm^3 and viral loads of less than 20 000 copies/mL were at the least risk of HR-HPV infection in a study in the USA (Palefsky, Minkoff. et al. 1999). Moscicki et al (2000) found that the rates of HPV infection and disease were higher among HIV-positive women than HIV-negative women despite the fact that they had similar risky sexual behaviour histories and that very few of the HIV-infected women had very low CD counts. In a study done on women intravenous (IV) drug users, HIV-seropositive status was also associated with an increased risk of HR-HPV infection (Dev, Lo. et al. 2006). The prevalence of HIV infection in South Africa has increased from 1% in 1990 to 29.5% in 2004 as shown in the annual antenatal HIV surveillance data (Department of Health of South Africa. 2005). Khayelitsha has one of the highest rates of HIV infection in the Western Cape (Medicins sans frontiers. 2003), and therefore HIV infection could be an important risk factor for HPV infection in this community.

There has been controversial evidence in the literature on the association of Herpes Simplex virus type-1 (HSV-1), type-2 (HSV-2) and *Chlamydia trachomatis* infection and cervical cancer. Infection with HSV-2 has been found to increase the risk of cervical cancer in some women already infected with HR-HPV (Smith, Herrero. et al. 2002). However more recently, a study done by Finan. et al. 2006, showed that HR-HPV-positive women who also had an infection with *Chlamydia trachomatis* or HSV-1, were

at a greater risk of developing cervical cancer than those infected with HR-HPV and HSV-2 (Finan, Musharrafieh. et al. 2006). In Argentina, women infected with HSV-1 or HSV-2 were not at an increased risk of cervical cancer and neither of these infections were associated with HPV positivity in women with normal cytology (Perez, Barbisan. et al. 2005). In Jamaica, co-infection with *Chlamydia trachomatis* or HSV-2 was not associated with the risk of HSIL lesions after controlling for HPV (Castle, Escoffery. et al. 2003). Infection with *Chlamydia trachomatis* has also been associated with persistent genital HPV infection (Samoff, Koumans. et al. 2005).

It has been hypothesized that the local "cervicovaginal milieu" is important for a women being susceptible to HR-HPV infection (Watts, Fasarri. et al. 2005:1129). Watts. et al (2005) showed that Bacterial Vaginosis (BV) infection was associated with prevalent HR-HPV infection (OR; 1.17; 95% CI; 1.08; 1.27) but was not associated with duration of HR-HPV infection (Watts, Fasarri. et al. 2005:1133). Trichomonas Vaginalis was also shown to be associated with a decreased prevalence of HR-HPV infection (OR; 0.82, 95% CI; 0.71; 0.96) but positively associated with incident HR-HPV infection (OR; 1.36, 95% CI; 1.14; 1.62) (Watts, Fasarri. et al. 2005:1134).

A depressed immune system (e.g. patients on immunosuppressive drugs) is also associated with higher rates of HR-HPV infection (Wiley, Masongsong. 2006). Research has also shown that there are some immunogenetic factors that are important for clearance of the HPV infection and the presence of for example some human leukocyte antigen (HLA) markers may be linked to the development of cervical cancer (Wiley, Masongsong. 2006)

1.6.5 Prevention of HPV infections

A prophylactic HPV vaccine was licensed in 2006. One of the largest pharmaceutical companies have been running vaccine efficacy trials which have culminated in their vaccine becoming FDA and European Union (EU) approved and commercially available in the USA and Europe. The vaccine that is available is active against two HR-HPV types

namely 16 and 18 and two low-risk types namely 6 and 11. It is registered for use in girls and women aged 10-25 and is available as a course of 3 injections. So far the vaccine has been found to be nearly 100% effective and extremely safe to use. Although the licensure of the vaccine is very exciting there are many programmatic problems that still need to be addressed. The best time to vaccinate girls and boys would be before the onset of sexual intercourse and this would require parents and guardians to give consent for this vaccine. There have been numerous debates on whether this strategy will be seen to be promoting sexual promiscuity and if parents would accept this vaccine (Brewer, Fazekas. 2007, Davis, Dickman. et al. 2004). Another programmatic issue would be the duration of efficacy and when booster shots would be needed. The current HPV vaccine will not be shortly available on a mass scale in developing countries due to the high cost of these vaccines. Even with the vaccine, the need for cervical screening programs and preventative programs would still need to exist as the vaccine will only prevent about 70% of all cervical cancers. A second vaccine by a competitor company, which is only active against the HR-HPV types 16 and 18 should be licensed soon and it is hoped that market forces will bring down the prices in developing countries. To date neither of these vaccines have been licensed in Africa so secondary preventative strategies and programmes are still vitally important in such countries like South Africa.

HPV infection is a sexually transmitted infection and preventative strategies have focused on promoting monogamous sexual relationships and use of condoms. Even though a meta-analysis on the efficacy of condom use preventing HR-HPV infection, concluded that there is no consistent evidence that using condoms reduces the risk of HR-HPV infection (Manhart, Koutsky. et al 2002), preventative programs continue to promote the use of condoms due to the benefits of reducing other sexually transmitted infections.

The fact that a persistent infection with HR-HPV types puts women at a high risk for cervical cancer allows different preventative strategies to be used in the public sector to prevent HR-HPV infection and ultimately cervical cancer. Screening for HR-HPV is an option but further research needs to be done in order to bring down the cost of the HR-HPV screening tests. Other primary preventative measures for HR-HPV infection such as

abstinence or the use of condoms is not possible in many populations and therefore knowledge of other risk factors for HPV infection besides sexual behaviour risk factors for different age groups of women is important in the South African context for HPV preventative programs to work. Women at a higher risk for HR-HPV infection could be targeted for preventative programs and more regular cervical screening services could be incorporated into the national screening program in South Africa. A systematic review by Shepard et al, found that educational programs, which have educational information along with the teaching of sexual negotiation skills, can encourage short term sexual reduction behaviour and this could possibly reduce the transmission of HPV infection (Shepherd. Peersman. et al. 2000). Further research is needed to see the effect of these type of programs on HPV transmission in developing countries such as South Africa.

Chapter two

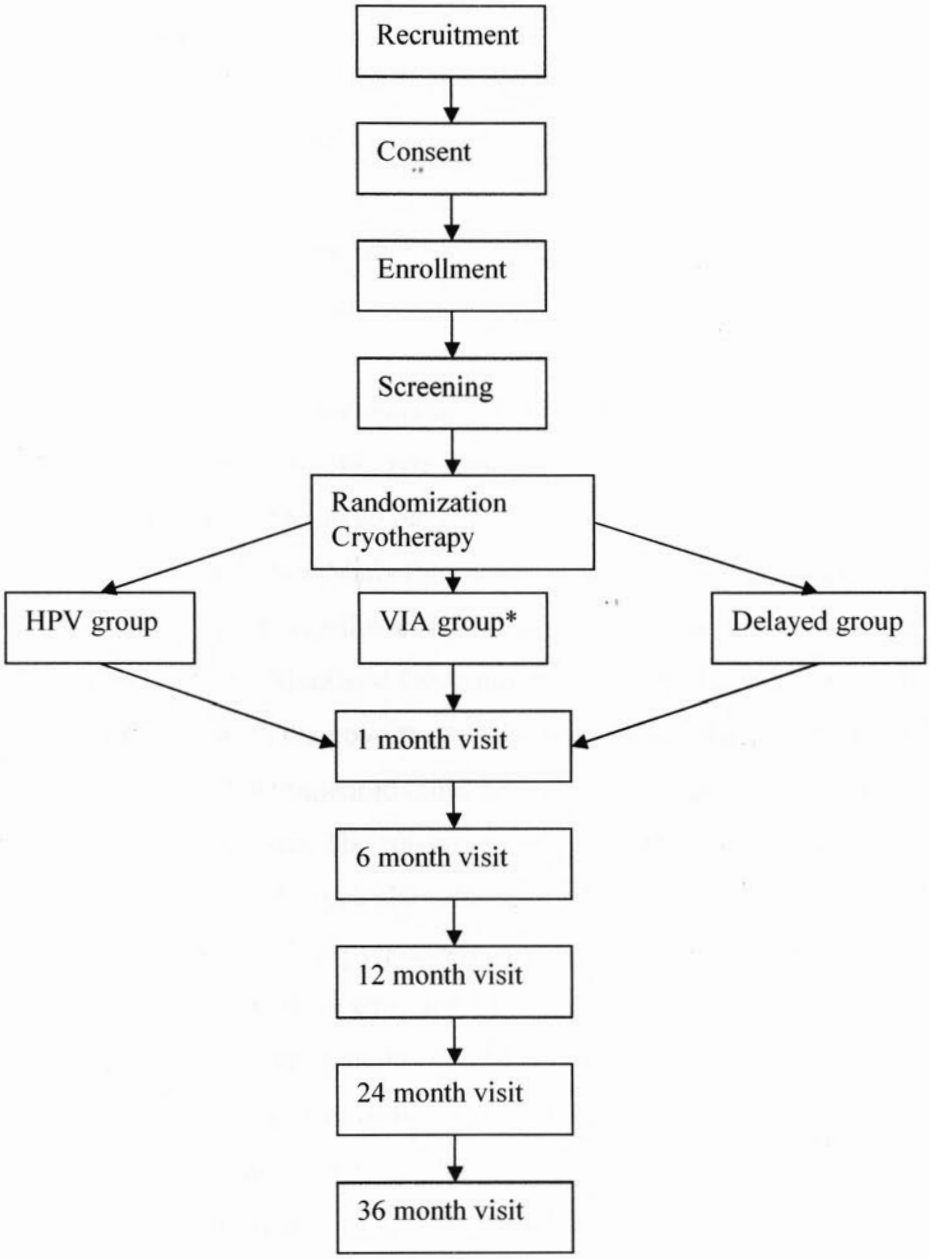
Methods

2.1 Background

2.1.1 Introduction

The data for this study was taken from a study called the "Screen and Treat" (SAT) Study which was a randomized controlled trial that was completed in Khayelitsha in December 2006, and which had been reported on previously (Denny, Kuhn. et al. 2005). The SAT study was a three-arm, randomized clinical trial, comparing two "screen and treat" approaches to a delayed control arm. The objective of the SAT study was to determine the safety, efficacy and acceptability of two "screen and treat" (SAT) approaches for cervical cancer that were designed to be more resource-appropriate for low resource settings. An overview of the study is presented in an algorithm in figure 1. This thesis analysis has used data from the SAT study enrollment visit and only baseline data has been used in this cross-sectional study.

Figure 1: Algorithm of SAT study



* **VIA**- Visual inspection with acetic acid

2.1.2 Setting of the SAT study

2.1.2.1 Khayelitsha

Khayelitsha was chosen for the SAT study site as it was an area where many people had relatively poor access to health care services. It was expected that there would be many women who had previously been unscreened and who may have had pre-invasive cervical disease. The SAT study was able to evaluate different screen and treat approaches in a low-resource setting.

Khayelitsha is a peri-urban settlement situated 25 kilometers outside of Cape Town in the Western Cape Province. This township was established in 1983 by the South African apartheid government who, with forced removals, sent many African people to live outside of the City of Cape Town. Many thousands of people were displaced from their homes and forced to settle in Khayelitsha. The total population of Khayelitsha grew rapidly and in 1986 when the Apartheid Government abolished the "pass laws", there was a further influx of people from the poverty-stricken homelands. Since 1994, there have been many efforts by the Government to build houses and services for the thousands of people now living in Khayelitsha. The census in 2001 estimated that 329 002 people lived in 85 614 households in Khayelitsha (Statistics SA. 2003), although Statistics SA acknowledged that this was probably an underestimated value. There had also been a 5.3% growth in population numbers since the 1996 census. About 52% of the population living in Khayelitsha in 2001 was female. The 2001 census data showed that 23.7% of the population was between the ages of thirty-five and sixty-four. Most of the community was Xhosa-speaking (98%) and there were thousands of people leaving the Eastern Cape on an annual basis to live in Khayelitsha (Statistics SA. 2003).

Khayelitsha has many problems with poverty, high crime levels, unemployment and poor living conditions (Statistics SA. 2003). In 2001, 50.8% of the population was unemployed compared to 1996 when 40.2 % of the population was unemployed. Nearly two-thirds of the population lived in informal dwellings although this had decreased from

80.2% in 1996. About 62% of the community had piped water either in the dwelling or on the site of the dwelling compared to 1996 when 73.1% had access to piped water in the dwelling or on the site (Statistics SA. 2003). Nearly a quarter of the population still had no access to formal sanitation including the bucket latrine service in 2001, although 76% of the community had electricity in the dwelling (Statistics SA. 2003).

The health services in the Khayelitsha area have been overwhelmed by the many health problems which characterized this area. The HIV prevalence in women attending anti-natal clinics had risen from 22% (95% CI; 18; 27) in 2001 to 24.9% (95% CI; 20.7; 29.1) in 2002 (Department of Health. 2002). A report by the Medical Research Council done in 2001, on Mortality in Cape Town, reported that the crude premature mortality rates were 1.5 times higher in Khayelitsha compared to Cape Town (Scott, Sanders. et al. 2003) and this could be attributed to the "quadruple burden of disease" that this community experienced (Scott, Sanders. et al. 2003). The community of Khayelitsha had a higher than average mortality rate from causes such as communicable diseases, maternal and perinatal deaths and nutritional deficiencies. There was also a significant burden of deaths from non-communicable diseases and injuries, but there was also the additional problem of HIV and AIDS (Scott, Sanders. et al. 2003). The report also stated that there was a very high rate of homicidal deaths (120/100 000) and deaths from road traffic accidents which led to Khayelitsha having a disproportionately higher premature mortality rate compared to other areas. (Scott, Sanders. et al. 2003).

2.1.2.2 Study sites

Michael Mapongwana Day Hospital in Harare, Khayelitsha was chosen as the first SAT study site in May 2000. This site had already been developed as a cervical screening site as part of other research studies and reported on previously (Denny, Kuhn. et al. 2000). The provincial government runs this Community health care center that is only open during the day.

A second SAT study site was set up in September 2000 at Site B Day hospital, site B Khayelitsha. Due to overwhelming numbers at this study site, a third study site on the same premises was set up in January 2001 to accommodate the overflow of participants. Site B Day hospital is a 24-hour unit which serves Site B and the surrounding areas and is next to a very busy central shopping district and large informal settlement.

2.1.3 Preparations for the study

2.1.3.1 Community approval

The study team for the SAT study had been working in Khayelitsha on other research studies since 1996. They had previously received community acceptance of the study and once again, the community health forums (CHF) were approached for their approval of the SAT study. Members of the community of Khayelitsha are elected onto these CHF and are mandated to monitor the provision of health services in their areas. The CHF hold many meetings at which health provision in the community is discussed. The principal investigator of the SAT study attended many CHF meetings to discuss the proposed study and written approval was obtained from the head of the CHF in Khayelitsha.

2.1.3.2 Training

All staff employed to work on the SAT study underwent intensive training for four months prior to the initiation of the study. The community health workers were women employed from the Khayelitsha community and all staff except for the medical doctors were fluent in Xhosa, the predominant language of the women living in Khayelitsha.

2.1.3.3 Study site facilities

At each of the three clinics, steel second-hand shipping containers were renovated into clinic rooms. Each site had a patient waiting area where participants could watch television and videos while waiting for their appointments. The participants also had

access to refreshments in the waiting areas. A consent video and educational videos were shown for small groups of women in these waiting rooms. Each clinic room was separate, which allowed for privacy and confidentiality. At both Michael Mapongwana (site MM) and Site B day hospital, a room inside the day hospital was allocated to us for the entire study period. Screening of the participants and cryotherapy was performed in these rooms as these were closer to the emergency facilities if they were ever necessary.

The primary health care facilities of the two day hospitals were used when necessary and in the advent of specialist care being necessary for the participants, GF Jooste Hospital and Groote Schuur Hospital were used for secondary and tertiary care respectively.

2.1.4 Study participants for the SAT study

The target population for the SAT study was women living in Khayelitsha who had never been screened for cervical cancer and who were between thirty-five and sixty-five years old. Women were recruited from clinics, taxi ranks, train stations, sewing clinics, shopping centers, churches, and from the general neighbourhood of Khayelitsha.

Inclusion criteria for the SAT study were:

* Women aged 35-65 years
* Women who had never had a pap smear previously.

Exclusion criteria for the SAT study were:

* Pregnant women
* Previous treatment for cervical disease
* Previous hysterectomy
* Women unable to give informed consent.

Exclusion criteria for cryotherapy were:

* A lesion suspicious for cancer of the cervix
* A lesion greater than 75% of the cervix

* A lesion extending near the vaginal walls
* A severely atrophied cervix.

2.1.5 Recruitment for the SAT Study

Women were recruited into the SAT study between May 2000 and December 2002. Trained community health workers (CHW) would go out into the different areas of the community and gather women together to explain the nature of the study. A CHW would go into the waiting areas of the day hospitals with a loudhailer and tell the people about the prevention of cervical cancer. Fliers were handed out as well as educational materials on cervical cancer. The sister in charge of the sites would attend radio interviews on a weekly basis and many phone-in sessions were held where women could phone in and question the nursing sister. Many topics were covered in these interviews and many participants were recruited through this method. Adverts were also placed in the local newspapers as well as information articles on different aspects of women's health which were written by the principal investigators and published in these newspapers. Health festivals were also held in the site B community hall which attracted both men and women to the hall to hear about cervical cancer.

2.1.6 Informed consent for the SAT study

All potential participants for the SAT study attended a group session where a senior nursing sister would explain the study to a group of about 10 women. They would then watch a consent video which was specially produced by the project team. This 10-minute video, which was in Xhosa, showed the senior sister and principal investigator discussing the study in detail. This video ensured that all participants heard the same detailed explanation of the study and that no aspects of the study were forgotten. Each participant would then attend a one-on-one session with the consent sister who would go though the written informed consent form by means of a check list. Two copies of the consent form were signed and the patient was allowed to keep one copy while the other copy was filed

in a confidential study file. The informed consent form was written in English and Xhosa and participants could choose which language they preferred. (see Appendix 1)

The sister would then conduct HIV pre-test counselling for all participants. Each participant had already consented to having an anonymous linked HIV blood test taken during the consenting process, but could also agree to having a separate blood sample taken in order for her to obtain her HIV result. Once the counselling was complete the sister would take the bloods and complete the blood logs. All HIV blood logs were kept in a locked cupboard and only the screening sister had access to these logs. HIV serostatus was determined using two commercial antibody enzyme-linked immunosorbent assay (ELISA) tests at the virology laboratory at the University of Cape Town, by a pre-determined study protocol (Abbott HIV 1/2 0 Kit on the Abbott AXSYM system, Abbott Laboratories, Chicago, IL; Oregan Teknika, Durham, NC, Vironosticka HIV Uni-form 2 plus 0 kit, Oregan Teknika, Boxtel, The Netherlands). The staff at the Virology laboratory would send the anonymous linked HIV blood test results to an independent third party in the USA. The main database was then sent to the independent party and this was merged with the anonymous linked HIV blood results database. All participant numbers were removed from the combined database before being sent back to the study office for analysis. This ensured that no study personnel could link an HIV result to a particular participant, but the HIV result was linked to all the other study results.

