A STUDY OF GENITAL HUMAN PAPILLOMAVIRUS (HPV) INFECTION AND ANTIBODY RESPONSE IN HETEROSEXUALLY ACTIVE SOUTH AFRICAN COUPLES

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Thesis presented for the degree of DOCTOR OF PHILOSOPHY in the Division of Medical Virology, Department of Clinical Laboratory Science,

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DEDICATION

This thesis is dedicated to my father (**H.D. Mbulawa**), my mother (**E.N. Mbulawa**).

And to the Church of the Lord Jesus Christ Youth
"But seek his kingdom and his righteousness and all these things will be given to you as well"
Matthew 6:33

DECLARATION

The work described in this thesis was done in the Division of Medical Virology, Department of Clinical Laboratory Sciences and the Institute of Infectious Disease and Molecular Medicine (IIDMM), University of Cape Town, under the supervision of Professor Anna-Lise Williamson and Dr Dianne Marais. The work is my own and where use has been made of others, their contribution has been acknowledged.

Signed by candidate

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Zizipho ZA Mbulawa
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TABLE OF CONTENTS

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Participants were partners recruited to investigate genital HPV transmission from among those screened but ineligible for an HIV transmission trial or who had completed the HIV transmission trial, in Gugulethu, Cape Town, South Africa. The initial recruitment of couples who participated in this study was funded by the Bill and Melinda Gates Foundation. The South African Research Chairs Initiative of the Department of Science and Technology, Medical Research Council, National Health Laboratory Services, UCT University Research Committee, Cancer association of South Africa, Swedish International Development cooperation Agency, National Research Foundations and Poliomyelitis Research Foundation are sincerely thanked for funding various parts of the project. Bursaries from National Research Foundations, UCT based scholarship, Poliomyelitis Research Foundation and Department of Science and Technology Women in Science Award are also thanked for funding my studies.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome	INF	Interferon
AIS	Adenocarcinoma *in situ*	LCR	Long control region
AOR	Adjusted odds ration	LSIL	Low grade squamous intraepithelial lesion
ARV	Antiretroviral	MFI	Median fluorescence intensity
ASCUS	Atypical squamous cell of undetermined significance	MHC	Major histocompatibility complex
BSA	Bovine serum albumin	Min	Minutes
CI	Confident interval	mL	Millilitre
CIS	Carcinoma *in situ*	µl	Microlitre
CIN	Cervical intraepithelial neoplasia	Nm	Nanometre
CMI	Cell mediated immunity	OD	Optical density
COPV	Canine oral papillomavirus	OR	Odds ratio
CRPV	Cottontail rabbit papillomavirus	ORF	Open reading frame
C_t	Threshold cycle	PCR	Polymerase chain reaction
CTL	Cytotoxic T lymphocytes	PVA	Polyvinyl alcohol
DNA	Deoxyribonucleic acid	PVP	Polyvinyl pyrrolidone
ELISA	Enzyme-linked immunosorbent assay	Rb	Retinoblastoma
GST	Glutathione S-transferase	RLU	Relative light unit
HC2	Hybrid capture 2	RR	Relative risk
HIV	Human immunodeficiency virus	Rt-PCR	Real-time polymerase chain reaction
HLA	Human lecocyte antigen	SD	Standard deviation
HMBS	Homo sapiens hydroxymethylbilane synthase	SEAP	Secreted alkaline phosphatase
HPV	Human papillomavirus	SHPC	Streptavidin-horseradish peroxidase conjugate
HR-HPV	High-risk human papillomavirus	SIL	Squamous intraepithelial lesions
HSIL	High-grade squamous intraepithelial lesion	STD	Sexually transmitted disease
HSV	Herpes simplex virus	STI	Sexually transmitted infection
IgA	Immunoglobulin A	Th	T-helper cell
IgG	Immunoglubulin G	TMB	3,3', 5,5'-tetramethylbenzide
IgM	Immunoglobulin M	URR	Upstream regulatory region
ICC	Invasive cervical cancer	VIN	Vulvar intraepithelial neoplasia
IL	Interleukin	VLP	Virus-like particles
LR-HPV	Low-risk human papillomavirus	WHO	World Health Organisation
LSIL	Low-grade squamous intraepithelial lesion	ºC	Degrees Celsius

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study constitutes the first report on type-specific human papillomavirus (HPV) concordance and transmission in heterosexually active couples that are human immunodeficiency (HIV)-seronegative, HIV-seropositive or HIV-discordant and in which 71% of female participants have normal cervical cytology.

Aims: The aims of the study were to investigate the aspects of human papillomavirus (HPV) infection in a cohort of South African heterosexual couples including HIV-negative, HIV-concordant and HIV-discordant couples:

i. The prevalence of HPV types according to age, HIV status and CD4 count
ii. The impact of HIV co-infection on HPV viral load, the natural history of HPV infection and the type-specific HPV concordance in couples.
iii. The impact of cervical disease on HPV viral load, the natural history of HPV infection and the type-specific HPV concordance in the couples.
iv. The distribution HPV antibodies to 9 HPV types and predictors of HPV sero-conversion over a 12 month period.
v. Factors associated with HPV acquisition, clearance and transmission.

Methodology: A total of 486 black heterosexually active couples were recruited from the Manyanani clinic, Empilisweni centre, Gugulethu, Cape Town and followed for 24-months. Cervical and penile HPV types were determined by Roche Reverse Linear Array HPV genotyping assay. HPV viral loads of 12 high-risk (HR) HPV types in cervical and penile cells were determined by *hpVIR* real-time polymerase chain reaction (Gustavsson *et al*., 2009). HPV antibodies (combined IgG, IgA and IgM) to the major capsid protein L1 of 9 HPV types were determined by Luminex-based multiplex serology (Waterboer *et al*., 2005 and 2006). HPV prevalence, acquisition, clearance and transmission in couples were analysed using STATA 11.0 (StataCorp, College Station, TX, USA).

Results: HPV infection in women and men was significantly influenced by HIV status, age and CD4 count. HIV-positive women had significantly more HPV compared to HIV-negative women (74% 205/277 and 37% 76/207 respectively, P<0.0001). HIV-positive men also had significantly more HPV compared to HIV-negative men (81% 121/149 and 49% 159/322 respectively, P<0.0001). Women were found to have more HPV antibodies to single and

multiple HPV types than men. In men, type-specific HPV concordance was associated with their own HIV-positive status (P=0.05) and their female partner's HIV status (P=0.005). In women type-specific HPV concordance was associated with their HIV-positive status and low CD4 count (P<0.0001) but not their male partner's HIV-positive status (P=0.08). Women or men with high HR-HPV viral load were more likely to have HPV concordance compared to women or men with low viral load. Among couples with a female partner with abnormal cervical cytology the type-specific HPV concordance was significantly higher compared to couples where the female partner had normal cervical cytology (64% 84/131 compared to 34% 108/314, P<0.0001).

Natural history studies showed the rate of clearing of any HPV was higher in men (95.12, 95% confident interval (CI): 83.3-108.1 per 1000 person-months) compared to women (66.95, 95% CI: 57.0-78.5 per 1000 person-months, P=0.001). Women with abnormal cervical cytology had lower clearance HPV rates compared to women with normal cervical cytology. The HPV antibody responses were not associated with HPV clearance in either women or men. The risk of acquiring any HPV type in men was higher compared to women (3.07, 95% CI: 2.65-3.54 compared to 1.82, 95% CI: 1.53-2.14 per 1000 person-months).

In both women and men, HIV-infection, having an HIV-positive partner and having a sexual partner infected with an HPV type similar to the one acquired were significantly associated with increased risk of acquiring new HPV types during follow-up. In women, a HIV viral load ≥10 000 copies per mL (relative risk (RR): 1.46, 95% CI: 0.99-2.16), living with the study partner (RR: 1.45, 95% CI: 1.02-2.07), more lifetime sex partners (P=0.015), more sexual acts with study partner in the last month prior to the study visit (P<0.001) and the presence of genital warts in the last 6-months before the study (RR: 3.07, 95% CI: 1.06-2.93), were also associated with an increased risk of acquiring new HPV types during follow-up but not in men.