For those participants who wanted to know their HIV test result, their blood tubes were also sent to the virology laboratory at the University of Cape Town and their HIV status was determined using two ELISA antibody tests (Abbott Laboratories, Chicago, IL; Oregan Teknika, Durham, NC). The results were sealed in an envelope and hand-delivered to the consent sister who would give the result to the participant during a pre-organized post-test counselling session. These results were kept in a confidential study file which was kept in a locked cupboard. No other staff members had access to these HIV results.

2.1.7 Enrollment of the SAT study participants

Each participant was interviewed and an enrollment questionnaire was completed by an enrollment community health worker. Three community health workers received intensive training on the enrollment questionnaire and all questionnaires were administered in Xhosa. The questionnaire covered demographic questions such as age, marital status, job status, housing, as well as behavioural information on number of partners and use of contraception. (see Appendix 2).

2.1.8 Screening of the SAT study participants

The screening nursing sister would explain the screening process to each participant individually. The sister would check that all the samples had been labelled correctly with the unique patient number and that the clinical examination form (see Appendix 3) had the same corresponding patient number.

Before the gynaecological examination, the nursing sister explained to the participant how to take a self sample HR-HPV test. This consisted of a cotton-tipped swab which the participant inserted into her vagina and then turned 180 degrees. This swab was then put in special transport medium and was later shipped to Columbia University for HR-HPV testing. This sample was in order to compare self testing for HR-HPV to physician testing for HR-HPV for another sub-study of the main SAT study.

The sister would then examine the patient in lithotomy in a clinic room with a CHW assisting her at all times. The external genitalia were examined prior to the insertion of a pre-warmed bivalve speculum. The presence and amount of discharge and vaginitis were then recorded. The sister would then take the PH of the vaginal area by wetting a PH strip in the surrounding mucous membranes and immediately reading the answer. She would then take a sample of the discharge present and place the discharge on three different slides. On one slide she would add a drop of 10% Potassium Hydroxide (KOH) and

perform a whiff test. On the second slide she would place a drop of normal saline solution and cover with a cover slip. This slide, called a wet preparation, would later be viewed under the clinic microscope. The third slide was allowed to air dry after the discharge was smeared thinly over the slide and then the slide was shipped to Columbia University where it was used for a gram stain in the laboratory.

The sister would then view the cervix and decide if there was any suspicion of any cervical cancer present. She would also look for other exclusion criteria for cryotherapy like a severely atrophied cervix or a malformed cervix. The nursing sister would evaluate for cervical stenosis and cervicitis and then take a pap smear using a plastic Ayer's spatula, first for an ectocervical specimen and then an endocervical brush for the endocervical sample. Both of these were then placed in the Thin prep (Thin prep Pap test, Cytec Corporation) bottle and all material was "shook" off the devices before the devices were removed and the bottle was tightly shut. A specially designed cytobrush from Digene was then inserted into the cervical os, turned 180 degrees and then placed in a Digene HR-HPV testing transport medium. A second sample was then taken in the same way which was then used to test for *Neisseria gonorrhoea/ Chlamydia trachomatis* at Columbia University.

The Digene Hybrid Capture II (HC-II) assay and high-risk probe mixture (Digene Corporation, Gaithersburg, Md) was used at the University of Cape Town for HR-HPV testing. The Hybrid capture II detects the presence of one or more of a group of 13 oncogenic types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Testing for *Neisseria gonorrhoeae and Chlamydia trachomatis* (Hybrid capture for CT/GC, Digene Corporation) was also done using a DNA probe and this was conducted at Columbia University. Both conventional Papanicolaou tests and liquid-based cytology were processed in the same laboratory in the USA (Health Networks Laboratory, Allentown, PA) and it was reported in a blinded manner using the Bethesda Terminology.

Once the woman was off the bed and getting dressed, the sister would view the prepared wet preparation slide under the microscope to exclude the presence of *Trichomonas*

vaginalis and clue cells. Amsel's criteria for Bacterial Vaginitis were used to diagnose and treat for the presence of *Gardnerella vaginalis.* The sister would treat the diagnosed infections according to pre-determined treatment protocols and discuss her findings with the participant. The participant was reminded to return 2-6 days later for her randomization visit.

Study participants with a lesion suspicious for cancer (n=46), or who had a cervix that was not suitable for cryotherapy (n=405) were excluded from the study and were not randomized to receive cryotherapy.

2.2 Ethics and research committee approval for the SAT study

Approval for the study was obtained by the ethics and research committee of the University of Cape Town and the Institutional Review Board of Columbia University, New York, NY. A data safety monitoring board reviewed the project regularly and two study audits were done in 2006.

2.3 Study design for HR-HPV risk factor analysis

For the purposes of this risk factor analysis, information was taken from the SAT study enrollment screening visit before randomization and before cryotherapy was performed. The study design for this analysis is a cross-sectional analytical design.

2.4 Population and sampling of the HR-HPV risk factor analysis

The participants in the study were previously unscreened African women, aged 35 to 65 years, living in Khayelitsha, Cape Town. The sample of participants that was analyzed was all participants who were eligible for enrollment and underwent HR-HPV testing.

2.5 Sample size of the HR-HPV risk factor analysis

6645 participants were eligible for enrollment and underwent HR-HPV testing. All of these participants were included in this analysis.

2.6 Measurements for the HR-HPV risk factor analysis

2.6.1 Instruments

The measurements have been described in the explanation of the SAT study. The SAT study enrollment questionnaire (see Appendix 2) relied on self-reporting from the participants during an interview with a trained enrollment CHW.

2.6.2 List and definition of variables for the HR-HPV risk factor analysis

Tables 2.1a-d, list the variables that were used in the analysis. Only baseline data from the enrollment and screening visit were used for this analysis.

Table 2.1a: List of socio-demographic variables and definitions

Variable	Definition	Comments
Age	Age in years	Continuous and categorical variable 35-39; 40-49; 50-65 years
Married	Marital status at the enrollment visit	Binary variable 1=yes, 0=no
Smoking status	Current smoking status	Binary variable 1=yes, 0=no
Alcohol use in last month	The number of units of alcohol consumed on an occasion in the last month	Categorical variable Moderate/large (4+ units) Small amount(1-3 units) None at all
Education level	Highest educational level obtained	Categorical variable No schooling Some primary school Some high school High school graduate
Employment status	Current employment status	Binary variable 1=yes, 0=no
Type of housing	Type of house lived in by participant.	Categorical variable Other house Brick house

Table 2.1b: List of behavioural characteristic variables and definitions

Variable	Definition	Comments
Age at first intercourse	Age in years at first intercourse	Continuous variable and categorical variable (in years) <15 15-19 20-24 >25
Number of lifetime sexual partners	Number of sexual partners in entire lifetime	Continuous variable and categorical variable 0-1 2-4 5-9 >9
Number of sexual partners in last month	Number of sexual partners in last month	Continuous variable
Sexual activity in last month	The numbers of sexual partners in the previous month	Categorical variable Not sexually active Sexually active with one partner Sexually active with two or more partners
Age at first pregnancy	The age at first pregnancy	Continuous and categorical variable (in years) Never been pregnant 10-14 years 15-19 years 20-24 years >24 years
Number of pregnancies	Number of pregnancies	Continuous variable
Number of live births	Number of babies that were born alive	Continuous and categorical variable 0-1 2-3 4-5 >5

Table 2.1c: List of variables for use of contraception and definitions

Variable	Definition	Comments
Previous contraceptive use	What contraception had ever been used?	Categorical variable COC LAIP Condoms Sterilization Other
Current contraceptive use	What contraception was used in the 3 months before enrollment?	Categorical variable No current use Previously sterilized Condoms COC DMPA Net-EN
Current contraceptive use	Was any contraception used in the 3 months before enrollment?	Binary variable 1=yes, 0=no
Current hormonal contraceptive use	Was any hormonal contraception used in the 3 months before enrollment (including COC and LAIP)	Binary variable 1=yes, 0=no

Table 2.1d: List of biological variables and definitions

Variable	Definition	Comments
HR-HPV infection	Hybrid capture II positive result	Binary variable 1=pos, 0=neg
N.gonorrhoeae and *C.trachomatis* positive test result	Hybrid capture II positive result (second sample)	Binary variable 1=pos, 0=neg
T.*vaginalis* positive test result	Trichomonas *vaginalis* organism seen under microscopy	Binary variable 1=yes, 0=no
Human immunodeficiency virus	ELISA test positive	Binary variable 1=yes, 0=no
Moderate to severe vaginal discharge at screening	Assessed by the nursing sister at screening	Binary variable 1=yes, 0=no
Cytology result	The results of the cytology result at screening as reported by the laboratory	Categorical variable Unsatisfactory Normal ASCUS* LSIL** HSIL***/cancer
Menopausal status	Self-reported at enrollment interview	Categorical variable Pre-menopausal Post-menopausal
Age of menopause	Self-reported at enrollment interview	Continuous variable
Previously treated for STI	Self-reported at enrollment interview	Binary variable 1=yes, 0=no
Previously treated for genital warts	Self-reported at enrollment interview	Binary variable 1=yes, 0=no

* ASCUS- atypical squamous cells of unknown significance

** LSIL – Low grade squamous intra-epithelial lesion

*** HSIL – High grade squamous intra-epithelial lesion

2.6.3 Validity and reliability of instruments

Questionnaires for the SAT study

All three interviewers received standard training on how to administer the enrollment questionnaire according to a set protocol. Random checks were performed during the study whereby the interview was observed to check for protocol adherence. All staff members received training in good clinical practice in 2004 and 2006.
Although it is understood that self-reporting can be problematic, the enrollment questionnaire relied upon self-reporting of answers. The CHWs had been trained to explain to the participants about the confidentiality issues around the questions and the need for honesty in research studies. Self-reporting of menopausal status was asked during the enrollment interview and this was not validated by any other means.

Laboratory testing for the SAT study

The laboratory technologists at the University of Cape Town, who performed the HR-HPV testing, received training from the Digene Corporation before the onset of the study. Throughout the study, random samples were sent to Columbia University for validation of the HPV test results. The wet preparation results for *Trichomonas vaginalis* were randomly checked by sending a sample to the microbiology laboratory at the University of Cape Town for culture of *Trichomonas vaginalis*. All laboratory technicians had to abide to a strict laboratory protocol and observed standard operating procedures.

2.6.4 Pilot study

A pilot study was done before commencement of the SAT study in April 2000. Forty participants were enrolled in the pilot project and no changes were made to the protocol.

2.7 Statistical methods

All data for the SAT study were entered into coded data sheets and then entered into an access database by a trained data capturer. The data was sent to Columbia University for cleaning and transformation into an excel spreadsheet. The anonymous linked HIV data were entered into the database once all identifying patient numbers were deleted from the database. This database was then sent to the University of Cape Town.

Data analysis for the HR-HPV risk factor analysis was performed by using Stata 9 statistical package (Stata Corporation, College Station, Texas, USA). All women that had complete enrollment data and who had a HR-HPV test result were included in the analysis. Three age category variables were programmed from variable for "mean age in years", namely age category 35 to 39 years, age category 40 to 49 years and age category 50 years to 65 years. The educational level variable was categorized from the level of schooling that was captured on the enrollment form. The categories were the following: some primary school was coded as yes if any primary school level was passed; some high school was coded as yes if any level of high school was passed, not including grade 12/matric, and high school graduate was coded as yes if grade 12 or matric was passed. The type of housing was categorized into two levels either brick house or other type of house which included shacks on serviced and un-serviced sites. The following variables were also transformed into category levels as per table 2.1 a-d: age at first intercourse, number of lifetime partners, age at first pregnancy and number of live births. The use of contraception was categorized from data on the enrollment form into previous contraceptive use and current contraceptive use. Current contraceptive use included any method that had been used in the three months prior to enrollment in the SAT study. If there was any hormonal injectable method that was unknown (n=9) then this was categorized as DMPA, as most women in the Western Cape in this age group use DMPA more than Net-EN (Morroni, Myer. et al 2004).

The dependent variable is a binary variable with "1"= a positive HR-HPV test result and "0" = a negative HR-HPV test result. This was used for the univariate and multivariate

logistic regression analysis as the dependent variable. The independent variables and their definitions are listed in table 2.1 a-d.

Summary statistics determining the nature of the distribution of the variables was performed and the appropriate summary measures were calculated (means, standard deviations, confidence intervals for normally distributed variables and medians and inter-quartile ranges for non-parametric variables). The socio-demographic characteristics, behavioural characteristics, use of contraception variables and the biological characteristics were described by calculating the proportions with specific characteristics for categorical variables and means (and/or medians) for continuous variables. All differences between proportions were tested using the Chi-squared tests and Fisher's exact test if there were too few observations. Differences between means were tested using t-tests and Kruskall-wallis tests for categorical variables. Statistical significance was set at a level of 0.05. The summary statistics were presented for the full dataset and for the three age categories: age 35-39, age 40-49 and age 50 to 65 years.

Risk factors for HR-HPV infection were first analyzed in a univariate logistic regression analysis to see if there was an overall association between the dependent variable and the exposure or independent variables. The variables found to be statistically significant on the univariate analysis were then entered into the multivariate logistic regression analysis. Maximum likelihood estimates were used for fitting the logistic model. This analysis compared models by comparing the likelihood ratio tests and the Aikaike's information criterion (AIC) during model building. The multivariate logistic regression modelling process began with the first model with just the dependent variable HPV status. Then the potential confounders were added to the model with only the response variable. Secondly all the risk factors were added individually to check for the effects on the model and the model with the lowest AIC was chosen at each step. Interaction variables were then included to determine the effect on the final model.

Model checking was performed by looking at the linear combination of the "x-variables" and assessing the link function. The outlying and influential observations were assessed

by comparing Pearson and deviance residuals. Pearsons-goodness-of-fit statistics were calculated as well as the measure of the leaverage of the co-variate pattern (h_l).

The multivariate logistic regression analysis was done for the full dataset and for the three age categories previously defined. The variable "sexual activity in the previous month" was included in the multivariate model regardless of the significance level, to adjust for the participant's sexual activity which is a known risk factor for HR-HPV infection. Adjusted odds ratios (OR) (which provide the measure of the strength of the association between the exposure variable and the outcome variable adjusted for all the other risk factors in the model simultaneously) were calculated with 95% confidence intervals (CI). The multivariate regression analysis was then stratified for the three age categories and HIV status to determine the effect of these risk factors on the model. The method for checking heterogeneity of effect between the variables was done using a multiplicative scale and by creating a cross-product variable for each variable and the hypothesized interaction variable and then comparing the final model with a model with the interaction variable included. The likelihood ratio test and AIC values were compared to check for the model that was the most significant. If the cross-product interaction term improved the significance of the model, then this variable was included as an interaction variable.

The design of this study is a cross-sectional analytic study design. In the analysis of data from cross-sectional studies two measures of effect, namely the prevalence odds ratio (POR) and the prevalence ratio (PR), can be calculated. There has been much debate on which is the best measure to use (Pearce, N. 2004, Thompson, Myers. et al. 1998, Zocchetti, Consonni. et al. 1997). The PR is often used as the measure of effect when one is interested in the "public health burden of disease" along with the prevalence difference (Pearce, N. 2004:1050). In our study we are more interested in the disease aetiology and Pearce. N (2004) argues that the POR should be used in this situation because a) fewer assumptions are needed for the POR than the PR when estimating the incidence rate ratio, b) the POR can be estimated using logistic regression and the Mantel-Haenszel method which are used to estimate the odds ratio (OR) in case-control studies and c) the POR

provides a "practical, analytical and theoretical consistency" if a prevalence study and a prevalence case-control study is done on the same study population (Pearce, N. 2004:1050). In this study the POR has been calculated with the multivariate logistical regression analysis using the Stata 9 statistical package (Stata Corporation, College Station, Texas, USA). Throughout the results, the OR which are reported are the POR as this is a cross-sectional analytic study.

Chapter three
Results

3.1 Study group

A total of 7088 participants were evaluated between 14 June 2000 and 3 December 2002 for the SAT study. HR-HPV testing results and enrollment data were available for 6645 participants from the SAT study and all of these were included in this HR-HPV risk factor analysis.

3.2 The prevalence of HR-HPV infection

Of the 6645 participants included in the analysis, 21.3% (95% CI; 20.3; 22.3) tested positive for HR-HPV infection. In the age group 35-39 years, 25.4% (95% CI; 23.8; 27.1) tested positive for HR-HPV infection, in the age group 40-49 years, 18.4% (95% CI; 17.0; 19.9) were HPV positive and in women aged 50 to 65 years, 19.3% (95% CI; 17.1; 21.4) were HPV positive.

3.3 Socio-demographic characteristics

The socio-demographic characteristics of the study population for the full dataset are presented in table 3.1a. The stratification by the three age categories 35-39, 40-49 and 50-65 years is also shown in the table which shows the comparisons in the distribution of the exposure variables by each age category.

The median age for the whole study population was 42 years (inter-quartile range (IQR), 37-48). Most women, (41.7%, n=2768) were in the 40-49 year age category, followed by the 35-39 year age category, (38.9%, n=2575) and the smallest number of women were in the 50-65 year age category (19.6%, n=1302).

Half of all the study participants were married, with the oldest age category having the

least percentage of married women (46.5%, n=606) compared to the age category 40-49, where 52.2% (n=1445) were married (p=0.003).

Current smoking was reported by 7.7% (n=509) of the women in the entire population and 14.1% (n=939) had used alcohol in the previous month. In total, 9.5% (n=629) of all the women reported no schooling. In the age category 50-65 years, 19.4% (n=252) reported no schooling compared to 4.8% (n=123) of the youngest age category (p<0.001). Nearly half (n=3011) of the youngest age category had attended some high school compared to just over a third (n=459) of all women in the oldest age category (p<0.001). In the age category 50 to 60 years, 1.7% (n=22) had completed high school compared to 14% (n=360) of the youngest age category (p<0.001).

A quarter (n=1661) of all the women were formally employed with the middle age category age 40-49, having the highest percentage of employed women (27.2% n= 754, P<0.001). Nearly three-quarters of the youngest age group (n=1887) lived in informal housing areas compared to 65.2% (n=849) of women aged 50-65 years (p<0.001).

Table 3.1a Socio-demographic characteristics of the study population stratified by age category (n=6645)

	Full dataset	Age 35-39	Age 40-49	Age 50-65	p-value
Variable	**6645**	**2575 (38.9)**	**2768 (41.7)**	**1302 (19.6)**	
Age, mean (SD), y	43.3 (7.1)	36.8 (1.3)	43.9 (2.8)	55.0 (4.1)	<0.001
Age, median (IQR), y	42 (37-48)	37 (36-38)	43 (41-46)	54 (51-58)	
Age , [N, (%)], y					
35-39	2575 (38.8)				
40-49	2768 (41.7)				
50-65	1302 (19.6)				
Married, [N, (%)]	3364 (50.6)	1313 (51.0)	1445 (52.2)	606 (46.5)	0.003
Current smoker, [N, (%)],	509 (7.7)	160 (6.2)	240 (8.7)	109 (8.4)	0.002
Alcohol use in last month, [N, (%)],					
4+ drinks on an occasion	236 (3.6)	81 (3.2)	112 (4.1)	43 (3.3)	<0.001
1-3 drinks on an occasion	703 (10.6)	224 (8.7)	314 (11.3)	165 (12.7)	
None at all	5706 (85.9)	2270 (88.2)	2342 (84.6)	1094 (84.0)	
Educational level, [N, (%)],					
No schooling	629 (9.5)	123 (4.8)	254 (9.2)	252 (19.4)	<0.001
Some primary school	2474 (37.2)	749 (29.1)	1156 (41.8)	569 (43.7)	
Some high school	3011 (45.3)	1343 (52.2)	1209 (43.7)	459 (35.3)	
High school graduate	531 (8.0)	360 (14.0)	149 (5.4)	22 (1.7)	
Currently employed, [N, (%)],	1661 (25.0)	650 (25.2)	754 (27.2)	258 (19.8)	<0.001
Type of housing, [N, (%)],					
Other house	4700 (70.7)	1887 (73.3)	1964 (71.0)	849 (65.2)	<0.001
Brick house	1945 (29.3)	688 (26.7)	804 (29.1)	453 (34.8)	

The socio-demographic characteristics stratified by HR-HPV status are shown in Table 3.1b. Mean age, marital status and employment status were significantly different between those women that were HR-HPV-positive and those that were HR-HPV-negative.