Female to male HPV transmission was more common compared to male to female HPV transmission (28.0 compared to 11.7 rates per 1000 person-months). HIV-positive women were found to be at high risk of HPV transmission from their male partners compared to HIV-negative women (RR: 2.31 95% CI: 1.08-4.92, P=0.03). HIV-positive men with <350mL CD4 counts had high risk of HPV transmission from female partners compared to HIV-positive men ≥350/mL CD4 counts (RR: 3.17, 95% CI: 1.05-9.55, P=0.04). In male to female transmission events, LR-HPV transmission was more common while in female to male transmission events,

HR-HPV transmission rate was similar to LR-HPV transmission rate. HPV transmission to women or men was not associated with increased number of sexual act with study partner a month prior the visit. These findings suggest that the observed transmissions were not just HPV deposited by the partner from previous sexual act but true infection.

Conclusion: Data from this study indicate that HIV co-infection increases HPV prevalence, multiple infections and viral load in both women and men. HIV co-infection in couples or a female partner increased type-specific HPV concordance and transmission. In men, type-specific HPV concordance with their female partner is influenced by their own HIV status and that of their female partner while in women type-specific HPV concordance is influenced by their own HIV status but not that of their male partner. Couples with type-specific HPV concordance were found to have higher HPV viral load compared to couples with no type-specific HPV concordance, suggesting that high HPV viral load may play a role in HPV transmission between partners. Female to male HPV transmission was more common compared to male to female HPV transmission. HPV transmission was not influenced by increased number of sexual act a month before study visit, indicating the observed HPV transmissions are true infections not just HPV deposited by the partner during previous sexual act. Men demonstrated a higher HPV acquisition and clearance during follow-up compared to women. The data from this study adds to very limited data on the natural history of HPV in men and sexually active couples and assist to inform the government on HPV vaccine policy.

This study resulted to the following published articles

1. **Zizipho Z. A Mbulawa,** Dianne J. Marais, Leigh F Johnson, David Coetzee, Anna-Lise Williamson. The impact of human immunodeficiency virus on the natural history of human papillomavirus genital infection in South African men and women. Journal of Infectious Disease in Press.

2. **Zizipho Z. A Mbulawa,** Dianne J. Marais, Leigh F Johnson, Andrew Boulle, David Coetzee, Anna-Lise Williamson. Influence of human immunodeficiency virus and CD4 count on the prevalence of human papillomavirus prevalence in heterosexual couples. Journal of General Virology 91(Pt12): 3023-31. 2010

3. **Zizipho Z. A. Mbulawa,** David Coetzee, Dianne J. Marais, Mercy Kamupira, Eugene Zwane, Bruce Allan, Deborah Constant, Jennifer R. Moodley, Margaret Hoffman, Anna-Lise Williamson. Genital human papillomavirus (HPV) prevalence and HPV concordance between heterosexual couples is positively associated with human immunodeficiency virus co-infection. Journal of Infectious Disease 199: 1514-1524, 2009.

CHAPTER 1: LITERATURE REVIEW

1. INTRODUCTION

1.1 The burden of cervical cancer and HPV-related cancers world-wide

Cervical cancer is the second most common cancer in women worldwide (Lowy and Schiller, 1998) and the most common cancer in South African women (Mqoqi *et al*., 2004). A total of 530,000 cases of cervical cancer and 275,000 deaths caused by cervical cancer were reported in 2008 (Ferlay *et al*., 2010). It has been reported that 85% of these cases and subsequent deaths occur in developing countries (Ferlay *et al*., 2010). Eastern, Western and Southern Africa are the high risk regions. South Africa have a 26.6 per 100,000 age standardised incidence rate and 14.5 per 100, 000 age standardised mortality rate for cervical cancer (Ferlay *et al*., 2010). The incidence of cervical cancer is higher than mortality due to cervical cancer. Survival rates differ between countries and are higher in developed countries than in developing countries where cervical cancer cases are often presented at health care services in an advanced stage (Parkin & Bray, 2006).

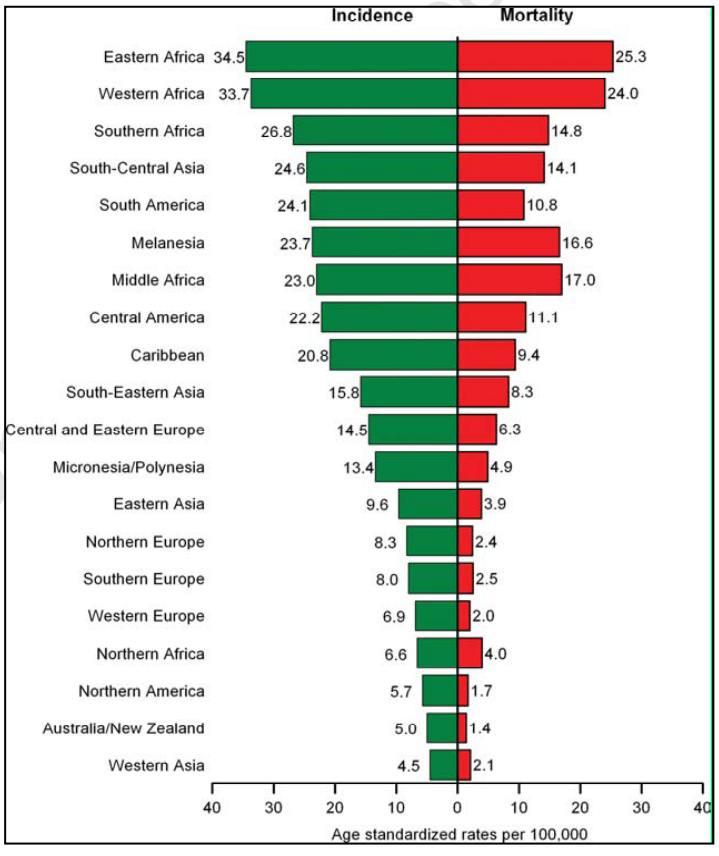

Figure 1.1. Worldwide age standardized incidence and mortality rates of cervical cancer (Ferlay *et al*., 2010)

Figure 1.2 demontrate the cervical incidence and mortality rate in countries in Africa. Guinea has the highest incidence and mortality rate of cervical cancer while Egypt has the lowest rate. South African has 27 per 100,000 women-years incidence rate and 14 per 100,000 women-years mortality rate of cervical cancer in 2008.

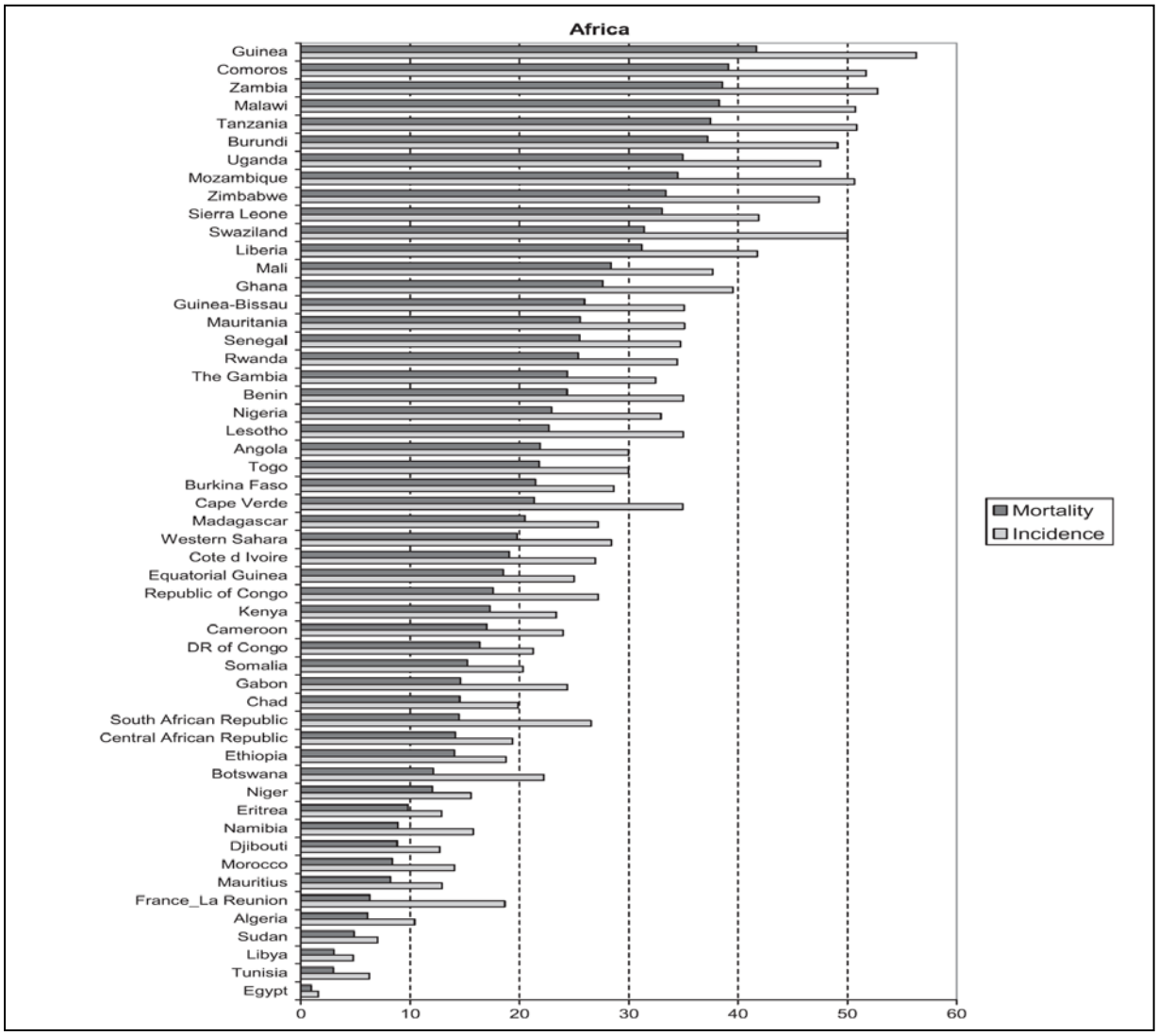

Figure 1.2. Age standardized incidence and mortality rates of cervical cancer (per 100,000 women-years) in African countries (Arbyn *et al*., 2011).

Human papillomavirus (HPV) persistent infection is essential for the development of precancerous lesions and cervical cancer (Walboomers *et al*., 1999; Parkin *et al*., 1999; Munoz *et al*., 2006; Castellsague *et al*., 2006; Parkin & Bray, 2006). HPV DNA is found in almost all cervical cancers at 99.7% (Walboomers *et al*., 1999; Bohmer *et al*., 2003). HPV is also responsible for 40-50% cases of penile cancer worldwide (WHO, 2006; Parkin and Bray, 2006). A total of 26,000 penile cancer cases are reported annually (Parkin & Bray, 2006). Whereas the peak prevalence of penile cancer occurs in the elderly around the age of 60 years,

it also occurs occasionally in young men (Bleeker *et al*., 2008). High-risk (HR) HPV DNA is detected in 30%-100% of penile carcinoma cases (Bleeker *et al.,* 2008). A history of genital warts is reported to be strongly associated with penile cancer, indicating that genital warts are a risk marker for penile cancer (Daling *et al.,* 2005). Low-risk (LR) HPV, HPV-6 and -11 are associated with 95% of genital warts (Bleeker *et al.,* 2008). Table 1.1 demonstrates cancer cases attributable to HPV in both developed and developing countries. According to Parkin & Bray (2006), HPV is associated with 100% cancer cases of the cervix, 40% of the penis, 40% of vulva and vagina, 90% of anus, 3% of mouth and 12% of oro-pharynx.

Table 1.1. HPV infection-attributable cancer in developing and developed countries (Parkin & Bray, 2006)

Site	Attributable to HPV (%)	Developed countries			Developing countries		
		Total cancers	Attributable to HPV	% all cancer	Total cancers	Attributable to HPV	% all cancer
Cervix	100	83,400	83,400	1.7	409,400	409,400	7.0
Penis	40	5200	2100	0.0	21,100	8400	0.1
Vulva, vagina	40	18,300	7300	0.1	21,700	8700	0.1
Anus	90	14,500	13,100	0.3	15,900	14,300	0.2
Mouth	3	91,200	2700	0.1	183,100	5500	0.1
Oro-pharynx	12	24,400	2900	0.1	27,700	3300	0.1
All sites		5,016,100	111,500	2.2	5,827,500	449,600	7.7

Men with penile high grade lesions are reported to have a higher prevalence of penile HR-HPV infection and also a higher HPV viral load compared to men with no penile lesions (Bleeker *et al.,* 2002; Bleeker *et al.,* 2005b). This shows the positive association between HR-HPV and penile lesions in men. Men with female sexual partners with cervical or vulval HPV associated cancers are at increased risk of becoming HPV infected and later to develop penile lesions (Bleeker *et al.*, 2002; Bleeker *et al.,* 2005b). According to Bleeker *et al.,* (2005b) the rate of flat penile lesion regression is low in males with female sexual partners that are HPV positive with the same HPV type compared to those men whose partners are HPV negative, suggesting that the HPV re-infection between partners plays an important role in this scenario.

1.2 HPV structure and genome organisation

HPVs are small non-enveloped DNA viruses of approximately 55nm in diameter classified within the *Papillomaviridae* family (Bernard *et al*., 2010). Structurally, their genetic material is enclosed within a capsid comprising 72 capsomeres each containing 5 L1 proteins. Figure 1.3 shows a three-dimensional model of HPV.

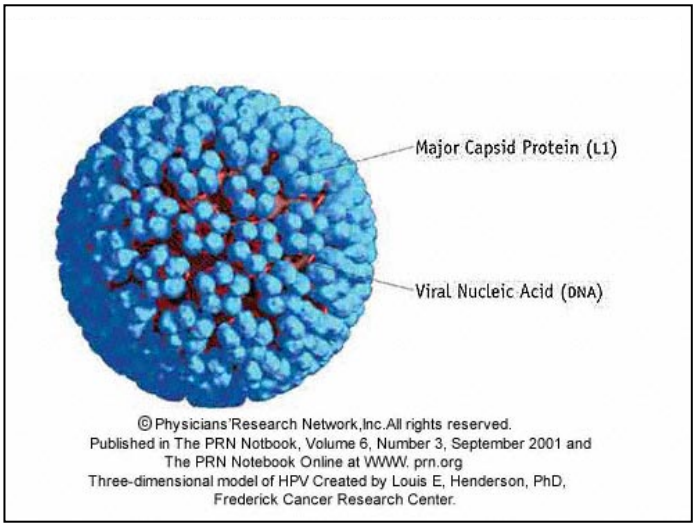

Figure 1.3. A 3-D (dimensional) model of human papillomavirus L1.

Genomically, HPVs have a double stranded circular DNA genome. Their genome is approximately 8 kilobase pairs in size with eight open reading frames (ORFs, Doorbar, 2007). These ORFs are further divided into three regions; the early, late and noncoding or upstream regulatory region (URR). The URR is also known as long control region (LCR); it encodes no proteins but contains the origin of replication and promoter (Settheetham-Ishida *et al*., 2005).