The mean age in the HR-HPV-positive group was 42.4 years compared to 43.6 in the HR-HPV-negative group ($p<0.001$). Most of the participants who were HR-HPV-positive, were in the age group 35-39 (46.3%), while the HR-HPV-negative participants

were mostly in the age group 40-49 years (43.2%).

Table 3.1b Socio-demographic characteristics at the enrollment visit for participants aged 35-65 years stratified by HR-HPV status (n=6645)

	HR-HPV positive	HR-HPV negative	p-value
Variable	**1416 (21.3)**	**5229 (78.7)**	
Age, mean (SD), y	42.4 (7.0)	43.6 (7.1)	<0.001
Age, median (IQR), y	40 (37-46)	42 (38-48)	
Age , [N, (%)], y			
35-39	655 (46.3)	1920 (36.7)	<0.001
40-49	510 (36.0)	2258 (43.2)	
50-65	251 (17.7)	1051 (20.1)	
Married, [N, (%)]	609 (43.0)	2755 (52.7)	<0.001
Current smoker, [N, (%)]	123 (8.7)	386 (7.4)	0.102
Alcohol use in last month, [N, (%)]			
4+ drinks on an occasion	55 (3.9)	181 (3.5)	0.389
1-3 drinks on an occasion	161 (11.4)	542 (10.4)	
None at all	1200 (84.7)	4506 (86.2)	
Educational level, [N, (%)]			
No schooling	133 (9.4)	496 (9.5)	0.142
Some primary school	548 (38.7)	1926 (36.8)	
Some high school	608 (42.9)	2403 (46.0)	
High school graduate	127 (9.0)	404 (7.7)	
Currently employed, [N, (%)]	315 (22.2)	1346 (25.7)	0.007
Type of housing, [N, (%)]			
Other House	1030 (72.7)	3670 (70.2)	0.061
Brick house	386 (27.3)	1559 (29.8)	

3.4 Behavioral characteristics

The behavioral characteristics at the enrollment visit for all women stratified by age categories are presented in table 3.2a. The "p-value" in the table refers to a global comparison between the age groups presented in the table and looks at the significant differences between each age group and the other age groups.

The median age at first intercourse was 16 years (IQR 15-18) for all women, 16 (IQR 15-18) in women aged 35-39 years and 17 (IQR 15-18) in women aged 50-65 years ($p<0.001$).

The majority of all women had between 0-4 lifetime partners with 7.4% (n=489) having 10 or more lifetime partners. In the age category 50-65 years, 4.7% (n=61) reported 10 or more lifetime partners compared to 9.4% (n=242) of the youngest age category 35-39 years ($p<0.001$). Nearly three-quarters of all women (71.0%) reported that they were sexually active with one partner in the month prior to being interviewed. Over half (52.1%) of the women aged 50-65 years reported no sexual activity in the month before enrollment compared to 16.5% of the women in age category 35-39 years ($p<0.001$).

Most of the women had been pregnant with a median of 4 pregnancies (IQR 3-5) and similarly, median parity was 4.0 (IQR 2-5) for all women. The median age of first pregnancy was 19.0 years (IQR 17-21) for all women and 56.9% of all the women had their first pregnancy below the age of 20 years. Over a third of the women aged 50 to 65 years had given birth to 5 or more children while 4.3% of the youngest age group of women had 5 or more live births ($p<0.001$).

Table 3.2a Behavioral characteristics at the enrollment visit for all participants stratified by age category (n=6645)

	Full dataset	Age 35-39	Age 40-49	Age 50-65	p-value
Variable	**6645**	**2575(38.9)**	**2768(41.7)**	**1302(19.6)**	
Age at first intercourse*					
Mean, (SD), y	16.6 (2.2)	16.4 (2.2)	16.6 (2.2)	17.0 (2.4)	<0.001
Median, (IQR), y	16 (15-18)	16 (15-18)	16 (15-18)	17 (15-18)	
<15, [N, (%)]	869 (13.1)	384 (14.9)	352 (12.7)	133 (10.2)	<0.001
15-19	5187 (78.1)	1990 (77.3)	2173 (78.5)	1024 (78.6)	
20-24	550 (8.3)	189 (7.3)	230 (8.3)	131 (10.1)	
>25	38 (0.6)	12 (0.5)	13 (0.5)	13 (1.0)	
Number of lifetime partners					
Mean, (SD)	4.4 (3.9)	4.9 (4.1)	4.3 (4.2)	3.7 (2.9)	<0.001
Median, (IQR)	3 (2-5)	4 (3-6)	3 (2-5)	3 (2-5)	
0-1, [N, (%)]	600 (9.0)	165 (6.4)	266 (9.6)	169 (13.0)	<0.001
2-4	3785 (57.0)	1380 (53.6)	1604 (58.0)	801 (61.5)	
5-9	1771 (26.7)	788 (30.6)	712 (25.7)	271 (20.8)	
>9	489 (7.4)	242 (9.4)	186 (6.7)	61 (4.7)	
Number of sexual partners in last month					
Mean, (SD)	0.7 (0.5)	0.9 (0.4)	0.7 (0.5)	0.5 (0.5)	<0.001
Median, (IQR)	1 (0 -1)	1 (1-1)	1 (0-1)	0 (0-1)	
Sexual activity in last month [N, (%)],					
Not sexually active	1835 (27.6)	425 (16.5)	732 (26.5)	678 (52.1)	
Sexually active with one partner	4716 (71.0)	2091 (81.2)	2006 (72.5)	619 (47.5)	<0.001
Sexually active with two or more partners	94 (1.4)	59 (2.3)	30 (1.1)	5 (0.4)	
Age at first pregnancy					
Mean, (SD), y	18.9 (4.4)	18.6 (5.0)	18.9 (4.1)	19.0 (4.0)	<0.001
Median, (IQR), y	19 (17- 21)	19 (17-21)	19 (17-20)	19 (18-21)	
Never been pregnant, [N, (%)]	161 (2.4)	95 (3.7)	50 (1.8)	16 (1.2)	<0.001
10-14	120 (1.8)	60 (2.3)	49 (1.8)	11 (0.8)	
15-19	3659 (55.1)	1437 (55.8)	1551 (56.0)	671 (51.5)	
20-24	2188 (32.9)	761 (29.6)	929 (33.6)	498 (38.3)	
>24	517 (7.8)	222 (8.6)	189 (6.8)	106 (8.1)	
Number of pregnancies					
Mean, (SD)	4.0 (2.0)	3.1 (1.6)	4.2 (1.9)	5.1 (2.4)	<0.001
Median, (IQR)	4 (3– 5)	3 (2-4)	4 (3-5)	5.(4-7)	
Number of live births					
Mean, (SD)	3.7 (2.0)	2.9 (1.5)	4.0 (1.9)	4.8 (2.2)	<0.001
Median, (IQR)	4 (2 -5)	3 (2-4)	4 (3-5)	5 (3-6)	
0-1, [N, (%)]	785 (11.8)	440 (17.1)	255 (9.2)	90 (6.9)	<0.001
2-3	2427 (36.5)	1291 (50.1)	859 (31.0)	277 (21.3)	
4-5	2324 (35.0)	734 (28.5)	1117 (40.4)	473 (36.3)	
>5	1109 (16.7)	110 (4.3)	537 (19.4)	462 (35.5)	

*Missing data for one participant

Behavioral characteristics for all participants aged 35-65 years stratified by HR-HPV status is shown in table 3b.The median age of first sexual intercourse (16) was the same for both groups (p=0.846). However the mean number of lifetime sexual partners was 4.7 in the HR-HPV-positive group compared to 4.2 in the HR-HPV-negative group (p=0.009). Sexual activity in the month prior to being interviewed was similar between the two groups (p=0.503) with 71.4% (n=1011) of the HR-HPV-positive women being sexually active with one partner and 70.9% (n=3705) of the HR-HPV-negative women being sexually active with one partner.

The median age at first pregnancy, the median number of pregnancies and median number of live births was similar for HR-HPV-positive and HR-HPV-negative women (p=0.847, p=0.313, p=0.924 respectively). However there was a significant difference between the two groups when the number of live births is categorized at different levels (p=0.001). In the HR-HPV-positive women, 9.5% (n=134) had between 0 and 1 live birth compared to HR-HPV-negative women, where 12.4% (n=651) had between 0 and 1 live birth.

Table 3.2b Behavioral characteristics at the enrollment visit for participants aged 35-65 years stratified by HR-HPV status (n=6645)

	HR-HPV positive N (%) 1416 (21.3)	HR-HPV negative N (%) 5229 (78.7)	p-value
Age at first intercourse*			
Mean, (SD), y	16.6 (2.2)	16.6 (2.2)	0.846
Median, (IQR), y	16 (15-18)	16 (15-18)	
<15, [N, (%)]	195 (13.8)	674 (12.9)	0.107
15-19	1081 (76.3)	4106 (78.5)	
20-24	132 (9.3)	418 (8.0)	
>25	7 (0.5)	31 (0.6)	
Number of lifetime partners			
Mean, (SD)	4.7 (5.1)	4.2 (3.6)	0.009
Median, (IQR)	4 (3-5)	4 (2-5)	
0-1, [N, (%)]	114 (8.1)	486 (9.3)	0.042
2-4	788 (55.7)	2997 (57.3)	
5-9	389 (27.5)	1382 (26.4)	
>9	489 (7.4)	364 (7.0)	
Number of sexual partners in last month			
Mean, (SD)	0.8 (0.5)	0.8 (0.5)	0.333
Median, (IQR)	1 (0-1)	1 (0-1)	
Sexual activity in last month [N, (%)]			
Not sexually active	381 (26.9)	1454 (27.8)	0.503
Sexually active with one partner	1011 (71.4)	3705 (70.9)	
Sexually active with two or more partners	24 (1.7)	70 (1.3)	
Age at first pregnancy			
Mean, (SD), y	18.9 (4.1)	18.9 (4.5)	0.847
Median, (IQR), y	19 (17-19)	19 (17-21)	
Never been pregnant, [N, (%)],	23 (1.6)	138 (2.6)	0.074
10-14	33 (2.3)	87 (1.7)	
15-19	794 (56.1)	2865 (54.8)	
20-24	452 (31.9)	1736 (33.2)	
>24	114 (8.1)	403 (7.7)	
Number of pregnancies			
Mean, (SD)	3.9 (2.0)	4.0 (2.0)	0.313
Median, (IQR)	4 (3-5)	4 (3-5)	
Number of live births			
Mean, (SD)	3.7 (1.9)	3.7 (2.0)	0.924
Median, (IQR)	4 (2-5)	4 (2-5)	
0-1, [N, (%)]	134 (9.5)	651 (12.4)	<0.001
2-3	562 (39.7)	1865 (35.7)	
4-5	505 (35.7)	1819 (34.8)	
>5	215 (15.2)	894 (17.1)	

* Missing data for one participant

3.5 Use of contraception by the study sample

The use of contraception at the enrollment visit for all participants and stratified by age category is presented in table 3.3a. The "p-value" in the table refers to a global comparison between the age groups presented in the table and looks at the significant differences between each age group and the other age groups.

Some form of contraception had been used previously by 84.9% of all women in the study. Nearly 80% of all women had previously used long-acting intra-muscular progestogens (LAIP). In the older age category 50-65 years, just over half (56.5%) had used LAIP compared to 89.2% in the women in the age category 35-39 years ($p<0.001$). Only 1.0% of the oldest category of women had ever used male condoms previously compared to 12.9% of the youngest age group ($p<0.001$). A quarter of all women (26.1%) had been sterilized previously while in the younger age category 35-39 years, 20.8% had surgical sterilization ($p<0.001$). Other types of contraception included intra-uterine devices and herbs given by traditional healers.

More than half (56.0%) of all women were not currently using any contraception (defined as use of contraception within the three months prior to being interviewed). In the older category of women age 50-65 years, this increased to 75.7% and in the women aged 35-39 years, 46.9% were not currently using any contraception ($p<0.001$). None of the women aged over 50 years were currently using male condoms while only 1.5% of the women aged 35-39 years were currently using condoms ($p<0.001$). Most of the women currently using contraception besides previous sterilization, were using DMPA. Nine woman reported they did not know which LAIP they were using and these women were classified as using DMPA in the analysis. When grouping any current hormonal contraceptive use (defined as use of either a COC or a LAIP within the three months prior to enrollment), 31.6% of women aged 35-39 years, were currently using a hormonal method of contraception compared to 0.8% ($n=10$) in women over the age of 50 years. ($p<0.001$).

There was no association between current condom use and current use of the combined oral contraceptive (p=0.248) but there was an association between the current use of condoms and the use of LAIP (p<0.001) and previous sterilization (p=0.031) (data not shown).

Table 3.3a Use of contraception at enrollment visit for participants stratified by age category (n=6645)

	Full dataset	**Age 35-39**	**Age 40-49**	**Age 50-65**	**p-value**
Variable	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
	6645 (100)	**2575 (38.9)**	**2768 (41.7)**	**1302 (19.6)**	
Previous birth control use					
OC	2497 (35,6)	927 (36.0)	1142 (41.3)	428 (32.9)	<0.001
LAIP	5296 (79.7)	2297 (89.2)	2263 (81.8)	736 (56.5)	<0.001
Condoms	501 (7.5)	331 (12.9)	157 (5.7)	13 (1.0)	<0.001
sterilization	1734 (26.1)	536 (20.8)	892 (32.2)	306 (23.5)	<0.001
other	196 (2.9)	45 (1.7)	96 (3.5)	55 (4.2)	<0.001
Current contraceptive use					
No current use	3722 (56.0)	1208 (46.9)	1528 (55.2)	986 (75.7)	<0.001
Previously sterilized	1707 (25.7)	515 (20.0)	886 (32.0)	306 (23.5)	
Condoms	57 (0.9)	38 (1.5)	19 (0.7)	0	
COC	127 (1.9)	85 (3.3)	40 (1.5)	2 (0.2)	
DMPA**	784 (11.8)	539 (20.9)	238 (8.6)	7 (0.5)	
Net-EN	248 (3.7)	190 (7.4)	57 (2.1)	1 (0.1)	
Current contraceptive use					
Yes	2923 (44.0)	1367 (53.1)	1240 (44.8)	316 (24.3)	<0.001
No	3722 (56.0)	1208 (46.9)	1528 (55.2)	986 (75.7)	
Current hormonal contraceptive use					
Yes	1159 (17.4)	814 (31.6)	335 (12.1)	10 (0.8)	<0.001
No	5486 (82.6)	1761 (68.4)	2433 (87.9)	1292 (99.2)	

** unknowns (n=9) classified as DMPA
Net-EN = norethindrone enanthate; DMPA = depot medroxyprogesterone acetate; COC = combined oral contraceptive.

Use of contraception at the enrollment visit for participants aged 35-65 years stratified by HR-HPV status is presented in table 3.3b.

Similar proportions of HR-HPV-positive and HR-HPV-negative women had previously used combined oral contraceptives (COC) (p<0.062), LAIPs (p=0.194) and condoms (p=0.141) in their lifetimes but significantly more HR-HPV-negative women had been sterilized previously (p<0.001) compared to HR-HPV-positive women.

The current use of any contraception was similar between HR-HPV-positive and HR-HPV-negative women (p=0.667). More HR-HPV-positive women had used a hormonal method of contraception in the 3 months prior to enrollment than HR-HPV-negative women (p<0.001). In HR-HPV-positive women, 16.2 % (n=230) had used DMPA currently compared to 10.6% (n=554) in HPV-negative women (p<0.001). Significantly more HR-HPV-positive women (1.6%) than HR-HPV-negative women (0.7%) were currently using condoms (p=0.001).

Table 3.3b Use of contraception at enrollment visit for participants aged 35-65 years stratified by HR-HPV status (n=6645)

	HR-HPV positive N (%) 1416 (21.3)	**HR-HPV negative N (%) 5229 (78.7)**	**p-value**
Previous birth control use (n=5642)	1217 (85.9)	4425 (84.6)	0.218
OC	510 (41.9)	1987 (44.9)	0.062
LAIP	1152 (94.7)	4144 (93.6)	0.194
Condoms	121 (9.9)	380 (8.6)	0.141
Sterilization	29(2.4)	1437 (32.5)	<0.001
Other	37 (3.0)	159 (3.6)	0.351
Current contraceptive use			
No current use	786 (55.5)	2936 (56.2)	<0.001
Previously sterilized	285 (25.7)	1422 (27.2)	
Condoms	22 (1.6)	35 (0.7)	
COC	25 (1.8)	102 (2.0)	
DMPA	230(16.2)	554 (10.6)	
Net-EN	68 (4.8)	180 (3.4)	
Current contraceptive use			
Yes	630 (44.5)	2293 (43.9)	0.667
No	786 (55.5)	2936 (56.2)	
Current hormonal contraceptive use			
Yes	323 (22.8)	836 (16.0)	<0.001
No	1093 (77.2)	4393 (84.0)	

Use of contraception at the enrollment visit for participants aged 35-39 years stratified by HR-HPV status is presented in table 3.3c. This was done to see the use of contraception in the younger women stratified by HR-HPV status.

Similar proportions of HR-HPV-positive and HR-HPV-negative women had previously used combined oral contraceptives (COC) ($p<0.653$), LAIPs ($p=0.449$) and condoms ($p=0.679$) in their lifetimes but significantly more HR-HPV-negative women had been sterilized previously ($p<0.001$) compared to HR-HPV-positive women.

The current use of any contraception was similar between HR-HPV-positive and HR-HPV-negative women ($p=0.351$). More HR-HPV-positive women had used a hormonal method of contraception in the 3 months prior to enrollment than HR-HPV-negative women ($p<0.001$). In HR-HPV-positive women, 25.8 % ($n=230$) had used DMPA currently compared to 19.3% ($n=370$) in HR-HPV-negative women ($p<0.001$). Significantly more HR-HPV-positive women (8.9%) than HR-HPV-negative women (6.9%) were currently using Net-EN ($p<0.001$).

Table 3.3c Use of contraception at enrollment visit for participants aged 35-39 years stratified by HR-HPV status (n=2575)

	HR-HPV positive N (%) 655 (25.4)	HR-HPV negative N (%) 1920 (74.6)	p-value
Previous birth control use (n=2361)	613 (93.6)	1748 (91.0)	0.042
OC	236 (38.5)	691 (39.5)	0.653
LAIP	599 (97.7)	1698 (97.1)	0.449
Condoms	89 (14.5)	242 (13.8)	0.679
Sterilization	107(17.5)	429 (24.5)	<0.001
Other	14 (2.3)	31 (1.8)	0.426
Current contraceptive use			
No current use	297 (45.3)	911 (47.5)	<0.001
Previously sterilized	98 (15.0)	417 (21.7)	
Condoms	16 (2.4)	22 (1.1)	
COC	17 (2.6)	68 (3.5)	
DMPA	169(25.8)	370 (19.3)	
Net-EN	58 (8.9)	132 (6.9)	
Current contraceptive use			
Yes	358 (54.7)	1009 (52.6)	0.351
No	297 (45.3)	911 (47.5)	
Current hormonal contraceptive use			
Yes	244 (37.3)	570(29.7)	
No	411 (62.8)	1350 (70.3)	<0.001

3.6 Biological risk factors for HR-HPV infection

Biological risk factors for HR-HPV infection are presented in table 3.4a and these are stratified by age category. The "p-value" in the table refers to a global comparison between the age groups presented in the table and looks at the significant differences between each age group and the other age groups.

In all women, 5.2% (n=345) were positive on testing for *Neisseria gonnorrhoeae / Chlamydia trachomatis* and 10.7% (n=711) had *Trichomonas Vaginalis*. In total, 12.0% (95% CI; 11.2; 12.7) of the entire population in our study was HIV-positive. In women aged 35-39 years, 18.2% (95% CI; 16.7; 19.7) tested HIV-positive compared to 9.5% (95% CI; 8.4; 10.6) in women aged 40 to 49 years and 5.0% (95% CI; 3.8; 6.2) in women older than 50 years (p<0.001).

The results of cytological testing on all women are shown in table 3.4a. In total 3.2% (n=209) had high grade squamous intraepithelial lesion (HSIL) or cancer reported on their Pap smear result. Of the 209 women with HSIL or cancer, 42.6% (n=89) were aged 40-49 years, 42.1% (n=88) were aged 35-39 years and 15.3% (n=32) were 50 years and older (p=0.002). There were 3 cytology results missing, two from the age category 40-49 years and one from the age category 50-65 years.

In total, 24.9% (n=1654) of all the women self-reported that they were post-menopausal. The median age of menopause was 47 years (IQR 43-49) for the entire population. In the women aged 50 and older, 84.4% (n=1099) were post-menopausal compared to women aged 40 to 49 years where 18.6% (n=514) were post-menopausal (p<0.001).

Table 3.4a Biological risk factors for HR-HPV infection at enrollment visit for participants stratified by age category (n=6645)

	Full dataset	Age 35-39	Age 40-49	Age 50-65	p-value
variable	**6645**	**2575**	**2768**	**1302**	
HR-HPV positive, [N, (%)]	1416 (21.3)	655 (25.4)	510 (18.4)	251 (19.3)	<0.001
Neisseria gonorrhoeae /Chlamydia trachomatis positive, [N, (%)]	345 (5.2)	193 (7.5)	118 (4.3)	34 (2.6)	<0.001
Trichomonas vaginalis positive [N, (%)]	711 (10.7)	305 (11.8)	334 (12.1)	72 (5.5)	<0.001
Human Immunodeficiency virus positive [N, (%)]	795 (12.0)	468 (18.2)	262 (9.5)	65 (5.0)	<0.001
Moderate/severe vaginal discharge [N, (%)]	1531 (23.0)	710 (27.6)	663 (24.0)	158 (12.1)	<0.001
Cytology result*, [N, (%)],					
Unsatisfactory	221 (3.3)	84 (3.3)	99 (3.6)	38 (2.9)	0.002
Normal	5580 (84.0)	2124 (82.5)	2321 (83.9)	1135 (87.2)	
ASCUS	423 (6.4)	177 (6.9)	168 (6.1)	78 (6.0)	
LSIL	209 (3.2)	102 (4.0)	89 (3.2)	18 (1.4)	
HSIL/Cancer**	209 (3.2)	88 (3.4)	89 (3.2)	32 (2.5)	
Menopausal status, [N, (%)]					
Pre-menopausal	4991 (75.1)	2534 (98.4)	2254 (81.4)	203 (15.6)	<0.001
Post-menopausal	1654 (24.9)	41 (1.6)	514 (18.6)	1099 (84.4)	
Age of menopause, mean (SD), y	46.1 (4.8)	35.3 (2.8)	43.1 (3.4)	47.9 (4.2)	<0.001
Age of menopause, median (IQR), y	47 (43-49)	36 (34-37)	43 (41-46)	48 (46-50)	
Previously treated for STI, [N, (%)]	204 (3.1)	112 (4.4)	78 (2.8)	14 (1.1)	<0.001
Previously treated for warts, [N, (%)]	85 (1.3)	26 (1.0)	42 (1.5)	17 (1.3)	0.255

* 3 cytology results missing (2 in age 40-49, 1 in age 50-65).

** 14 Cancers included in HSIL category (4 in age35-39, 5 in age 40-49, 5 in age 50-65)

Biological characteristics for all women stratified by HR-HPV status is presented in table 3.4b. In the HR-HPV-positive participants, 5.9% were diagnosed with *Neisseria gonorrhoeae / Chlamydia trachomatis* and 10.9% with *Trichomonas vaginalis* compared to 5.0% and 10.7% respectively in the HR-HPV-negative participants (p=0.157, p=0.809 respectively).

There was a significant difference in HIV status between HR-HPV-positive and HR-HPV-negative participants, namely 27.1% (n=384) of HR-HPV-positive participants were co-infected with HIV while 7.9% (n=411) of the HR-HPV-negative participants were HIV-positive (p<0.001). The HR-HPV-positive participants had significantly more

abnormal cytology results on testing than the HR-HPV-negative participants (p<0.001). In HR-HPV-positive women, 13.2% (n=187) had either HSIL or cancer on their Pap smear compared to 0.4% (n=22) in the HR-HPV-negative women (p<0.001).

In HR-HPV-positive participants, 24.3% (n=344) were post-menopausal compared to 25.1% (n=1310) of HR-HPV-negative participants (p=0.558). The median age of menopause was 46 years in HR-HPV-positive women and 47 years in HR-HPV-negative women (p=0.055).

Table 3.4b Biological risk factors for HR-HPV infection at enrollment visit for participants aged 35-65 years stratified by HR-HPV status (n=6645)

Variable, [N, (%)]	**HR-HPV positive 1416 (21.3)**	**HR-HPV negative 5229 (78.7)**	**P-value**
Neisseria gonorrhoeae /Chlamydia trachomatis positive, [N, (%)]	84 (5.9)	261 (5.0)	0.157
Trichomonas vaginalis positive, [N, (%)]	154 (10.9)	557 (10.7)	0.809
Human Immunodeficiency virus positive [N, (%)]	384 (27.1)	411 (7.9)	<0.001
Moderate/severe vaginal discharge [N, (%)]	341 (24.1)	1190 (22.8)	0.294
Cytology result*, [N, (%)]			
Unsatisfactory	42 (3.0)	179 (3.4)	<0.001
Normal	815 (57.6)	4765 (91.1)	
ASCUS	203 (14.3)	220 (4.2)	
LSIL	169 (11.9)	40 (0.8)	
HSIL/cancer	187 (13.2)	22 (0.4)	
Menopausal status, [N, (%)]			
Pre-menopausal	1072 (75.7)	3919 (74.9)	0.558
Post-menopausal	344 (24.3)	1310 (25.1)	
Age of menopause, mean (SD), y	45.7 (5.0)	46.2 (4.8)	0.055
Age of menopause, median (IQR), y	46 (42-49)	47 (43-49)	
Previously treated for STI, [N, (%)]	51 (3.6)	153 (2.9)	0.191
Previously treated for warts, [N, (%)]	18 (1.3)	67 (1.3)	0.976

* 3 missing cytology results (all HR-HPV negative)

3.7 Association of socio-demographic factors with a positive HR-HPV test

The crude OR and 95% CI for associations between socio-demographic characteristics and a positive HR-HPV test are shown in table 3.5. There was a significant association with the mean age of the women, marital status, employment status and the risk of HR-HPV infection. For every one year increase in age, the odds of HR-HPV infection decreased by 2% (adjusted OR; 0.98, 95% CI; 0.97; 0.99). Women who were married had 32% decreased odds of HR-HPV infection compared to women who were not married (95% CI; 0.60; 0.76). Women who were employed had a 17% decreased odds of HR-HPV infection compared to women who were not employed (95% CI; 0.72; 0.95).

Table 3.5 Univariate logistic regression analysis for associations between the socio-demographic characteristics and a positive HR-HPV test.

	Odds ratio	**95% Confidence Interval**
Age, years *continuous*	0.98	0.97 ; 0.99
Age, y		
35-39	1.00	
40-49	0.66	0.58 ; 0.75
50-65	0.70	0.59 ; 0.82
Marital status		
Not currently married	1.00	
Currently married	0.68	0.60 ; 0.76
Currently smoking		
No	1.00	
Yes	1.19	0.97 ; 1.48
Alcohol use in last month		
4+ drinks on an occasion	1.00	
1-3 drinks on an occasion	0.98	0.69 ; 1.39
None at all	0.88	0.64 ; 1.19
Educational level		
No schooling	1.00	
Some primary school	1.06	0.86 ; 1.31
Some high school	0.94	0.76 ; 1.16
High school graduate	1.17	0.89 ; 1.55
Employment status		
Not currently employed	1.00	
Currently employed	0.83	0.72 ; 0.95
Type of housing		
Other house	1.00	
Brick house	0.88	0.77 ; 1.01

3.8 Association of behavioural factors with a positive HR-HPV test

The crude odds ratios and 95% CI for associations between behavioural characteristics and a positive HR-HPV test are shown in table 3.6.

Women who had more than nine lifetime partners, had a 50.0% (OR; 1.50, 95% CI; 1.10; 1.95) increased risk of HR-HPV infection compared to those women who had 0-1 lifetime partners. There was no significant association between the risk of HR-HPV infection and sexual activity in the previous month even when sexual activity was with two or more partners (OR; 1.31, 95% CI; 0.81; 2.11).

There was a significant association between the age at first pregnancy and the odds of HR-HPV infection in the univariate logistic regression analysis. Women who fell pregnant between the ages of 10-14, had more than two times the odds of HR-HPV infection compared to those women who had never been pregnant. (OR; 2.28, 95% CI; 1.25; 4.13) Women who had their first pregnancy after the age of 24 had a 70.0% increased odds of HR-HPV infection compared to women who had never been pregnant (OR; 1.70, 95% CI; 1.04; 2.76).

For women who had 2-3 live births, there was a 46.0% increased odds of HR-HPV infection compared to women who had 0-1 live births (OR; 1.46, 95% CI; 1.19; 1.80).

Table 3.6 Univariate logistic regression analysis for the associations between behavioural characteristics and an HR-HPV positive test.

	Odds ratio	95% Confidence Interval
Age at first intercourse*		
Mean, years *(Continuous)*	1.00	0.98 ; 1.03
<15	1.00	
15-19	0.91	0.77 ; 1.08
20-24	1.09	0.85 ; 1.40
>25	0.78	0.34 ; 1.80
Number of lifetime partners		
Mean *(continuous)*	1.02	1.00 ; 1.04
0-1	1.00	
2-4	1.12	0.90 ; 1.40
5-9	1.20	0.95 ; 1.51
>9	1.50	1.10 ; 1.95
Number of sexual partners in last month		
Mean *(Continuous)*	1.06	0.94 ; 1.12
Sexual activity in last month		
Not sexually active	1.00	
Sexually active with one partner	1.04	0.91 ; 1.19
Sexually active with two or more partners	1.31	0.81 ; 2.11
Age at first pregnancy		
Mean, years *(continuous)*	1.00	1.00 ; 1.02
Never been pregnant	1.00	
10-14	2.28	1.25 ; 4.13
15-19	1.66	1.06 ; 2.60
20-24	1.56	0.99 ; 2.46
>24	1.70	1.04 ; 2.76
Number of pregnancies		
Mean *(continuous)*	0.99	0.96 ; 1.01
Number of live births		
Mean (continuous)	1.00	0.97 ; 1.03
0-1	1.00	
2-3	1.46	1.19 ; 1.80
4-5	1.35	1.09 ; 1.66
>5	1.17	0.89 ; 1.55

3.9 Association of the use of contraception with a positive HR-HPV test

The crude OR and 95% CI for associations between the use of contraception and a positive HR-HPV test are shown in table 3.7. Women who had been sterilized previously had a 33.0% decreased odds of HR-HPV infection compared to women who had never been sterilized (OR; 0.67, 95% CI; 0.58; 0.78). Women who had used LAIP previously had no increased odds of HR-HPV infection compared to women who had not used LAIP previously (OR; 1.14, 95%CI; 0.98; 1.33).

Women who had been using condoms in the three months prior to enrollment into the study had more than two times the odds of HR-HPV infection compared to women who had not used any method (OR; 2.35, 95% CI; 1.37; 4.03). Women who had been using DMPA in the three months prior to enrollment had a 55.0% increased odds of HR-HPV infection compared to women who had not used any method (OR; 1.55, 95% CI; 1.30; 1.84).

Table 3.7 Univariate logistic regression analysis for associations between the use of contraception and a positive HR-HPV test.

	Odds ratio	95% Confidence interval
Previous birth control use	1.11	0.94 ; 1.31
COC	0.91	0.81 ; 1.04
LAIP	1.14	0.98 ; 1.33
Condoms	1.19	0.96 ; 1.48
Sterilization	0.67	0.58 ; 0.78
Current contraceptive use		
No current use	1.00	
Previously sterilized	0.75	0.64 ; 0.87
Condoms	2.35	1.37 ; 4.03
COC	0.92	0.59 ; 1.43
DMPA	1.55	1.30 ; 1.84
Net-EN	1.41	1.06 ; 1.89
Any current contraceptive use		
No	1.00	
Yes	1.03	0.91 ; 1.16
Current hormonal contraceptive use		
No	1.00	
Yes	1.55	1.34 ; 1.80

3.10 Association of biological factors with a positive HR-HPV test

The crude OR and 95% CI for associations between the biological characteristics and a positive HR-HPV test are shown in table 3.8. Women who were HIV-infected had more than four times the odds of HR-HPV infection compared to women who were HIV-negative (OR; 4.36; 95% CI; 3.74; 5.09). There was no significant association in the univariate logistic regression analysis between women who were menopausal and odds of HR-HPV infection compared to women who were not menopausal (OR; 0.96, 95% CI; 0.84; 1.10). Women who had *Neisseria gonnorrhoeae/Chlamydia Trachomatis* genital infections did not have significant increased odds of HR-HPV infection compared to women who did not have these sexually transmitted infections (OR; 0.99; 95% CI; 0.78;1.25).

Table 3.8 Univariate logistic regression analysis for associations between biological characteristics and a positive HR-HPV test

	Odds ratio	95% Confidence interval
Neisseria gonorrhoeae /Chlamydia trachomatis		
Negative	1.00	
Positive	0.99	0.78 ; 1.25
Trichomonas vaginalis		
Negative	1.00	
Positive	1.02	0.85 ; 1.24
Human Immunodeficiency virus		
Negative	1.00	
Positive	4.36	3.74 ; 5.09
Moderate/severe vaginal discharge	1.08	0.94 ; 1.24
Menopausal status		
Pre-menopausal	1.00	
Post-menopausal	0.96	0.84 ; 1.10
Previously treated for STI	1.24	0.90 ; 1.71
Previously treated for warts	0.99	0.59 ; 1.67

3.11 Multivariate logistic regression analysis

A multivariate logistic regression analysis was performed and the results are presented in table 3.9. See Appendix 5 for the results of the model checking.

HIV-positive women were four times more likely in unadjusted analysis to be HR-HPV positive compared with HIV-negative women (unadjusted OR; 4.36, 95% CI; 3.74; 5.09) This association persisted in a multivariate model to predict HR-HPV infection after adjusting for demographic, behavioural variables, use of contraception and other biological variables (adjusted OR; 4.08; 95% CI; 3.47; 4.80).

The association between the current use of DMPA and the odds of HR-HPV infection remained significant in a multivariate model when adjusted for variables (adjusted OR; 1.37, 95% CI; 1.13; 1.65). For the full dataset, women who used Net-EN in the previous three months had a 39.0% increased odds of HR-HPV infection compared to women who

had not used any current method of contraception (adjusted OR;1.39, 95% CI; 1.02; 1.88). There was no significant association between the use of the COC and the odds of HR-HPV infection when the variables in the model were adjusted for other demographic, behavioural and biological characteristics (adjusted OR; 0.84, 95% CI; 0.53; 1.33). In an unadjusted analysis, the current use of condoms was significantly associated with an increased odds of HR-HPV infection (OR; 2.35, 95% CI; 1.37; 4.03). This association remained significant in the model adjusted for by socio-demographic, behavioural, use of contraception and biological characteristics for the full dataset (OR; 2.15, 95% CI; 1.22; 3.80). Women who had been sterilized had a decreased odds of HR-HPV infection compared to women who had not used any current method of contraception (adjusted OR; 0.72, 95% CI; 0.61; 0.85).

Married women had significant decreased odds of HR-HPV infection in an unadjusted analysis compared with unmarried women (unadjusted OR; 0.68, 95% CI; 0.60; 0.76). The association persisted in a multivariate model to predict HR-HPV infection after adjusting for other variables (adjusted OR; 0.71; 95% CI; 0.62; 0.81).

In a crude analysis, between 2 and 5 live births was significantly associated with an increased odds of HR-HPV infection (OR; 1.46, 95% CI; 1.19; 1.80) (OR; 1.35, 95% CI; 1.09; 1.66). The association between mean number of live births and the odds of HR-HPV infection remained significant in the model adjusted for by socio-demographic, behavioural and biological characteristics for the full dataset (adjusted OR; 1.10, 95% CI; 1.06; 1.14).

For every one year increase in age, the odds of HR-HPV infection decreased by 1% in all women in the study (adjusted OR; 0.99, 95% CI; 0.98; 0.997). For this sample, women who were employed had a 14% decreased odds of HR-HPV infection compared to women who were not employed (adjusted OR; 0.86, 95% CI; 0.74; 0.99).

In the regression analysis, sexual activity in the month before enrollment was not significant, but was kept in the model so that other variables were adjusted for by sexual activity.

Table 3.9 Adjusted OR and 95% CI from a logistic regression analysis modelling the relative odds of HR-HPV infection according to participant socio-demographic characteristics, behavioural variables, use of contraception and biological characteristics.

Variable	Full dataset (n=6645)
	OR (95% CI)
HIV-positive	4.08 (3.47; 4.80)
Current contraception	
No current use	1.00
Previously sterilized	0.72 (0.61; 0.85)
Condoms	2.15 (1.22; 3.80)
COC	0.84 (0.53; 1.33)
DMPA	1.37 (1.13; 1.65)
Net-EN	1.39 (1.02; 1.88)
Married	0.71 (0.62; 0.81)
Number of live births	1.10 (1.06; 1.14)
Age in years	0.99 (0.98; 0.997)
Currently employed	0.86 (0.74; 0.99)
Sexual activity in last month	
Not sexually active	1.00
Sexually active with one partner	1.13 (0.97; 1.31)
Sexually active with two or more partners	1.06 (0.63; 1.76)

3.12.1 Effect of age on the risk factors for HR-HPV infection

Adjusted OR and 95% CI from a multiple logistic regression analysis modelling the relative odds of HR-HPV infection according to participant socio-demographic characteristics, use of contraception and biological characteristics, stratified by age category is presented in table 3.10.