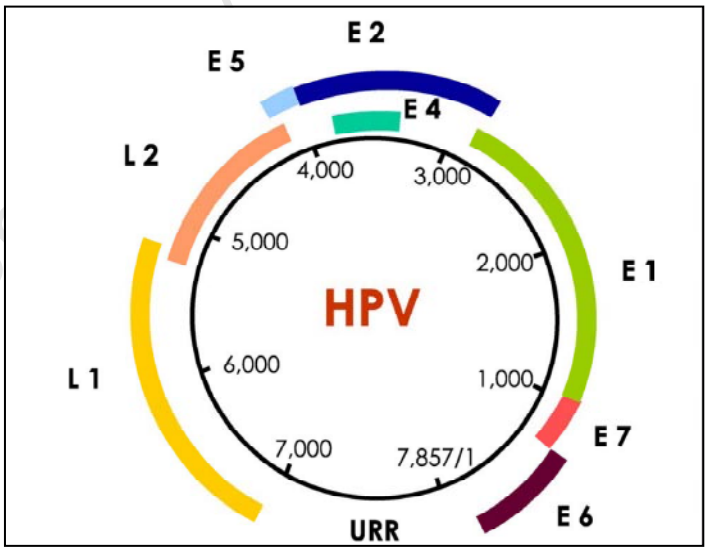

Figure 1.4. Human papillomavirus (HPV) genome, presenting late region, early region genes and upstream regulatory region (URR; Munoz *et al*., 2006).

The URR is reported to play a role in determining the host range and tissue tropism of each HPV type. It is also important in regulating viral gene expression after HPV infection (Doorbar *et al*., 2007; Nishimura *et al*., 2007). The early region encodes six ORFs that express non-

structural proteins namely, E1, E2, E4, E5, E6 and E7. The early proteins control the DNA replication and the assembly of virus particles. The late region encodes two ORFs expressing structural proteins, namely, L1 a self assembling major protein, and the minor protein L2. (Figure 1.4). The E1 protein is highly conserved when compared with other proteins and is responsible for HPV replication. E2 proteins also play a role in HPV replication and in HPV transcription as well as in segregation of the HPV genome after cell division. It is interesting to note that although E4 is encoded in the early region it is not expressed during the early phase of the HPV life cycle, however the exact function of E4 is not yet clear but it is reported to play a role in the collapse of the cellular cytokine rating network and this process can facilitate viral release (Doorbar, 2007; Hamid *et al*., 2009).

The E5 protein play a role in transformation and sometimes it can be referred to as an oncogene because it is reported to have the ability to transform mouse fibroblasts and keratinocytes (Hamid *et al*., 2009). Both E6 and E7 are oncogenic; they play a role in viral replication and in tumourigenesis and they both have transforming and immortalization properties (Hamid *et al*., 2009). E6 and E7 act by inactivating the functions of two tumor suppressor proteins. The oncogenic function of E6 and E7 from high-risk (HR) and low-risk (LR) HPV differ (Hamid *et al*., 2009).

E7 protein binds and degrades retinoblastoma (Rb) tumor suppressor protein while E6 protein binds and degrades p53 tumor suppressor protein (Seavey *et al*., 2006). Nakagawa *et al*., (1999) reported that HPV positive women showed p53 gene mutations which may disrupt important functions of p53 including regulation of cell division and DNA repair. Late proteins (L1 and L2 proteins) are structural proteins they are responsible for the formation of the viral capsid (Hamid *et al*., 2009; Table 1.2). Host cell proliferation is directly affected by E6 and E7 oncogenes and then E2 regulates E6 and E7 transcription. The disruption of the E2 gene results in loss of expression of E2 protein. The loss of E2 expression leads to the termination of proper HPV virus life cycle and deregulation of E6 and E7 protein. The loss of E2 expression is very important in tumourigenesis (Hamid *et al*., 2009).

Table 1.2. The HPV proteins and functions (Hamid *et al*., 2009)

Protein	Function
E1	Replication
E2	Transcription, replication
E4	Disruption of cytokeratin networks/cell growth arrest
E5	Transformation
E6	Transformation (binds to p53 amongst other proteins)
E7	Transformation (to pRb amongst other proteins)
L1	Major capsid protein
L2	Minor capsid protein

1.3 HPV classification

The papillomaviruses are a diverse family of viruses and have been detected in more than 20 different mammalian species, birds and reptiles (Doorbar, 2007; Bravo *et al*., 2010). The papillomaviruses belong to the *Papillomaviridae* family with 29 different papillomavirus (PV) genera identified; these include Alpha, Beta, Gamma, Nu, Mu etc. (Figure 1.5). Within the genera there are HPV species; an HPV species refers to the HPV types that belong to the same evolutionary branch. For example the Alpha genus contains HPV species, namely α1, α2 up to α14 HPV species (Schiffman *et al*., 2009a; Bernard *et al*., 2010). More than 200 genomically different HPV types have been identified (Jung *et al*., 2004). HPV types are defined as having more than a 10% L1 gene sequence difference, HPV subtypes have 2% to 10% homology from that of the original HPV type and then a HPV variant must have less than 2% homology (de Villiers *et al*., 2004).

HPV types are divided into two groups, cutaneous and mucosal HPV types (Gross & Pfister, 2004; Jung *et al*., 2004). Cutaneous HPV are those that infect squamous epithelium and cause skin warts; while, mucosal HPV infect mucous membranes of the genital tract, oral cavities, conjuctiva and respiratory tract (Gross & Pfister, 2004). There are forty genital-mucosal HPVs that have been identified and grouped as HR, probably HR or LR-HPV types according to their link with cervical cancer. HR-HPV types are associated with the development of precancerous lesions and cervical cancer; while LR-HPV types are associated with the development of genital warts and/ or low grade cervical neoplasia (Munoz *et al*., 2003; Munoz *et al*., 2006). Probable HR-HPV types are possibly carcinogenic however they are rarely detected in cervical cancers (Schiffman *et al*., 2009a). HPV types that belong to α7 and α9 HPV species are known