HIV-positive women were four times more likely in an adjusted analysis to be HR-HPV positive compared with HIV-negative women (adjusted OR; 4.08, 95% CI; 3.47; 4.80). This association remained significant after stratification for each age category.

The association between the current use of DMPA and the odds of HR-HPV infection remained significant in a multivariate model when adjusted for other variables (adjusted OR; 1.37, 95% CI; 1.13; 1.65). The association remained significant for all categories except for women aged 50-65 years and this could be due to sparse numbers of older women using DMPA. For the age category 35-39 years, women who used Net-EN in the previous three months had a 49.0% increased odds of HR-HPV infection compared to women who had not used any current method of contraception (OR; 1.49, 95% CI; 1.05; 2.15). There was no significant association in the full dataset and the different age categories, between the use of the COC and the odds of HR-HPV infection when the model was stratified by the three age categories. The association between the current use of condoms and the increased odds of HR-HPV infection remained significant in the model stratified by age category only for the women aged 35 to 39 years (OR; 2.19, 95% CI; 1.09; 4.38). This association was not significant in women older than 39 years, possibly due to a limited number of women using condoms in the older age groups.

Women who had been sterilized had a decreased odds of HR-HPV infection compared to women who had not used any current method of contraception (adjusted OR; 0.72, 95% CI; 0.61; 0.85). This significant association remained in women aged 35 to 39 years (OR; 0.72, 95% CI; 0.54; 0.96) and women aged 50 to 65 years (OR; 0.56, 95% CI; 0.39; 0.82) when the model was stratified by age category.

Married women in all age categories had decreased odds of HR-HPV infection in the stratified analysis compared with unmarried women. The number of live births of between 2 and 5 was significantly associated with increased odds of HR-HPV infection in all age categories, after the final model was stratified by all three age categories. For the full dataset, women who were employed had a 14% decreased odds of HR-HPV infection compared to women who were not employed (OR; 0.86, 95% CI; 0.74; 0.99).

When stratified by age category, the association remained for women in age category 40-49 years and age category 50-65 years.

Table 3.10 Adjusted OR and 95% CI from a logistic regression analysis modelling the relative odds of HR-HPV infection according to participant socio-demographic characteristics, behavioural variables, use of contraception and biological characteristics, stratified by age category.

Variable	Age 35-39 (n=2575)	Age 40-49 (n=2768)	Age 50-65 (n=1299)
HIV-positive	4.01 (3.22; 4.98)	4.45 (3.38; 5.86)	3.16 (1.87; 5.34)
Current contraception			
No current use	1.00	1.00	1.00
Previously sterilized	0.72 (0.54; 0.96)	0.84 (0.66; 1.06)	0.56 (0.39; 0.82)
Condoms	2.19 (1.09; 4.38)	1.93 (0.69; 5.39)	*
COC	0.76 (0.43; 1.35)	1.03 (0.46; 2.34)	*
DMPA	1.33 (1.04; 1.69)	1.42 (1.01; 1.99)	1.50 (0.28; 7.97)
Net-EN	1.49 (1.05; 2.12)	0.92 (0.45; 1.89)	*
Married	0.66 (0.54 ; 0.81)	0.75 (0.61; 0.93)	0.75 (0.55; 1.02)
Number of live births	1.13 (1.05; 1.21)	1.08 (1.02; 1.15)	1.10 (1.04; 1.18)
Currently employed	1.04 (0.84; 1.30)	0.78 (0.62; 0.99)	0.66 (0.45; 0.99)
Sexual activity in last month			
Not sexually active	1.00	1.00	1.00
Sexually active with one partner	0.97 (0.76; 1.25)	1.26 (0.99; 1.60)	1.16 (0.86; 1.57)
Sexually active with two or more partners	0.82 (0.43; 1.59)	1.25 (0.48; 3.25)	2.25 (0.35; 14.24)

* dropped due to sparse numbers

3.12.2 Interaction of age and other variables in the final model

Heterogeneity of effect with age as a continuous variable and all the other variables in the final model for the full dataset was analyzed. A model which included the cross-product variable of mean age in years and current contraception significantly improved the model. The categorical variable current contraception was then programmed as a binary variable with No current contraception = "0" and current contraception = "1" for ease of interpretation. (See Appendix 6 for table of interaction variables.) There is a decrease in

the effect of age on HR-HPV status when women were currently using a form of contraception, compared to not using a current form of contraception. For every one year increase in age, the odds for HR-HPV infection decreased for those women that were currently on contraception ($p<0.001$).

3.13.1 Effect of HIV status on risk factors for HR-HPV infection

A multivariate logistic regression analysis stratified by HIV status is summarized in table 3.11. When the final model from the multivariate logistic regression analysis was stratified by HIV status, the mean lifetime number of live births remained a significant risk factor for HR-HPV infection in both HIV-positive (OR; 1.13, 95% CI; 1.03; 1.25) and HIV-negative women (OR; 1.09, 95% CI; 1.05; 1.14). This is the only variable which remained a risk factor in both groups of women.

In HIV-positive women, the only other significant risk factor was the current use of Net-EN compared to no current method (OR; 2.71, 95% CI; 1.22; 6.04). This association was not evident in HIV-negative women (OR; 1.23, 95%CI; 0.87; 1.74). In HIV-negative women, the significant risk factors for an increased odds of HR-HPV infection were current use of condoms (OR; 2.29, 95% CI; 1.22; 4.39) and current use of DMPA (OR; 1.49, 95% CI; 1.21; 1.84). In HIV-negative women there was a protective effect of previous sterilization (OR; 0.72, 95% CI; 0.60; 0.86) on the odds of HR-HPV infection, and married HIV-negative women were at a decreased odds of HR-HPV infection (OR; 0.68, 95% CI; 0.59; 0.78) compared to unmarried HIV-negative women. These protective associations were not seen in HIV-positive women. The increasing mean age in years was not a significant protective factor for HR-HPV infection for HIV-positive (OR; 0.97, 95% CI; 0.94; 1.00) or HIV-negative women (OR; 0.99, 95% CI; 0.98; 1.00). When the final model was stratified by HIV status, being currently employed was not a protective factor for HR-HPV infection in either HIV-positive (OR; 0.87, 95% CI: 0.62; 1.22) or HIV-negative women (OR; 0.86, 95% CI; 0.73; 1.01).

Table 3.11 Adjusted OR and 95% CI from a logistic regression analysis modelling the relative odds of HR-HPV infection according to participant socio-demographic characteristics, behavioural variables, use of contraception and biological characteristics, stratified by HIV status.

Variable	HIV-positive (n=795)	HIV-negative (n=5850)
	OR (95% CI)	
Current contraception		
No current use	1.00	1.00
Previously sterilized	0.74 (0.50; 1.11)	0.72 (0.60; 0.86)
Condoms	1.81 (0.51; 6.40)	2.29 (1.22; 4.30)
COC	1.00 (0.38; 2.58)	0.78 (0.45; 1.34)
DMPA	1.00 (0.66; 1.51)	1.49 (1.21; 1.84)
Net-EN	2.71 (1.22; 6.04)	1.23 (0.87; 1.74)
Married	0.88 (0.64; 1.22)	0.68 (0.59; 0.78)
Number of live births	1.13 (1.03; 1.25)	1.09 (1.05; 1.14)
Age in years	0.97 (0.94; 1.00)	0.99 (0.98; 1.00)
Currently employed	0.87 (0.62; 1.22)	0.86 (0.73; 1.01)
Sexual activity in last month		
Not sexually active	1.00	1.00
Sexually active with one partner	1.03 (0.75; 1.44)	1.16 (0.98; 1.37)
Sexually active with two or more partners	0.85 (0.37; 1.99)	1.24 (0.66; 2.32)

3.13.2 Interaction of HIV status and other variables in the final model

Heterogeneity of effect with HIV status and all the other variables in the final model for the full dataset was analyzed. No cross-product variables with HIV status significantly improved the final model.

Chapter four
Discussion

4.1 Summary of principal findings

In total, 6645 participants were included in the analysis of risk factors for HR-HPV infection in unscreened women aged 35 to 65 years in Khayelitsha, Cape Town. Of these women, 1416 (21.3%) (95% CI; 20.3; 22.3) tested positive for HR-HPV infection.

The multivariate logistic regression analysis showed that a positive HIV status (OR; 4.08, 95% CI; 3.47; 4.80), previous sterilization (OR; 0.72, 95% CI; 0.61; 0.85), current use of condoms (OR; 2.15, 95% CI; 1.22; 3.80), current use of DMPA (OR; 1.37, 95% CI; 1.13; 1.65), current use of Net-EN (OR; 1.39, 95% CI; 1.02; 1.88), currently married (OR; 0.71, 95% CI; 0.62; 0.81), mean number of live births (OR; 1.10, 95% CI; 1.06; 1.14), mean age in years (OR; 0.99, 95% CI; 0.98; 0.997) and currently employed (OR; 0.86, 95% CI; 0.74; 0.99) were significant in a model predicting the odds of infection with HR-HPV when adjusted for socio-demographic, behavioural variables, use of contraception and biological variables.

When the model was stratified by age category, similar results were obtained. In the age category 35 to 39 years current employment (OR; 1.04, 95% CI; 0.84; 1.30) was the only variable which did not remain significant when compared to the full dataset of all participants. In the age category 40- 49 years, previous sterilization (OR; 0.84, 95% CI; 0.66; 1.06), current use of condoms (OR; 1.93, 95% CI; 0.69; 5.39) and current use of Net-EN (OR; 0.92, 95% CI; 0.45; 1.89) were the variables which did not remain significant when compared to the full dataset of all participants. In the older women aged 50 and older, current use of DMPA (OR; 1.50, 95% CI; 0.28; 7.97) and currently married (OR; 0.75, 95% CI; 0.55; 1.02) did not remain significant. Current use of condoms and current use of Net-EN were variables with sparse numbers and were dropped in a multivariate model.

When the final model was stratified by a positive HIV status, the mean number of live births was the only variable which was significant in both HIV-positive (OR; 1,13, 95% CI; 1.03; 1.25) and HIV-negative participants (OR; 1.09, 95% CI; 1.05; 1.14). In HIV-positive participants, the current use of Net-EN (OR; 2.71, 95% CI; 1.22; 6.04) was the only other significant variable in the model. In HIV-negative participants, current use of Net-EN (OR; 1.23, 95% CI; 0.87; 1.74) and mean age in years were the only variables that were not significant compared to the model with all participants.

4.2 Prevalence of HR-HPV infection and the effect of age on HR-HPV infection

In this population of unscreened African women aged 35 to 65 years, the prevalence of HR-HPV infection was 21.3% (95% CI; 20.3; 22.3). In a review done by Bosch and de Sanjose in 2003, the prevalence of HR-HPV infection in women older than 30 years, from different areas around the world, ranged from 2% to 43.7% (Bosch, de Sanjose. 2003). The differences in prevalence were partly due to the different ages of women studied and the different tests used to detect infection with HR-HPV. Women who are over the age of 30 years and who are HR-HPV positive include women with new infections and those that have persistent infections. The prevalence of HR-HPV in our analysis was much higher compared to other studies from America, most parts of Europe and Asia in women aged 30 and older. However similar prevalences to our study was found in most parts of Africa, India and in Eastern Europe (Bosch, de Sanjose. 2003). In Africa, the prevalence of HR-HPV infection ranged from 21.6% in Morocco to 43.7% in Senegal (Bosch and de Sanjose. 2003). Other studies done in South Africa, have found the prevalence of HR-HPV infection to range from 17% (Allan, Marais. et al. 2006) to 21.3% (Wright, Denny. et al. 2000).

Most studies show a decreasing HPV prevalence with age, however some trials have shown a peak in women below age 25 years, then a decreased prevalence in women aged 35-54 and another peak in women older than 55 years (Herrero, Hildesheim. et al 2000). The prevalence of HR-HPV infection in our study was 25.4% (95% CI; 23.8; 27.1) in

women aged 35-39, 18.4% (95% CI; 17.0; 19.9) in women aged 40-49 years and 19.3% (95% CI; 17.1; 21.4) in women aged 50-65 years. Compared to studies done in the UK (Cuschieri, Cubie. et al 2004) and Costa Rico (Herrero, Hildesheim. et al 2000) our prevalence rates across all age groups are higher. Cuschieri et al found that 2.1% of women aged 35-45 were HR-HPV positive, 1.2% of women aged 45 to 55 years were positive and 0.7% of women aged older than 55 years were HR-HPV positive. A population-based study in Costa Rico showed a 9.7% prevalence of HR-HPV infection in women aged 35-44 years, a 9.8% prevalence in women aged 45-54 years and an 11% prevalence in women aged 55-65 years (Herrero, Castle. et al. 2005).

The study in Costa Rico also showed the second peak in women older than 55 years but this peak was not shown by Cuschiere et al. A population-based study in Thailand in 2003 also did not show the second peak in older women but it showed that the prevalence of HR-HPV infection was 6.5% among women aged 25-34 years, 4.5% in women aged 35-44 years, 4% in women aged 45-54 years, 3.5% in women aged 55-64 years and 2.9% in women aged 65 years and older (Sukvirach, Smith. et al. 2003). Our study did show a decrease in prevalence in HR-HPV infection in women aged 40-49 years with a slight increase in women older than 50 years of age. It has been postulated that the reasons for the second peak in prevalence in older women could be due to a combination of factors including: immune changes leading to longer duration of new infections or reactivation of latent infections; a population effect for sexual behaviour or other risk factors and age-related sexual behaviour for both men and women (Herrero, Castle. et al. 2005:1805). Castle, Schiffman. et al. (2005) investigated these postulates and found that viral persistence was more important than acquisition of new infections in older women. More prospective population studies needs to be done in older women to determine the reasons for this peak in prevalence in older women.

The prevalence of HR-HPV infection could be higher in our population for many reasons. All women had volunteered from the local community to be part of the study and this could have resulted in selection bias of the sample used. The women most at risk may have joined the study in larger numbers thus raising the prevalence of HR-HPV infection

in this sample. Many researchers have discussed the population effect of women of a certain age having a prevalence of HR-HPV infection due to the population all having similar high risk sexual practices at the time (Herrero, Castle. et al. 2005, Castle, Schiffman. et al. 2005). The women in our study seem to be as sexually active as the women in other parts of the world so the risks are comparable. What has not been investigated in our sample of women is the effect of the partner characteristics which could increase the community prevalence of HR-HPV infection. The prevalence of HR-HPV infection seems to be higher in poverty-stricken communities, which could indicate the effect of malnutrition, poor immune system and co-infection with other sexually transmitted infections such as HIV infection. Future prospective population studies which should include the investigation of the partner characteristics are paramount to determine the incidence and persistence of HR-HPV infection in our South African women. This will be valuable information for future HPV vaccine strategy initiatives in South Africa.

It is important to note that the prevalence of HR-HPV infection could be higher in our study than what we have reported, as we excluded women with either a history of treatment to their cervix, or with lesions that were suspicious of cancer.

4.3 The effect of HIV on the risk of HR-HPV infection

In total, 12.0% (95% CI; 11.2; 12.7) of the entire population in our study was HIV-positive. In women aged 35-39 years, 18.2% (95% CI; 16.7; 19.7) tested HIV-positive compared to 9.5% (95% CI; 8.4; 10.6) in women aged 40 to 49 years and 5.0% (95% CI; 3.8; 6.2) in women older than 50 years ($p<0.001$). There was a significant difference in HIV status between HR-HPV positive participants and those that were HR-HPV-negative. In a univariate analysis women who were HIV-positive had more than four times the odds of HR-HPV infection compared to women who were HIV-negative. In a multivariate logistic regression analysis HIV status was a significant predictor of HR-HPV infection and this remained after stratification by age category and menopausal status.

The 2001 Antenatal survey report by the Department of Health reported that the HIV prevalence was 19.3% (95% CI; 17.0; 21.5) in women aged 35-39 years, 9.1% (95% CI; 6.2; 11.9) in women aged 40-44 years and 17.8% (95% CI; 4.3; 31.4) in women aged 45-49 years (Department of Health, 2001). The 95% confidence intervals are wide for the older age groups due to the limited numbers of older women attending antenatal clinics. The HIV-prevalence rates in the women in our study were similar to these reported rates.

The Canadian Women's HIV study, similarly found that HIV-positive women had a nearly four times the odds of HR-HPV infection compared to HIV-negative women (OR; 3.75, 95% CI; 2.39; 5.89) (Ferenczy, Coutlee. et al. 2003). Other studies have shown that HIV infection is a risk factor of genital HPV infection and that HPV infection is detected more frequently in HIV-positive women and is more persistent in HIV-positive women (Moodley, Hoffman. et al. 2006, Jay, Moscicki. et al. 2000, Ferneczy, Coutlee. et al. 2003, Branca, Garbuglia. et al. 2003, La Ruche, You. et al. 1998, Laga, Icenogle. et al 1992).

The strong association between HIV infection and HR-HPV infection could be due to the women in our study having high viral HIV loads and low CD 4 counts. Although these measures were not taken in this study they have been researched in other studies and high viral loads and low CD 4 counts were found to activate HPV replication and increase HPV detection (Palefsky, Minkoff. et al. 1999). We can postulate that some of the women in our study might have had relatively high viral loads and low CD counts due to the timing of our study. In 2000, when this study was started there was no access to antiretroviral medication for state patients, unless they were enrolled in a research study. It will be imperative for future studies on the association of HIV on HR-HPV infection, to measure these parameters.

4.4 The effect of the use of contraception on HR-HPV infection

Nearly 85% of all the participants in the study had used some form of contraception before the study and the most common form used was the LAIPs. Similarly, the South

African Demographic Health survey (SADHS) done in 1998 reported that 73.5% to 86.8% of women in the age group of 35 to 65 years had used a form of contraceptive previously and that LAIPs were the most commonly-used contraceptive (Department of Health: SADHS, 1998).

Around half of all participants were currently not using any form of contraception in the three months before our study. In the SADHS, 61.2% of sexually active women were using a current method but this dropped to 57% in women aged 40-44 years and 46% in women older than 45 years (Department of Health: SADHS, 1998). In our study, less than 1.0% reported using any condoms in the three months before enrollment and in participants aged 35 to 39 years, 1.5% were currently using condoms. Comparing the use of condoms in the SADHS in 1998 and 2003, the current use of condoms decreased with the age of the women and was dependent on the type of current partner. The proportion of women currently using condoms had increased from 1998 to 2003 (Department of Health: SADHS, 2003, 1998). The current condom use in our study was lower than what was reported in the SADHS in 1998 and this is possibly due to the age of the women in the study and due to the nature of each partner.

In this study, we found that the current use of LAIPs increased the odds of HR-HPV infection. Another cross-sectional study done in 2001 at the United States-Mexico border found a similar effect (Giuliano, Papenfuss. et al 2001), however there are not many other clinical studies published on this effect. Laboratory studies have shown that progesterone has an effect on HPV cell transformation and transcription, which could increase the risk of pre-invasive cervical cancer (Pater, Bayatpour. et al. 1990, Yuan, Auborn. et al. 1999). Biologically HR-HPV prevalence is related to the incidence and duration of infection but in this cross-sectional study the temporal relationship of exposure between LAIPs and new versus persistent infections cannot be determined. The multivariate analysis adjusted for sexual activity which could have determined new infections, so we can possibly assume that the progesterone hormones exerted their effect on the persistence of the HR-HPV infection. However prospective population studies are still needed to answer this question.

It is also known that in the province in which this study took place, there is a strong demographic difference between the users of Net-EN, DMPA and other contraceptives. In a recent study in the Western Cape Province, women who used Net-EN were younger and more educated than women using DMPA (Morroni, Myer. et al. 2006). There is also a perception that Net-EN preserves future fertility while DMPA if often perceived by providers and users to be better for older women who have completed their families (Morroni, Myer. et al. 2006). Although we adjusted for all measured socio-demographic, behavioural and biological variables and use of contraception variables, there may be the residual effects of confounding with other characteristics that were not measured and adjusted for in our study. This possible association between the use of LAIPs and the odds of HR-HPV infection has important public health implications for South Africa and other countries where LAIPs are the most commonly used contraceptives. Prospective population studies on the incidence and persistence of HR-HPV infections will help to answer these questions.

Many studies (Winer, Lee. et al. 2003, Kataja, Syrjanen. et al. 1993) have shown no protective effect associated with condom use and HR-HPV infection and in a study in Washington in 1997, this was thought to be due to biased reporting on the number of times condoms have been used and possibly incorrect use of condoms (Winer, Lee. et al. 2003).

A study done by Kjaer et al in 1997, showed that that the current use of condoms compared to never using condoms, significantly increased the odds for HR-HPV infection nearly two-fold (adjusted OR; 2.2, 95% CI; 1.2; 4.3). A recent meta-analysis by Manhart and Koutsky, of over 20 trials which investigated the role of condoms in HPV transmission, concluded that the use of condoms did not decrease the acquisition of the HPV infection but the use of condoms may give some protection against HPV-associated disease like genital warts, precancer and cervical cancer (Manhart, Koutsky. et al. 2002). It is postulated that the use of condoms could aid in the clearance of the HPV infection, decreasing persistence and therefore decreasing the risk of HPV-associated diseases like pre-invasive cancer (Hogewoning, Bleeker. et al. 2003).

A longitudinal study done on Washington University students that was specifically designed to evaluate whether condom usage reduced the risk of HPV transmission found that women who had used condoms all the time in the study period were 70% less likely to acquire a new HPV DNA infection (Winer, Hughes. et al. 2006). This study precisely measured the frequency of condom use and due to the use of computer-assisted questionnaires instead of face-to-face interviews, the data is considered very accurate compared to other cross-sectional studies (Winer, Hughes. et al. 2006).

The number of women in our study sample who were currently using condoms was very low and this could have affected the results. It is postulated that the women who were using the condoms, were using them due to the perceived risk of their current partner and it is this factor that caused the risk of HR-HPV infection, and not the inherent use of the condom itself. Our study was limited in that we did not measure the consistent correct use of condoms, and because of the cross-sectional study design, we could not determine the temporality of the HPV infection and condom use.

4.5 Effect of other variables on the risk of HR-HPV infection

In this study, for every one extra live birth, there was a 10% increased odds of HR-HPV infection (OR; 1.10, 95% CI; 1.06; 1.14) when the model was adjusted for by all the other variables. When the model was stratified by a positive HIV status, the mean number of live births remained significant in both HIV-positive (OR; 1.13, 95% CI; 1.03; 1.25) and HIV-negative participants (OR; 1.09, 95% CI; 1.05; 1.14). Other studies have found conflicting results, Hildesheim et al, also found that the number of live births was associated with increased odds of HR-HPV infection but Kjaer et al reported that the number of live births in their study was associated with a decreased odds of HR-HPV infection compared to women who had never been pregnant (Hildesheim, Gravitt. et al. 1993, Kjaer, Van den Brule. et al. 1997). In all of these studies the confounding variables and number of sexual partners had been adjusted for in the final model. In our study these

variables were adjusted for and despite the number of recent sexual partners, the number of live births remained significant.

4.6 Strengths and limitations of the study

The strengths of the study are mainly the large sample size for this analysis. Although the sample size was calculated for the SAT study, the number of participants that were eligible for the risk-factor analysis gave the study enough power to determine the risk factors for HR-HPV infection in this population.

A further strength of the study is that community health workers (CHW) were employed to educate and to enroll participants. These CHW all lived in similar circumstances to the participants themselves and besides speaking the local language could understand the cultural influences pertinent in the participants' lives. There seemed to be a relationship of trust and the follow-up rates of the SAT study (of over 70% at 36 months) is a test to this fact. Focus groups were held for other studies and the participants acknowledged that the study staff were kind and helpful and that they would always recommend their friends and family to join the study. Although it is understood that some study participants may not answer sexual-related questions honestly in a formal study setting, it is felt that in this study the women gave honest answers to the various questions as there was an element of trust in the relationship between the participant and the CHW. The fact that no participants declined to answer the sexual questions supports this opinion.

A weakness of our study was that the study was not designed to investigate the risk factors for HR-HPV infection. The implication of this is that the volunteers of the SAT study may not have been representative of the community of Khayelitsha as a whole and our study sample could have been biased. Due to the convenience sampling, it is also difficult in this setting to make generalizations to the overall population in Khayelitsha. If we compare the socio-demographic characteristics of our study population to the 2001 census data taken from Khayelitsha, we find that a similar percentage of women had completed some secondary schooling but far more women had passed a matric (21.3%) in

the census results compared to only 8% of the study population having passed matric. The unemployment rate in our study was higher (75%) than the rate of 57.6% reported in the 2001 census. More women in our study (70.7%) lived in informal dwellings than what is reported in the 2001 census (64.6%) (Census 2001). Due to the differences in the socio-demographic characteristics we need to be cautious in making generalizations to the overall Khayelitsha population. It does seem that the women in our study were more disadvantaged than women living in the general Khayelitsha population and that these women would need to be targeted for further program research. A second implication is that the enrollment questionnaire was not designed to evaluate some of the risk factors, and more detailed information could have been collected on some of the risk factors. It is a limitation that not enough information was obtained on how long each contraceptive method was used.

A further limitation was that the study design was a cross-sectional analytic study design investigating the risk factors for prevalent HR-HPV infection and no analysis was done for persistent HR-HPV infection which is the main risk factor for cervical cancer. Prevalence is dependent on incidence of infection and duration and our data cannot determine which infections were new infections or which were persistent infections, as it was a cross-sectional study. If we look at the risk factors we could postulate which risk factors could be significant for incident or persistent infections. Younger women would be more at risk for incident infection but older women would have had more persistent infections. HIV infection increases the risk of new infections as well as the risk of persistent infections. The use of LAIPs probably increases the risk of persistent infections but may also have an effect on new infections. Women that are married are probably at a decreased risk of new infections due to behavioural characteristics but may possibly have more persistent infections. In cross-sectional studies, there is length-biased sampling whereby the cases in a cross-sectional study will over-represent cases with long duration on disease, and under-represent those cases with short duration of disease. In our study, the persistent cases of HR-HPV infection were probably more sampled than new infections of HR-HPV infections. A prospective population study on risk factors for

incident HR-HPV infections would assist in determining which risk factors are more important for incident or persistent infections.

A further problem with cross-sectional studies is the issue of temporality. In our study we could look at the significant risk factors individually to see which came first to determine the nature of the temporality. Regarding HIV infection, it is possible that HIV infection predisposes you to HR-HPV infection or that HR-HPV infection predisposes you to HIV infection, so it would be important to determine which came first, but this is impossible in a cross-sectional study design.

In this study, a multivariate logistic regression analysis was performed to report the measures of the exposure outcome association as being adjusted by other covariates that could have acted as confounding variables. In sexually-transmitted infections such as HR-HPV infection, the sexual history would possibly be a confounder. For example, if hormonal contraception is a risk factor for HR-HPV infection, but high-risk sexual behaviour is associated with contraception use and the risk of HR-HPV infection, then we would need to have adjusted for the high-risk sexual behaviour. However if this variable was not measured adequately, then the true confounding effect would not have been removed completely and although the results are adjusted associations, there may still be some residual effects of confounding. This phenomenon is sometimes referred to as residual confounding (Myer, Morroni. et al.2004) and is usually problematic when trying to measure behavioural dependent exposure variables. In this study, the sexual history was reported verbally to a CHW and as it would have been impossible to determine the "true" answer to the questions, there may be non-differential misclassification in the measurement of the variable, or if this was systematic for different groups in the study there could have been differential misclassification which could have attenuated the association between the exposure and outcome.

A further limitation is the lack of some important test measurements. The menopause status was self-reported and no other tests were done to validate this important variable. Secondly, no CD4 counts or viral loads were tested for; which are the important variables

to know in women that are HIV-positive. No measurements for infection with HSV-1 or HSV-2 were taken during the study. It would also have been useful to record the different types of HR-HPV infections but this information was not available for this analysis. In view of future HPV vaccine licensure in South Africa, it would be important to know the distribution of the types of HR-HPV infection in our populations.

4.7 Recommendations for new research

Expanding on the risk factors for HR-HPV infection shown in this study, further prospective population studies need to be done to investigate the risk factors of HR-HPV infection in younger women as well as the risks of HR-HPV persistence in older women. If these studies support the positive association of DMPA and Net-EN use and the risk of HR-HPV infection, then women using these methods would need to be educated and properly counselled on the risks of HR-HPV infection.

The commercially available HPV DNA tests are very expensive and not available in South Africa. The development of more affordable HPV tests should continue in countries such as South Africa and studies on how best to facilitate their use in primary care settings should be done. Cost effectiveness studies of establishing HPV based primary screening in poor countries in Africa and rural parts of South Africa where there are no established screening programmes should be done.

This study showed a high prevalence rate of HR-HPV infection in the Khayelitsha community and it will be extremely important to determine the role of other co-factors needed in the pathway from HPV-infection to invasive cervical carcinoma. Although much research has been done in other countries, more large scale population studies on HR-HPV positive women need to be done in South Africa. If these co-factors are determined specific to our women, then prevention of these co-factors could help in the prevention of pre-invasive lesions of the cervix.

With the imminent licensure of the HPV vaccine in this country, there will be much interest in the role of HPV vaccination in the prevention of cervical cancer. Much research will be needed on the programmatic issues around vaccinating young pre-adolescent girls before they become sexually active as well as the acceptability of the vaccine itself. However it is not expected that the vaccine will become widely available in the public sector due to its cost and the other competing needs that the government may need to cover. Therefore it is still important for HPV preventative programs to be put in place so that women at risk of HR-HPV infection could be educated and counselled.

4.8 Recommendations for policy in South Africa

This study has shown a positive association between HIV infection and the risk of HR-HPV infection. The policy of all HIV-positive women to have cervical screening as soon as a positive HIV diagnosis is made, regardless of her age, must be enforced. It is important that research on the appropriate intervals for pap smears in HIV-positive women in developing countries, is conducted. Cervical screening facilities need to be strengthened in areas where the HIV prevalence rate is high. All clinics providing voluntary testing and counselling and antiretroviral medication should have a sister on site doing cervical screening and education. Ongoing efforts for all women to have an HIV test must continue at all points of contact in the health care services to enable women to be counselled on their risks for other health problems such as HR-HPV infection.

4.9 Key findings and implications

This study showed that there is a positive association between HIV infection and HR-HPV infection. Ongoing efforts should be encouraged to prevent both HIV and HPV infection and safer sexual practices should be encouraged.

In this analysis there was a positive association between the use of LAIPs and HR-HPV infection. Further studies need to be done to verify this finding before women are counselled and educated on the potential implications of this finding.

Women in this study who were currently using condoms had increased odds of HR-HPV infection. However the correct and consistent use of condoms was not measured in this study and further studies need to be done.

In this study, women who were currently married had decreased odds of HR-HPV infection compared to those that were not married. This is a reflection of more stable monogamous relationships than those experienced when women are single. The implication would be to encourage healthy family relationships to prevent family break-ups and for services to be put in place for counselling around marital relationships.

Women in this study who were employed had decreased odds of HR-HPV infection than those that were not currently employed. This could be a reflection of employed women not having to rely on the male partner's income and thus have more choice in whether perhaps they stay in the relationship if there is any infidelity from the partner's side.

In conclusion, this study shows that there is a high prevalence of HR-HPV infection in African women aged 35 to 65 years living in Khayelitsha. The overwhelming association between HIV infection and HR-HPV infection in this study, has very important clinical and policy implications in the communities where HIV infection and cervical cancer are major health problems. It is hoped that recent efforts by our National Department of Health to address these problems will result in decreased levels of cervical cancer to levels currently seen in developing countries.

This study also adds onto the knowledge of risk factors for HR-HPV infection, but introduces the possibility of LAIPs having a significant effect on the prevalence of HR-HPV infection, and highlights the urgent need for further research into the risks of HR-HPV infection, until the HPV vaccine becomes widely available and affordable

Chapter five

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Appendix 1: SAT Study Informed consent form

UNIVERSITY OF CAPE TOWN AND
COLUMBIA UNIVERSITY, NEW YORK, USA

Consent to Join in a Research Project

This consent form provides you with what you need to know to decide if you want to join this research project.

Cape Town Cervical Cancer Project

WHY WE ARE DOING THE STUDY

You are invited to join a research project. This project will test how safe and effective are different screening programs for the prevention of cancer of the mouth of the womb (cervix).

SUMMARY OF WHAT WILL HAPPEN TO YOU

Joining this project means that:

1. You will be asked personal questions about your health and private life.
2. You will give blood that will be tested for a variety of things, including HIV.
3. The mouth of your womb (cervix) will be examined by a nursing sister and tested for cervical cancer precursors (that we call "white spots") using different tests.
4. A photograph will be taken of your cervix.
5. Some women with abnormal tests will be treated immediately and others will be observed for 6 months before treatment.
6. Many women who will receive treatment will not have cervical disease.
7. Treatment can have complications. If you receive treatment you will have a discharge and will be required not to have sex for one month. Sex during this month may increase your risk of getting infections, including HIV.
8. You will need to come back to the clinic 3-4 times during the next year.
9. Participating in this study gives you access to the best possible methods of cervical cancer prevention.

STUDY PROCEDURES

Screening Examination

If you agree to join the project, we will ask you to sign a document that says you agree to join and that you understand what is going to happen to you. You will then meet with a community health worker, who will ask you some personal questions about your health, your living situation and your sexual life. This will take about 5 minutes. You will then be asked to place a thin swab in your vagina (this you do yourself after removing your underpants). This will be tested for HPV (a virus that causes both the changes that can lead to cervical cancer, which we call the 'white spots' and to cervical cancer itself). You will then lie on an examination table and the nurse will place a speculum in your vagina. She will then use a soft brush to wipe skin from the cervix – this test is known as the Pap smear. This skin will be tested for the white spots and for cervical cancer and common cervical infections. If any of these diseases are found you will be treated. It takes about 4 weeks for the results to become available.

After the Pap smear has been taken, the nurse will wash your cervix with vinegar (this is the test that makes the 'white spots' visible). The nurse will first look to see if you have 'white spots' on the cervix and then she will use a magnifying glass. Finally, she will take a photograph of your cervix.

The entire examination will take about 5 minutes and should not cause any pain. If we see cancer, or something suspicious for cancer, you will be sent to see the doctor immediately. If this happens you cannot join the study.

We need to know if you are infected with HIV, the virus that causes AIDS. We will take about 4 teaspoons of blood from your arm and test it for HIV, the virus that causes AIDS, as well as other factors related to cervical disease. If you would like to know the results of your HIV test, we will give you the results. If you do not want to know the results, we will test you for HIV – but neither you nor the nurses will find out the results. However the project staff in New York will know the results, but will not be able to link the HIV test results to you directly.

Treatment Visit

You will need to return to the clinic 2-6 days after your first visit. At this visit you will be assigned to be either treated if your tests suggest disease, or to be re-examined in 6 months – even if your tests suggest a precancer is present. However if the tests suggest that cancer is present, you will be sent to see the doctor. Treatment will be by freezing the skin of the cervix. This will be done by the nurse in the clinic who will put a metal disc (like a coin) against the skin of the tip of the womb. This gets very cold and kills the skin.

Four-Week Follow-up Visit

At one month, you will come back to the clinic and we will ask you some questions.

Six-Month Colposcopy Visit

At six months, a doctor will examine you. At this visit you will again put a swab in your vagina and this will be tested for HPV. The doctor will then put a speculum in the vagina and once again a Pap smear will be performed using a soft brush to wipe the skin of the cervix. The doctor will then put vinegar on your cervix and examine your cervix using a special magnifying device. If anything abnormal is seen, a small piece of skin will be removed for further testing. Blood (4 teaspoons) will be taken from your arm and tested for HIV. You can either know the results of this test or not, depending upon your choice. You will be referred for treatment if any precancerous lesions (or 'white spots' that can grow into cancer) are found.

Twelve Month Colposcopy Visit

If you had an abnormal test result at your first visit, we want to see you again 12 months after you were enrolled in the study. This exam will be identical to the visit at six months, except no blood will be taken.

STUDY RISKS

You may feel some discomfort during the examination and feel slight pain when we take blood, and sometimes there is bruising. You may experience some brief pain, cramping, and/or bleeding if we take a piece of skin from your cervix.

If you join the project there could be a delay in the treatment of a precancer (the 'white spots' that can develop into cancer in the future) for up to 6 months. We feel that this is safe since it takes many years for a precancer (the 'white spots') to become a cancer. It is possible that you could need more extensive treatment because of this delay.

Most women who get treated will not actually have a cervical cancer or a precancer. The treatment that will be used in this project is called cryosurgery and involves freezing the skin of the tip of the womb. 'Freezing' has been used for 30 years and is considered safe. It often causes some cramping and some women feel hot or dizzy during freezing. Most women get a marked discharge that lasts for several weeks. In about 1 out of 100 women there are more serious complications including infection, bleeding, and narrowing of the mouth of the womb. It is very important you do not have sex for 4 weeks after freezing. Otherwise you may have bleeding or get an infection, ***including HIV***. If you are infected with HIV already, you may infect your partner with HIV if you have sex. You will get condoms to use if you must have sex during this 4 week period.

STUDY BENEFITS

The screening and treatment that you will receive in this project should prevent you from developing cervical cancer in the future.

You may also learn if you are infected with HIV. Knowing if you have HIV has both benefits and risks. If you are infected with HIV, you can take better care of yourself and see a doctor if you become sick. Being infected with HIV may effect whether you want to have more children and you may want to use condoms during sex to prevent you partner from becoming infected. The risks of knowing that you are infected with HIV include the stress associated with knowing that you have a serious illness, and the fact that some women find that their friends and family become afraid to have close contact with them.

ALTERNATIVES

If you do not wish to join this project, you can have a routine Pap smear taken.

COMPENSATION

All tests will be free. You will receive a R50 food voucher at each follow-up visit, but not at the first (screening) visit.

CONFIDENTIALITY

Your test results will be kept confidential and no information will be released or printed that would disclose that you are in the study, without your permission.