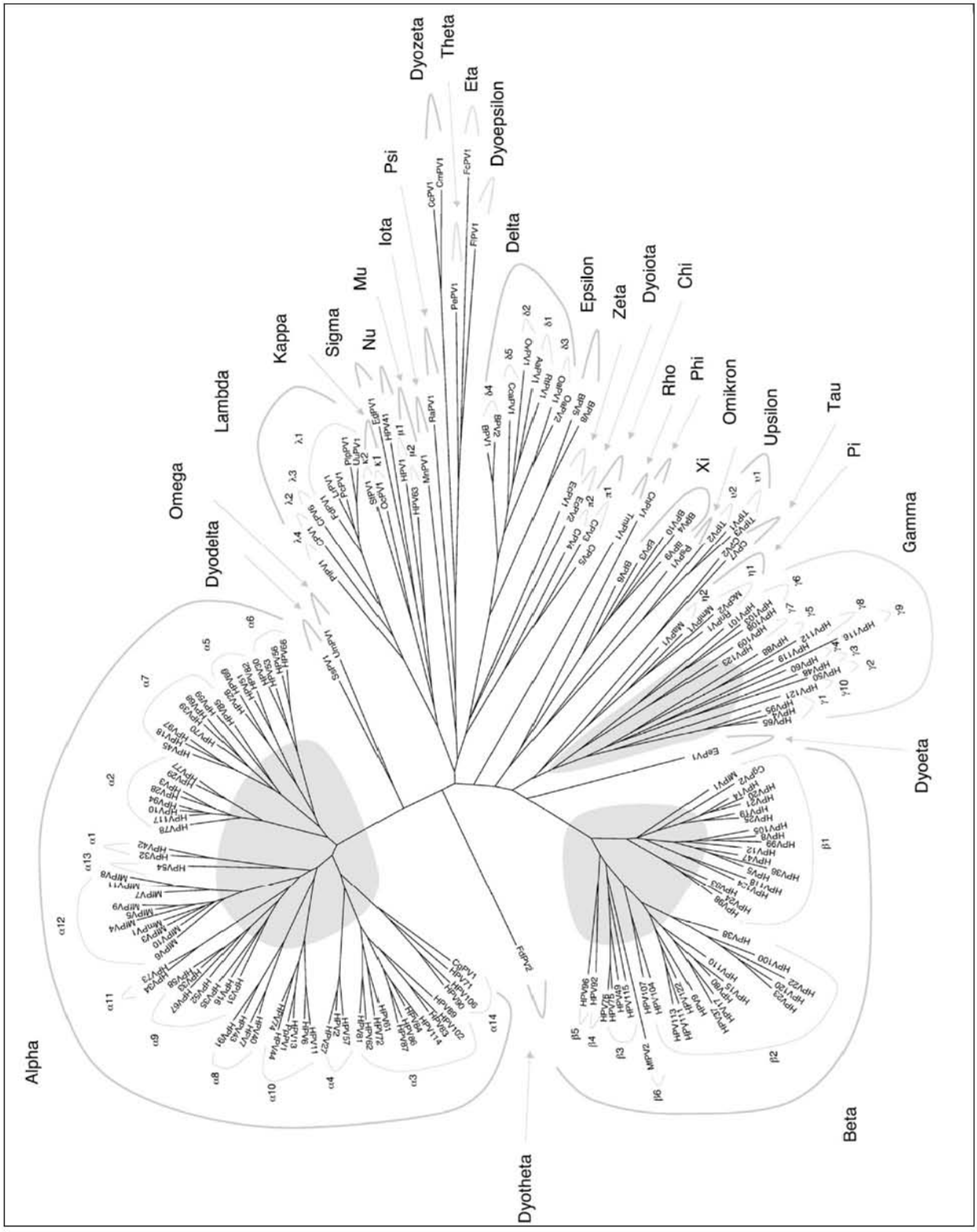

Figure 1.5. The phylogenetic tree of papillomavirus types based on L1 ORF sequence (Bernard *et al*., 2010).

to be the most common HR-HPV types (de Villiers *et al*., 2004; Schiffman *et al*., 2009a; Bernard *et al*., 2010, Figure 1.5). Fifteen HPV types have been classified as HR-HPV types, 3 have been classified as probable HR-HPV types, 12 have been classified as LR-HPV types and 3 have undetermined risk (Munoz *et al*., 2006; Table 1.3).

Table 1.3. Classification of Human papillomavirus (HPV) types by cervical oncogenicity (Adapted from Baseman & Koutsky, 2005)

Risk of classification	**HPV types**
High-risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Probable high-risk	26, 53, 66
Low-risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 83, 89

1.4 HPV life cycle

HPVs infect cutaneous and mucosal skin and their life cycle is linked to the stages of differentiation of the host epithelial cell. HPV enters the cells through microabrasions of epithelium. During HPV infection, HPV virions adsorb to cells via heparin sulphate on the heparin sulphate proteoglycan basement membrane receptors (Shafti-Karamat *et al*., 2003; Johnson *et al*., 2009; Sun *et al*., 2010). Successful HPV infection depends on the virus gaining access to the mitotically active cells (Culp *et al*., 2006). It is reported that HPV infection occurs in basal keratinocytes and these cells are reported not to be easily accessed by immune cells. During the early phase of the HPV life cycle HPV keeps a low profile. In this way the virus avoids the activation of the innate immune system (Stanley, 2006). As the cells differentiate, the expression of early proteins such as E6, E7, E1, E2, and E5 is initiated at a very low level and as the cell further differentiates the expression of viral genes is also up-regulated (Zheng & Baker, 2006, Figure 1.6).

It is estimated that approximately 3 weeks are required to release matured HPV virus after the time of infection (Grimes, 2006). There is no need for HPV to destroy the cells it infects because the keratinocytes have a short life span of around 3 weeks so at the time HPV matures; the keratinocytes are destined to die naturally as they are sloughed off into the lumen of the genital tract. This mechanism assists the virus to evade immune system. No inflammation is induced during cell death and the innate immune system is neither alerted nor adaptive immunity triggered to fight HPV infection. Due to the various degrees of oncogenicity of the

different HPV types, the time from HPV infection to the development of cytological lesion is found to vary from weeks to months in different studies (Oriel, 1971; Winer *et al*., 2005; Doorbar, 2006).

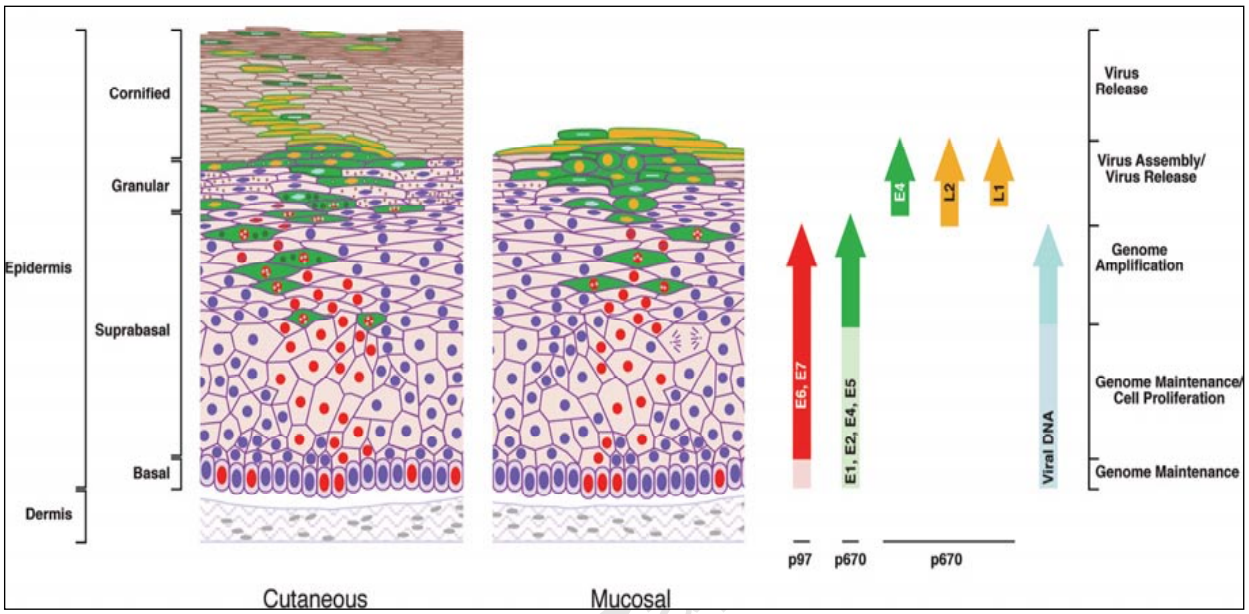

Figure 1.6. The human papillomavirus life cycle. (Doorbar, 2006).

1.5 Risk of HPV infection

Numerous studies have revealed that HPV is sexually transmitted and is the most common sexually transmitted virus (Kjaer *et al*., 2001; Schiffman & Castle, 2005; Rodriguez *et al*., 2007; Winer *et al*., 2008; Winer *et al*., 2010). HPV can also be transmitted by nonsexual routes, such as environmental fomite and vertical transmission but the sexual route is the most common type of transmission (Schiffman & Castle, 2005; Winer *et al*., 2008; Winer *et al*., 2010). Microscopic tears that commonly occur during sexual intercourse allow HPV access to the basal cells of the epithelium and to be transmitted successfully. Fifty percent of sexually active women and men will become HPV infected in their lifetime and the highest HPV incidence is in young individuals (Gross & Pfister, 2004; Cuschieri *et al*., 2004; Dunne *et al*., 2006; Chin-Hong *et al*., 2009). HPV infection is very rare in virginal women and genital HPV DNA appears as they initiate sexual intercourse and increases with an increasing number of sexual partners (Kjaer *et al*., 2001; Rodriguez *et al*., 2007; Winer *et al*., 2008).

Winer *et al*., (2008) conducted a study in which the participants were virgins at the time of enrolment or they had had their first intercourse with one male partner within the last 3 month period. The incidence of HPV infection in these women within a 1 year period was 28.5% after intercourse with one male partner, their first sexual partner, and HPV incident increased to 39.2% after 2 years and to 49.1% after 3 years. It was noted that in women with male partners who were sexually experienced with ≥2 female partners, the risk of HPV incidence was increased compared to those women with male partners that were not sexually experienced (Winer *et al.*, 2008). According to Trottier *et al*., (2010) individuals' sexual behaviour is associated with HPV infection and re-infection. Reeves *et al*., (1994) reported that HPV prevalence is high in individuals who first participated in sexual activity recently compared to those who are sexually experienced for years. Those who have been sexually active may have encountered the virus in the past and therefore immune memory plays a role in the clearance of the virus (Reeves *et al*., 1994). However, the role played by natural immunity in HPV natural history is not clear. Recently Trottier *et al*., (2010) reported that HPV re-infection is not controlled by natural immunity.

Infection with HR-HPV types is associated with a higher number of sexual partners and abnormal cervical cytology (Levi *et al*., 2002). A high HPV viral load may enhance HPV transmission between partners. Females with male sexual partners with HPV infection are more likely to acquire HPV infection and develop cervical disease (Heard *et al*., 2000). Men with four or more female sexual partners are more likely to have HPV infection, confirming that multiple sexual partners increase the risk of acquiring HPV infection (Kjaer *et al*., 2001; Svare *et al*., 2002; Rodriguez *et al*., 2007).

The number of lifetime and the number recent sexual partners are risk factors for HPV infection in women (Burk *et al*., 1996; Tarkowski *et al*., 2004) and in men (Hippelainen *et al*., 1993; Franceschi *et al*., 2002). Strong association between lifetime sexual partners and genital HPV acquisition has been reported in several studies (Winer *et al*., 2008). Sexual activity prior to the age of 16, sexual behaviour, sexual history of a partner as well as number of partners increases the risk of developing cervical cancer (Lehtinen *et al*., 1996; Silins *et al.,* 2002; Richardson *et al*., 2005; Winer *et al*., 2008). Kyo *et al*., (1994) reported that women with genital HPV DNA infected male partners are more likely to have cervical HPV DNA, indicating that the HPV status of a male partner influences HPV prevalence in women.

According to Richardson *et al*., (2005) the use of condoms during sexual intercourse increased the probability of clearing LR-HPV infection.

1.6 Natural history of HPV infection

After HPV infection the immune system and other mechanisms may clear the infection. If the immune response fails the infection will persist which increases the possibility of a cervical lesion progression to cancer (Koshiol *et al*., 2008). Different HPV types have different abilities to persist in genital epithelium (Tindle, 2002; Molano *et al*., 2003). Most women will still clear their precancerous lesion but if the lesions are not cleared or are left untreated they progress to cervical cancer after years or decades (Figure 1.7 and 1.8). There are other co-factors that enhance or speed up the steps to the development of cervical cancer such as smoking; parity; the presence of sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV), herpes simplex virus (HSV) and Chlamydia; immunosupression and infection with other micro-organisms (Schiffman *et al*., 2007; Schiffman & Castle, 2005). These cofactors will be further explored in next section.

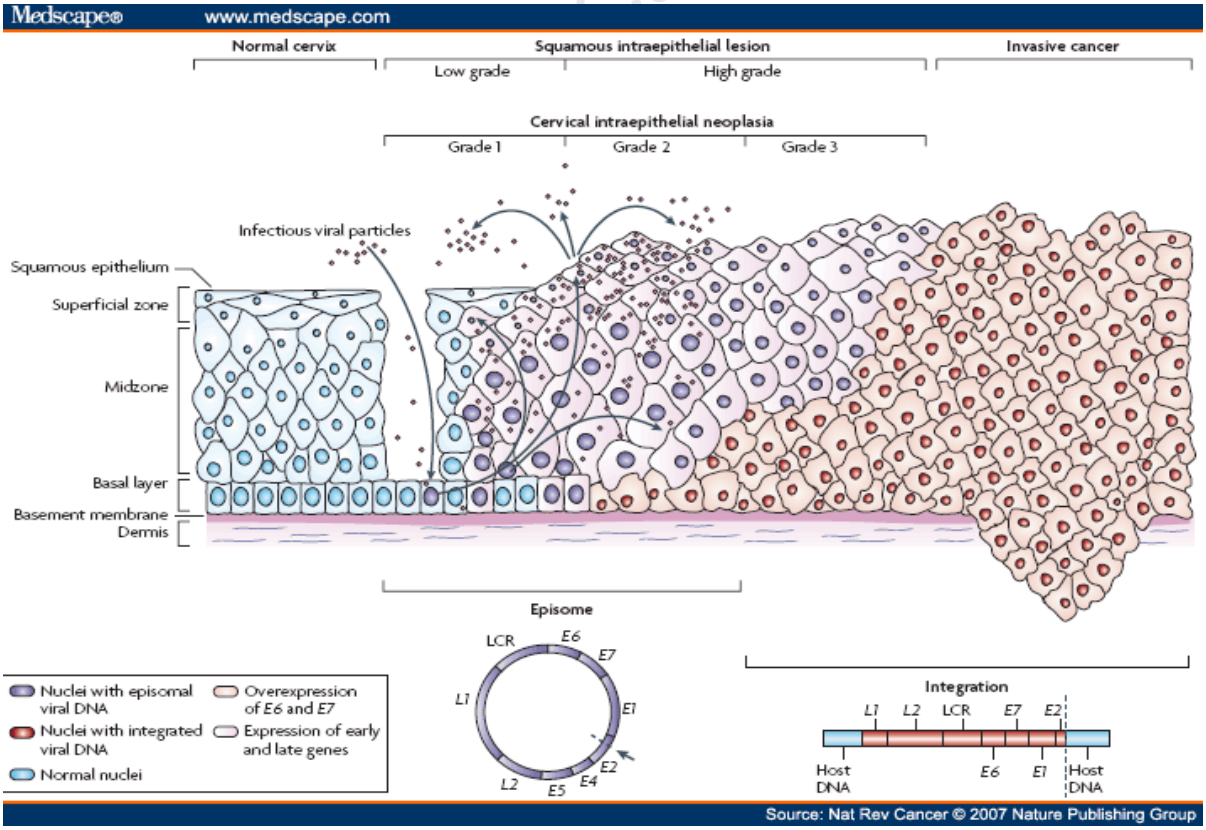

Figure 1.7. Development of cervical precancerous lesions and cancer after HPV infection (Woodman *et al*., 2007).

Peak prevalence of HPV is observed in young individuals soon after sexual debut and declines with increasing age even in highly exposed women such as sex workers. In some populations a second peak of HPV prevalence after menopause is been observed in all continents except in Asia (Castle *et al*., 2005; de Sanjosé *et al*., 2007). The observed second peak could be due to decreased immune and response hormonal changes at menopause resulting to reactivation of latent HPV infections (Molano *et al*., 2002; de Sanjosé *et al*., 2007). The change of sexual behaviour in women after menopause and that of their male partner could be the result of observed second peak (Molano *et al*., 2002; Sanjosé *et al*., 2007). The stages leading to development of cervical neoplasia and cancer are shown in Figure 1.7 and 1.8.