PARTICIPATION IS VOLUNTARY

Joining this project is completely voluntary. You can refuse to join - or stop being part of the project at any time.

QUESTIONS

If you have any questions, please ask. In the future if you have any questions you can reach Dr Lynette Denny who will do her best to answer them. If you have any questions on your rights as a research subject you can call the Institutional Review Board at 021-406-6911.

CONSENT TO PARTICIPATE IN THE PROJECT

I have discussed this study with the nurse to my satisfaction. I understand that I do not have to join the project, and that I can stop being part of the project at any time. I have read the above and agree to enter this research project. Signing this form does not waive any of my legal rights.

I have been informed that if I believe that I receive injury as a result of participating in a research study, I may contact the Principal Investigator; Dr Lynette Denny, Department of Obstetrics and Gynecology at Groote Schuur Hospital (21) 404-4488 so that I can review the matter and identify the medical resources which may be available to me. If I have any questions about my rights as a research subject I can call the Institutional Review Board at 021-406-6911.

I understand that:

a) I will receive a copy of this consent form.

Signature:_____

Participant Date

Signature: _____

Attending Clinician Date

Cape Town Cervical Cancer Project

I,_____(name of patient) agree to have 4 teaspoons of blood taken from me. I understand that these blood samples are for the purpose of testing different ways to prevent cervical cancer. I give permission for the blood to be tested for any factors considered relevant to the study, including HIV, antibodies against HPV and histocompatibilty antigens that may relate to how the body responds to HPV. I understand that the results of these tests will not be given back to me as they will not affect my treatment. I understand that the results of the blood tests will not be able to be linked to me personally but they will be linked to the other tests being performed on me. The blood results will be kept in the strictest confidence. I will also be offered HIV counselling. If I choose, I can have a confidential personal HIV test done and be told the results.

The information that is obtained from the analysis of my blood, bodily fluids and tissues, and photographs, may be used scientifically and may be used in other research. The analysis of my samples of blood and bodily fluids may contribute to the creation of new diagnostic tests, new medicines or other uses that may be commercially valuable to the sponsor. I will receive no financial benefits from such developments.

We would also like to store leftover portions of your body fluids and tissues for future testing. Such testing will probably include tests of other factors possibly related to the development of cervical cancer. The results of this testing will be reported to you or your doctor if they are medically useful, but it is likely that this will not be the case. Should you wish to have this material removed from storage for any reason in the future and destroyed you may contact Dr Lynette Denny at the Department of Obstetrics and Gynecology, University of Cape Town.

_____Yes, I agree to the storage and future testing of my blood, bodily fluids, and tissues.

_____No, I do not agree to the storage and future testing of my bodily fluids. Any leftover specimens will be destroyed at the end of the study.

_____ _____

Patient's Signature Date

_____ _____

Signature of Witness Date

_____ _____

Investigator's Signature Date

Appendix 2: Enrollment Questionnaire

Screen and Treat Study: Enrollment Questionnaire

Form 0 1

Site ... ☐☐

Patient Name _____ PATNO ..... ☐☐☐☐☐

Date of interview ☐☐ ☐☐ ☐☐
day / month / year .

Interviewer Name _____ ☐☐

1a. Year of birth _____ ☐☐

1b. How old are you? (age in years) _____ ☐☐

2. What is the highest level of education you have completed?
   - 88. No school
   - 1. Std 1 or less
   - 2. Std 2
   - 3. Std 3
   - 4. Std 4
   - 5. Std 5
   - 6. Std 6
   - 7. Std 7
   - 8. Std 8
   - 9. Std 9
   - 10. Std 10
   - 11. Any tertiary/university

3. Are you married?
   - 1. Yes
   - 2. No

4. Do you have a job?
   - 1. Yes → If yes, what is your job?
     - 1. Domestic worker
     - 2. Other _____
   - 2. No

5. Where are you living now?
   - 1. House
   - 2. Shack on serviced site
   - 3. Shack on an un-serviced site or other open land
   - 4. Other _____

6. How long have you been in Cape Town?
   88. Born here
   Number of years _____

7. Do you have a home in the rural area that you go to regularly?
   1. Yes
   2. No

PATNO | | | | | |

8. How old were you when you became pregnant for the first time ..... | |
   (Write 00 in blocks if never pregnant)

9. How many times have you been pregnant? ..... | |
   (Write 00 in blocks if never pregnant)

10. How many times have you had a live birth? ..... | |
    (Write 00 in blocks if never pregnant)

11. Have you had change of life?
    1. Yes
    2. No
       a. If YES, what was your age? | |
       b. If YES. are you taking hormone replacement therapy?
          1. Yes
          2. No

12. How old were you when you first had sexual intercourse? ..... | |
(This includes sexual intercourse with a man even if you were raped)

13. How many different men have you had sex with in the last month? .... | |
(Write 00 in the blocks if no sex in the last month)

14. How many different men have you had sex with in your entire life? ... | |

15. Have you ever been treated for a sexually transmitted disease?
    1. Yes
    2. No
       IF Yes, how long ago was the last time? In months if within the last year | |
       In years, if more than a year ago | |

16. Have you ever had genital warts?
    1. Yes
    2. No
       IF Yes, how long ago was the last time? In months if within the last year | |

In years, if more than a year ago ☐☐

17. Do you smoke <u>cigarettes</u> regularly? (Regularly means at least daily on average)
    1. Yes
    2. No

    IF ANY CIGARETTES, how many do you smoke per day? ... ☐☐

18. Do you smoke a pipe or use other forms of tobacco?
    1. Yes, pipe
    2. Yes, other_____
    3. No

19. Have you drunk any alcohol in the last month?
    1. Yes, moderate or large amount (4+ drinks on a single occasion)
    2. Yes, small amount (0-3 drinks on a single occasion)
    3. None at all

PATNO ☐☐☐☐☐

20. Have you **<u>EVER</u>** used any method of birth control?
    1. Yes
    2. No

20a. IF YES, have you EVER used the following

Birth control pills	1. Yes	2. No
Condom	1. Yes	2. No
Injection (Depo or other)	1. Yes	2. No
Sterilization	1. Yes	2. No
Other _____	1. Yes	2. No

20b. IF YES TO ANY ABOVE, have you used the following <u>IN THE LAST 3 MONTHS?</u>

Birth control pills	1. Yes	2. No
Condom	1. Yes	2. No
Other _____	1. Yes	2. No
Injection (Depo or other)	1. Yes	2. No

20c. IF YOU ARE USING INJECTION, How long have you been using it?

In months, if 1 year or less ☐☐

In years, if more than 1 year ☐☐

Which one are you taking now?

1. 2-month (Nuristerate)
2. 3-month (Depo)
3. Unknown

21. How did you hear about the project?

1. radio
2. health care worker with a loudhailer
3. health care worker in clinic
4. poster
5. flier
6. word of mouth

other_____

Appendix 3: Clinical Examination form

Screen and Treat Study: Clinical Examination Form

Form 0 2

Patient Name _____ PATNO | | | | | |

Bloods taken: 1. Yes 2. No

Confidential testing requested: 1. Yes 2. No

Date of clinical examination | | | | | | | | | | | |
day / month / year

Name of Clinician_____

1. Has self-sample for HPV been taken?
   1 Yes
   2 No

2. Is there a vaginal discharge?
   1 Yes
   2 No
   2a If yes, is the amount?
      1 Mild
      2 Moderate
      3 Severe

3. Does the patient have a significant vaginitis requiring treatment?
   1 Yes
   2 No

4. pH ___.___

5. Three glass slides prepared?
   1 Yes
   2 No

6. Whiff Test Result
   1 Positive
   2 Negative

View the cervix.

7. Is there any suspicion of cancer?
   1 Yes
   2 No

8. Is there another reason why this patient is not suitable for screen and treat?
   1 Yes
   2 No
   8a. If yes,
   specify_____

If yes to either 7 or 8, the patient must be excluded from the study and referred to the medical officer. Take a photograph and proceed to question 17.

9. Is there cervical stenosis?
   1 Yes
   2 No
   9a. If yes, is it?
   1. Absolute
   2. Relative

10. Does the patient have cervicitis?
   1 Yes
   2 No

11. Have you taken?

a.	Liquid Pap	1. Yes	2. No
b.	HPV1	1. Yes	2. No
c.	HPV2	1. Yes	2. No

First application of 5% acetic acid

12. Can you see a cervical lesion with the naked eye (no magnification)?
   1 Yes
   2 No

   12a. If yes, does the lesion cover more than 70% of the cervix?
   1 Yes
   2 No

If yes, the patient must be excluded from the study and referred to the medical officer. Take a photograph and proceed to question 17.

Second application of 5% acetic acid

13. Can you see a cervical lesion **with** magnification?
   1 Yes
   2 No

Third application of 5% acetic acid

14. Was a Cervigram taken?
    1 Yes
    2 No IF NO, Why _____

15. What is the wet mound result?
    1 No organisms
    2 Trichomonas seen
    3 Clue cells seen
    4 Trichomonas and clue cells seen
    5 No result

16. What medications did the patient receive?
    1 Flagyl
    2 Antifungal RX
    3 Triple RX (doxycycline, ciprofloxacin, flagyl)
    4 Flagyl & Antifungal
    5 Triple & Antifungal
    6 None

17. Category of patient in the study:
    1 DVI Positive (naked eye **OR** with magnification)
    2 DVI negative
    3 Excluded

Appendix 4: Descriptive statistics for the numerical variables

Descriptive statistics for all the numerical explanatory variables are summarized in table 1.

Table 1: Descriptive statistics for numerical explanatory variables

	minimum	maximum	median	IQR
Age (years)				
HPV-pos	35	65	40	37-46
HPV-neg	35	65	42	38-48
Age at first intercourse				
HPV-pos	10	30	16	15-18
HPV-neg	6	39	16	15-18
Number of lifetime partners				
HPV-pos	1	100	4	3-5
HPV-neg	1	60	3	2-5
Number of sexual partners in previous month				
HPV-pos	0	5	1	0-1
HPV-neg	0	5	1	0-1
Age at first pregnancy				
HPV-pos	0	38	19	17-19
HPV-neg	0	45	19	17-21
Number of pregnancies				
HPV-pos	0	14	4	3-5
HPV-neg	0	15	4	3-5
Number of live births				
HPV-pos	0	13	4	2-5
HPV-neg	0	15	4	2-5

Appendix 5: Multivariate logistic regression analysis – Model Checking

Model checking

The model was checked by performing a goodness of fit test. The Pearson chi2 test was not significant (p=0.699), indicating that the model is a good fit.

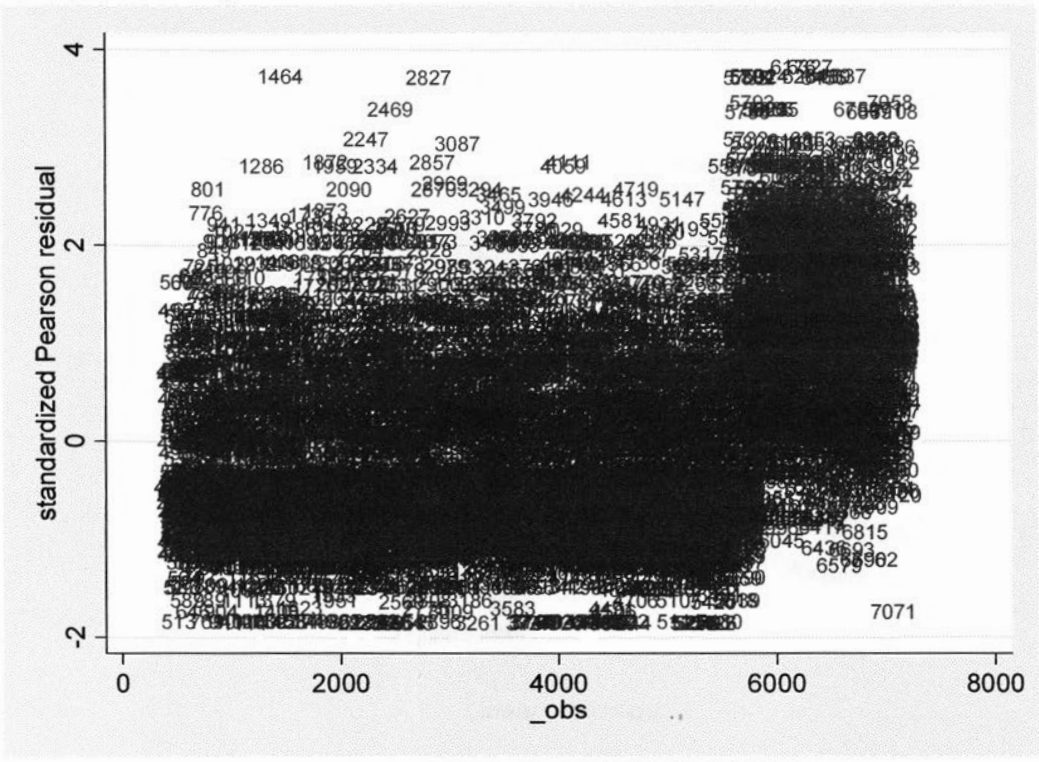
Form of the linear predictor

The form of the linear predictor was checked by plotting both the Pearson and deviance residuals versus the observation number and the residuals were plotted against the linear predictor (xb1). The graphs show that the residuals of some of the observations have large residuals but when the residuals are plotted against the linear predictor, there is a structure consistent with a binary response variable. The observation number was a randomly assigned number to each observation after the unique patient numbers were removed for the anonymous linked HIV test results. The scatter graphs show an interesting trend for the bigger observation numbers to have larger residuals. These were randomly assigned numbers so a further set of scatter graphs were plotted using the enrollment date instead of the observation number. The trend of larger residuals is not seen in these graphs and it is presumed that there was an anomaly with the observation numbers assigned to each observation.

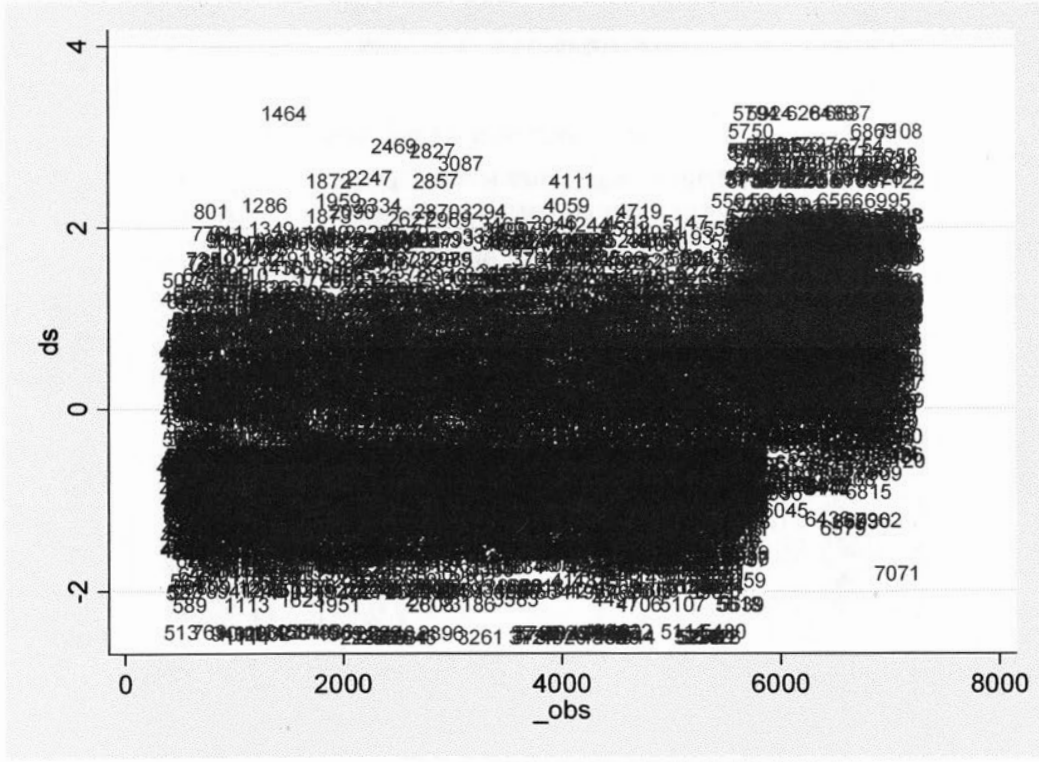
Adequacy of the link transformation

For this model there is a binary response variable and the logit transformation is always appropriate and does not need checking.