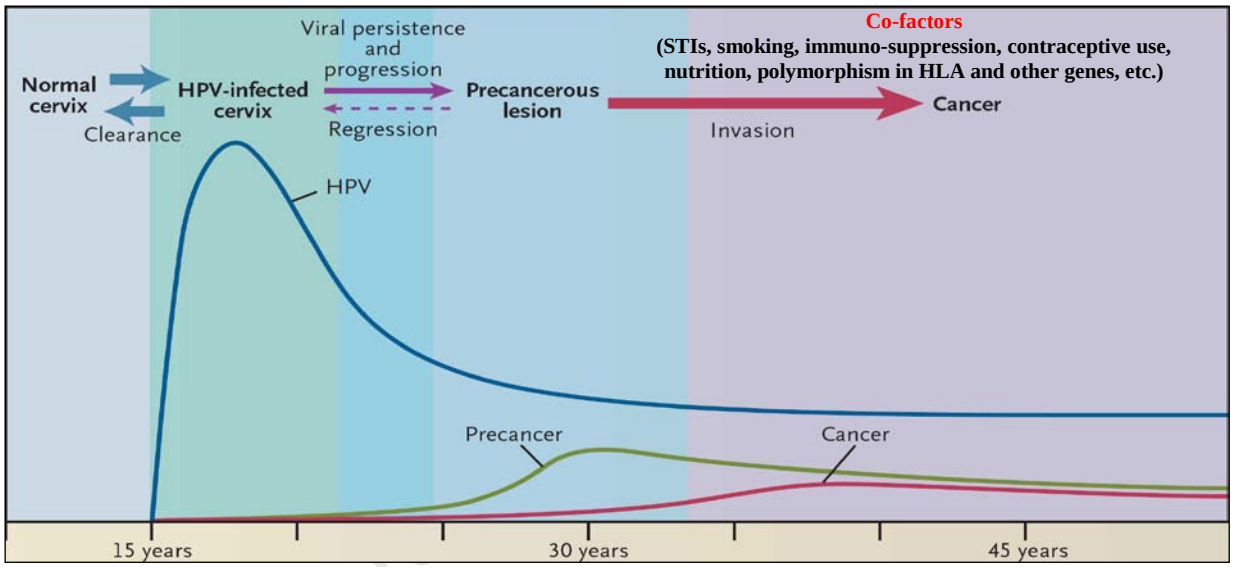

Figure 1.8. Stages leading to development of cervical cancer after HPV infection and cofactors enhancing its development. The peaks of the curves are not drawn to scale, HLA: human leukocyte antigen (Schiffman & Castle, 2005).

A large number of women are infected with HPV each year but only a few of them will develop precancerous lesions (Figure 1.9). Bosch, (2009) estimated that annually 300 million women are infected with genital HPV, with 30 million developing genital warts and 30 million developing low-grade cervical lesion over a number of years. It should be noted that women who develop low-grade cervical lesion, genital wart can also be detected as well. Of these, 10 million will develop high-grade cervical lesions over years to decades and then 0.5 million will develop cervical/genital cancer (Bosch, 2009). Ostor, (1993) reviewed all the scientific reports from 1950 to early 90s. Out of all women reported in these studies, 60% of low grade squamous intraepithelial lesion (LSIL) cases regressed, 30% were persistent, 10% were found

to progress to high-grade squamous intraepithelial lesion (HSIL) and 1% progressed to invasive cervical cancer (Ostor, 1993).

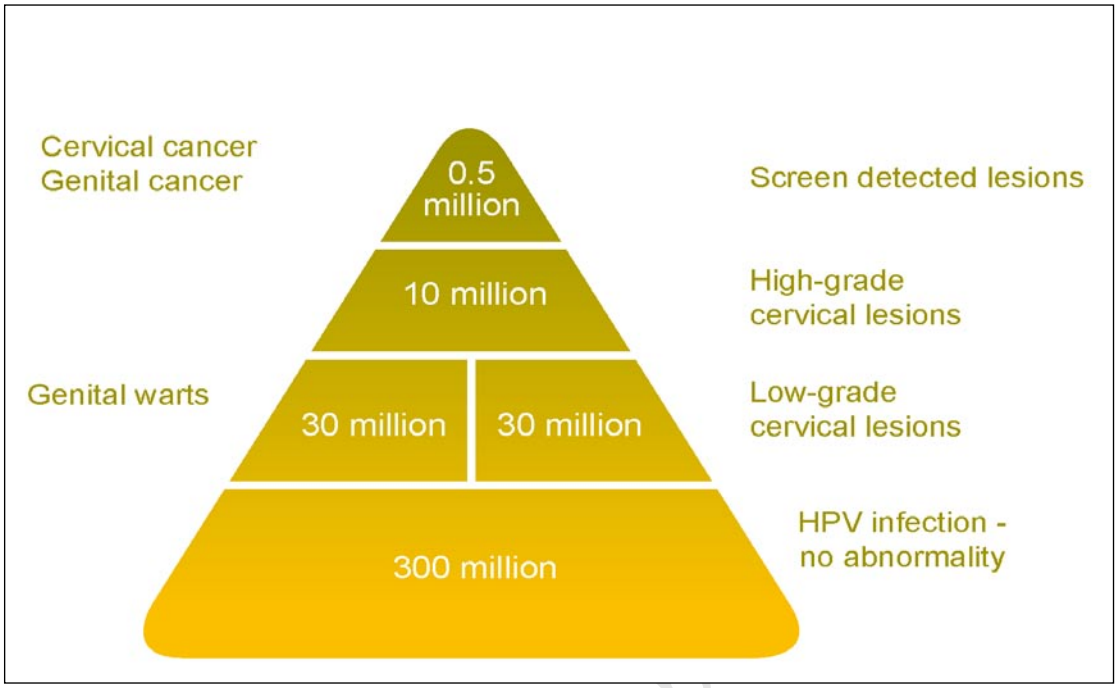

Figure 1.9. Worldwide estimates of the burden of human papillomavirus (HPV) and related genital disease (Bosch, 2008).

Although HPV infection has been studied extensively in women, data on male HPV infections and on transmission between couples are limited. The dearth of information on male HPV infection was related to the difficulty in sampling for genital HPV in men. However, recently more comprehensive sampling methods have enabled the more accurate determination of the presence of genital HPV in men (Weaver *et al*., 2004; Nicolau *et al*., 2005). A review by Dunne *et al.*, (2006) which included 40 publications, reported a genital HPV DNA prevalence in men from 1.3 to 72.9%. The sampling method, site sampled and processing method affected the prevalence of HPV infection in the men (Gross & Pfister, 2004; Dunne *et al.*, 2006). Men are HPV carriers and vectors. Although anogenital HPV infection in men is largely asymptomatic, men are believed to be responsible for the sustained transmission of HPV to their female partners and thus the perpetuation of HPV in the population (Castellsague *et al*., 2003).

1.7 HPV distribution in cervical cancer

Figure 1.8 demonstrates 15 high-risk (HR) HPV types that are associated with 94.5% cases of cervical cancer worldwide. There are eight most common HPV types, namely HPV-16, -18, -

31, -33, -35, -45, -52 and -58, frequently detected in cervical cancer in Africa, and HPV 16 and -18 are detected in approximately 70% of the cases (Clifford *et al*., 2006). In South Africa HPV-16 is detected in more than 50% cases of cervical cancers (Kay *et al*., 2003). HPV-16 is the most dominant HR-HPV type, its prevalence in cervical cancer cases varies between 52% and 58% in different countries. HPV-16 prevalence in HSIL ranges from 34% to 52%, in LSIL ranges between 16% and 29% (Clifford *et al.,* 2006). HR-HPV DNA is detected in 99.7% of cervical cancers, in 50% of women with ASCUS and in 80% of women with LSIL (Walboomers *et al.*, 1999; Solomon *et al*., 2001; Depuydt *et al*., 2003; Figure 1.10).