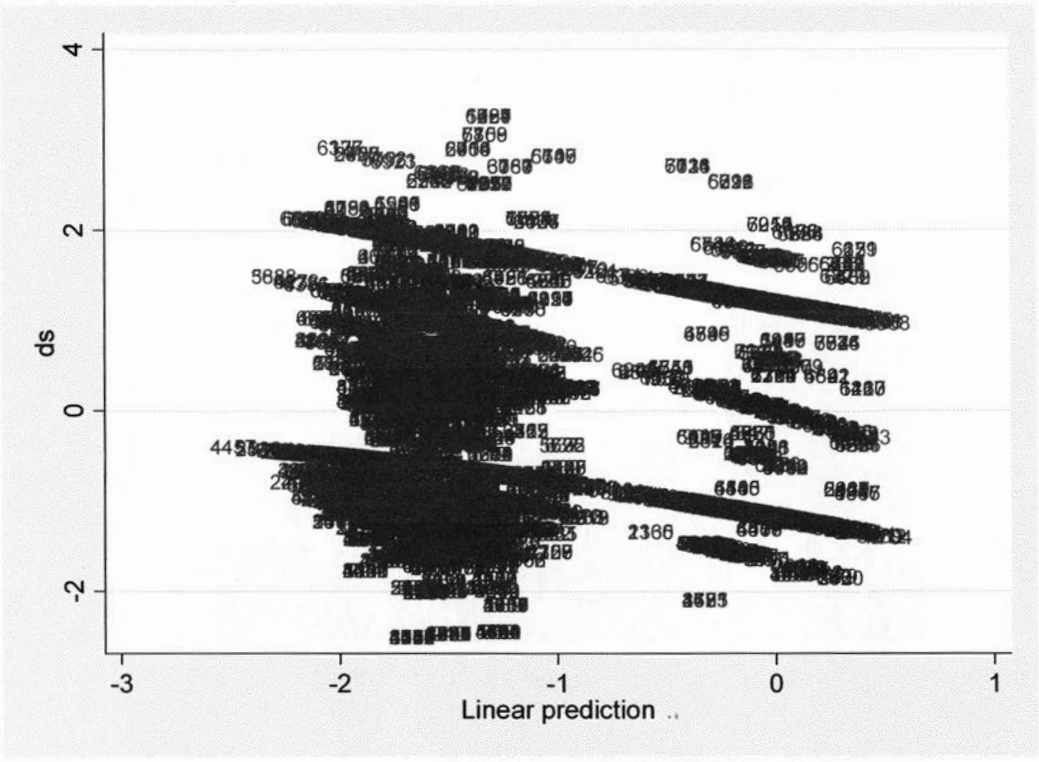
Graph 1: Scatter plot of standardized Pearson residuals



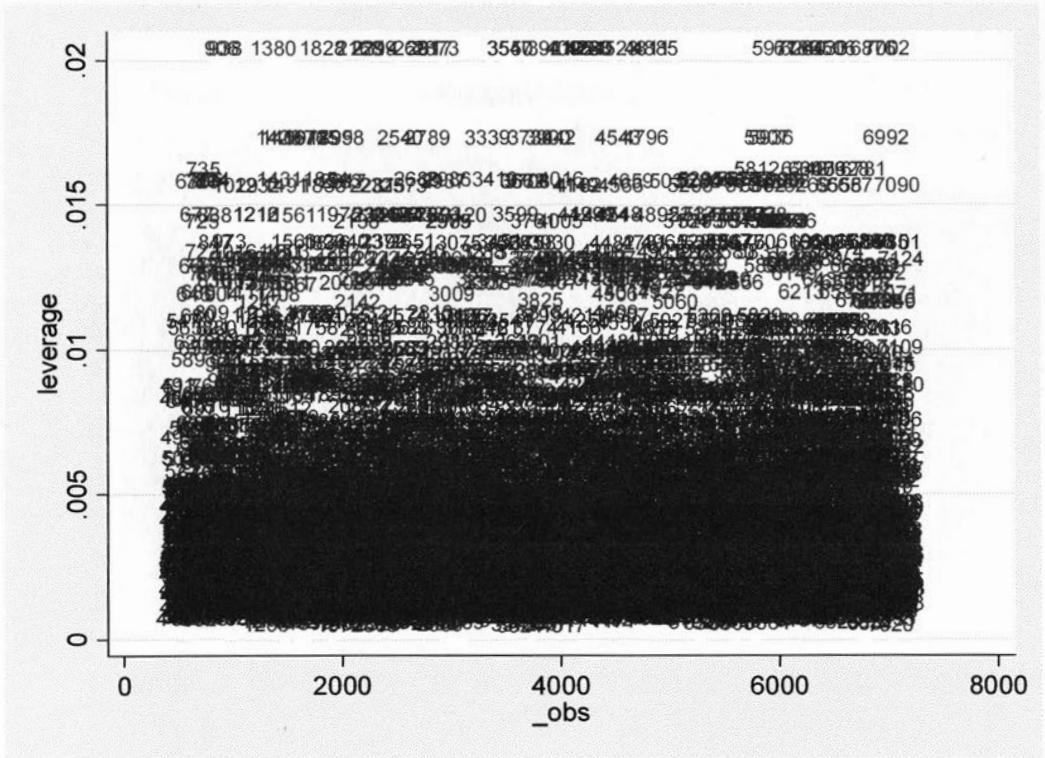
Graph 2: Scatter plot of standardized residuals



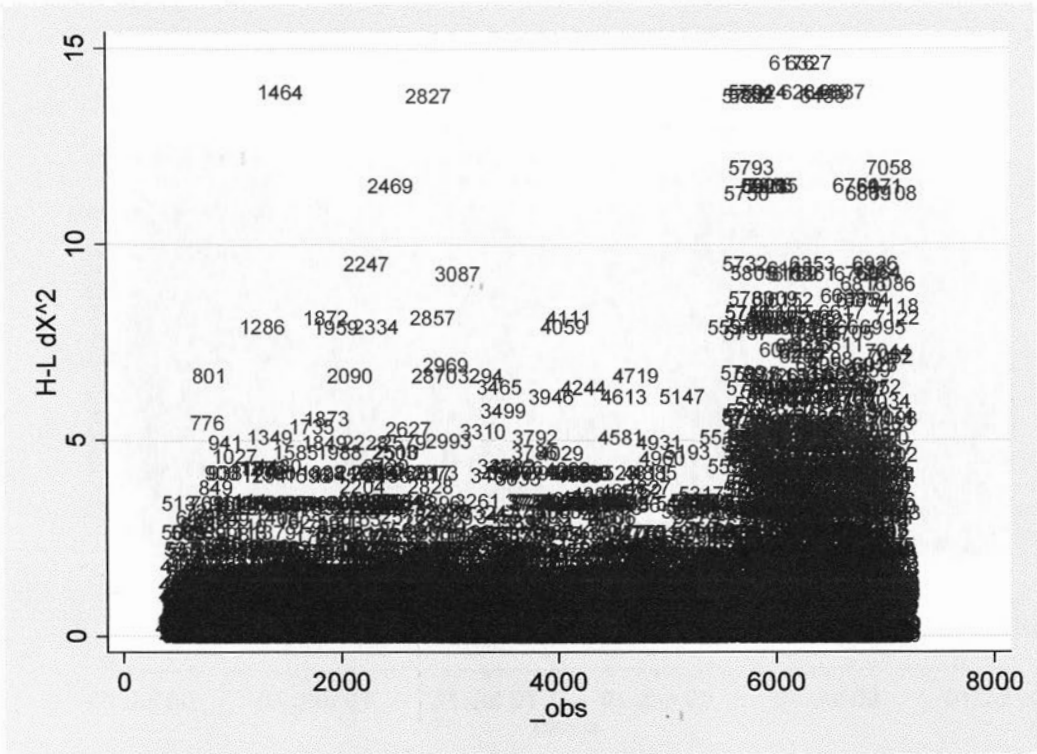
Graph 3: Scatter plot of linear prediction



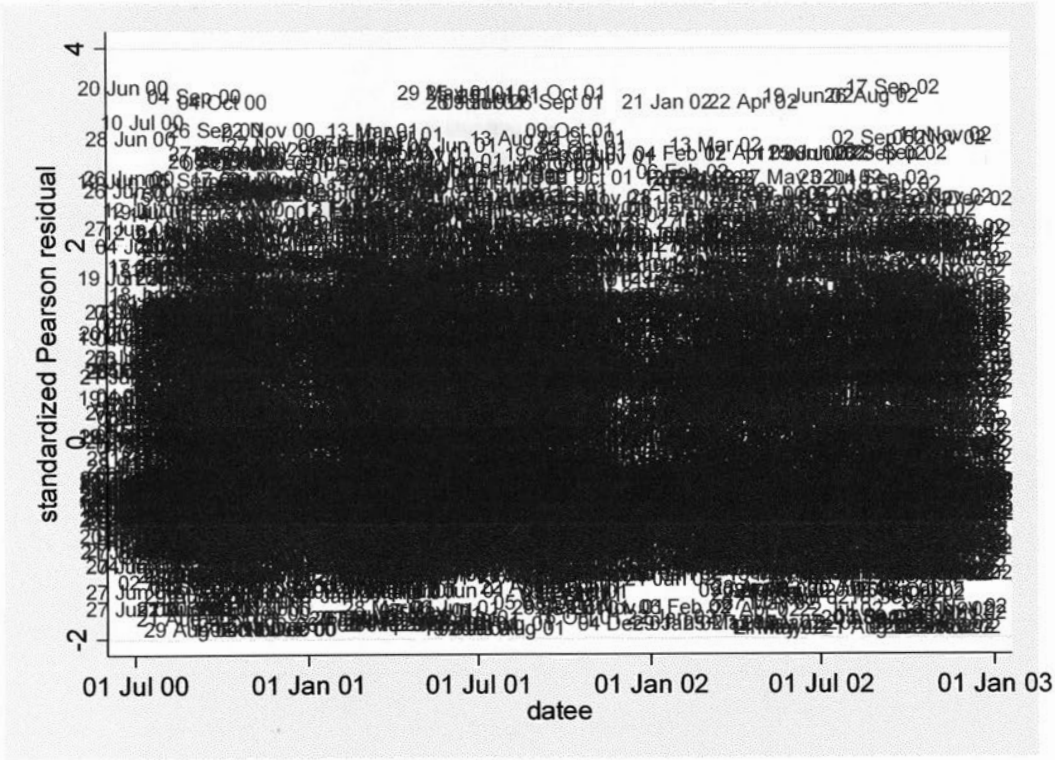
Graph 4: Scatter plot to detect influential observations



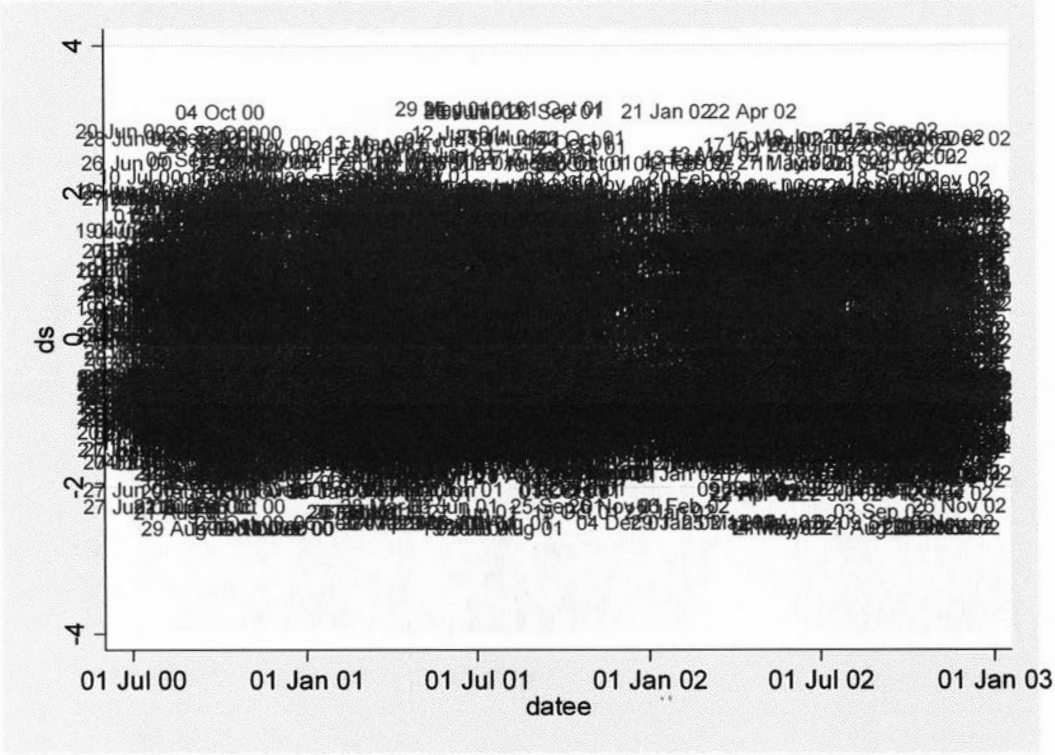
Graph 5: Scatter plot to detect outlier observations



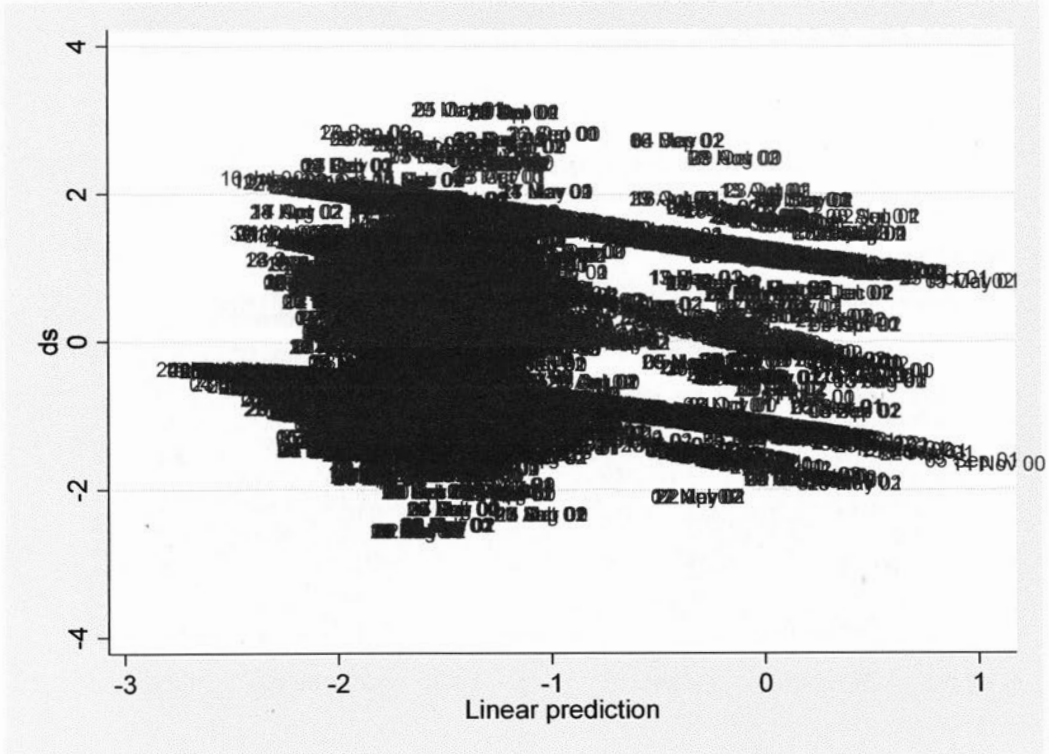
Graph 6: Scatter plot of standardized Pearson residuals (enrollment date)



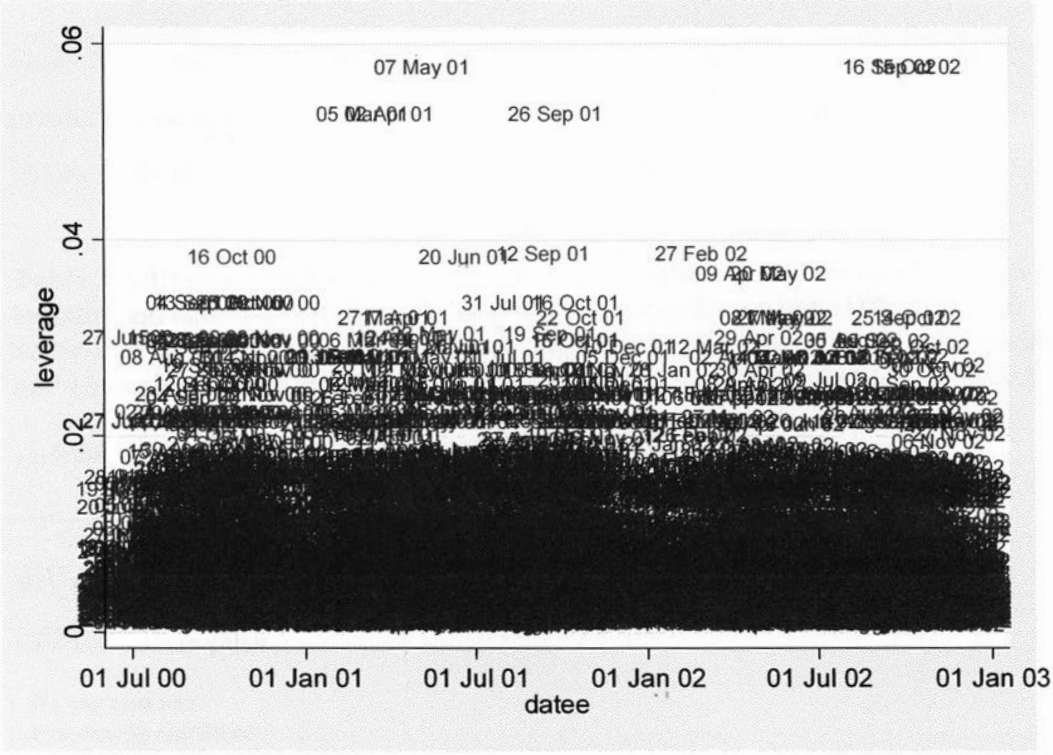
Graph 7: Scatter plot of standardized residuals (enrollment date)



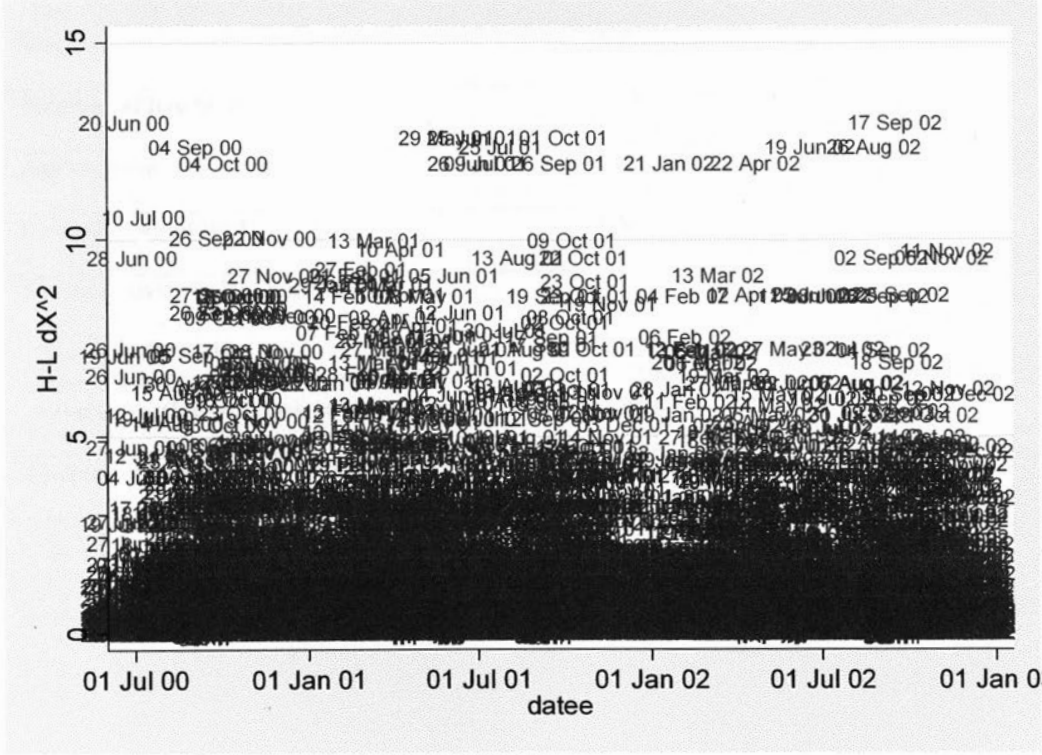
Graph 8: Scatter plot of linear prediction (enrollment date)



Graph 9: Scatter plot to detect influential observations (enrollment date)



Graph 10: Scatter plot of outlier observations (enrollment date)



Appendix 6: Interaction variables

Table 2 presents the final model with interaction variables included in the model. The interaction variable is a cross-product of the variables. Any current contraception and age in years was the only significant interaction variable.

Table 2 Adjusted Odds Ratio (OR) and 95% Confidence Intervals (CI) from a logistic regression analysis modelling the relative odds of HR-HPV infection according to participant socio-demographic characteristics, use of contraception and biological characteristics with the interaction variables within the model.

variable	Full dataset (n=6645)
	OR (95% CI)
HIV pos	4.09 (3.48; 4.80)
Current contraception	
No current use	
Previously sterilized	
Condoms	
COC	
DMPA	
Net-EN	
Married	0.71 (0.62 ; 0.81)
Number of live births	1.10 (1.06 ; 1.14)
Age in years	0.99 (0.98; 1.00)
Currently employed	0.86 (0.74 ; 0.99)
Sexual activity in last month	
Not sexually active	1.00
Sexually active with one partner	1.13 (0.97; 1.31)
Sexually active with two or more partners	1.05 (0.63; 1.76)
Currentanycontraceptionageyrs	0.98 (0.96; 1.00)