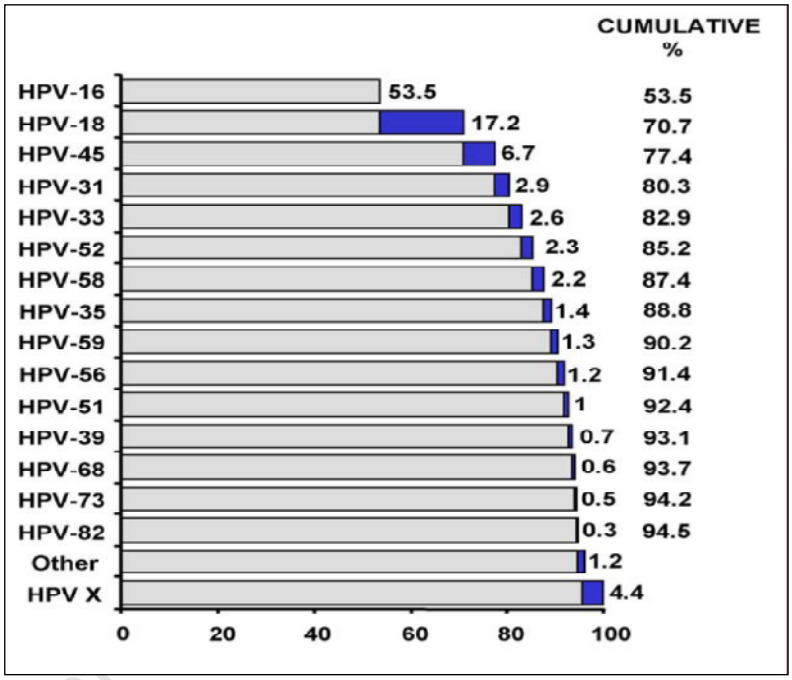

Figure 1.10. HPV types associated with cervical cancer from the IARC pooled-analysis of 3,085 cases Munoz *et al.* 2004. Adapted from Clifford *et al.*, 2006.

Miralles-Guri *et al*., (2009) conducted a literature review on penile cancer reports that were published between 1986 and 2008 evaluating HPV prevalence. Global HPV prevalence for penile cancer was found to be 46.9%. HPV-16 was the most dominant HPV types in penile carcinoma (28.3%) followed by HPV-18 (6.3%) and HPV-6/11 (3.8%, Figure 1.11).

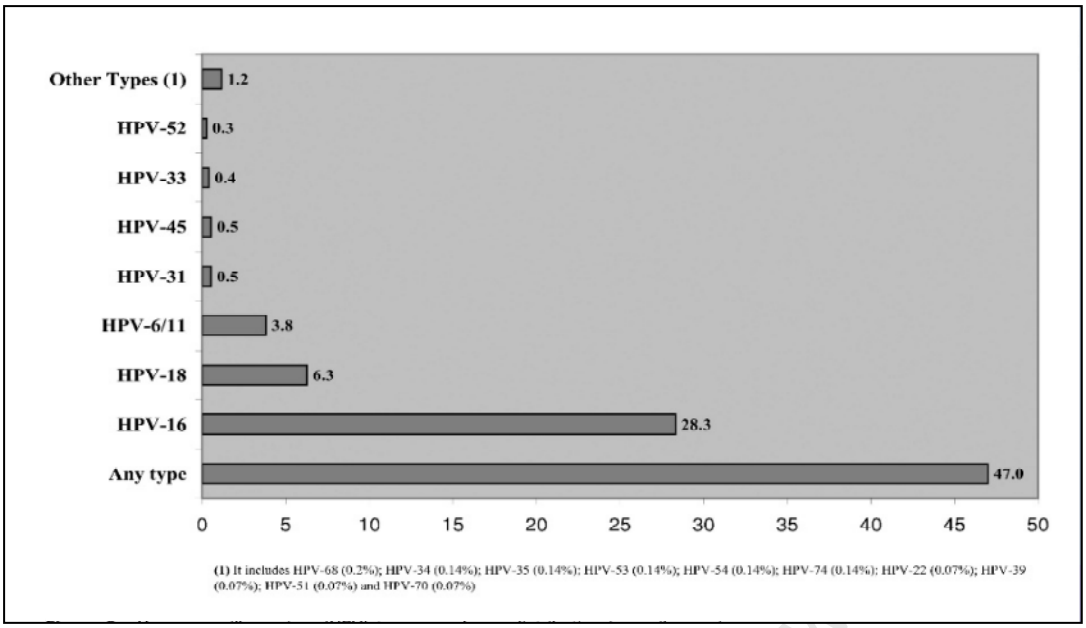

Figure 1.11. The distribution of HPV types in penile carcinomas (Miralles-Guri *et al*., 2009)

1.8 Risk factors associated with development of cervical and penile disease

Although cervical or penile HPV infection and persistence leads to the development of cervical or penile lesions, there are other cofactors that may enhance the development of these lesions (Castellsague *et al*., 2006). Figure 1.12 demonstrates overview of factors that are associated with cervical cancer. Different factors play role at different stages in the natural history of HPV and development of cervical cancer even though for some of the factors it is not clear where exactly they play role in the carcinogenesis process (Moscicki *et al*., 2006).

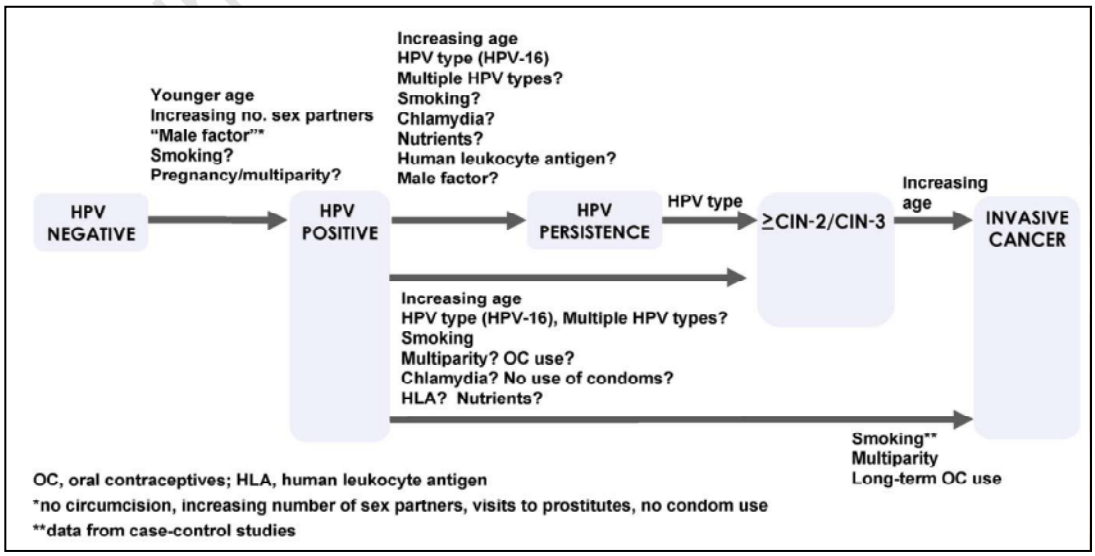

Figure 1.12. Factors associated with HPV natural history (Moscicki *et al*., 2006).

1.8.1 Smoking

Smoking is one of the risk factors in the development of cervical cancer. Recent smoking and past smoking are associated with high risk of HPV infection compared to non smokers (Rohan *et al*., 1991; Xi *et al*., 2009). Smoking is a risk factor for the development of SIL and invasive cancer in women and penile cancer in men (Maden *et al*., 1993; Daling *et al*., 2005). Women infected with HR-HPV types who smoke or who previously smoked are more likely to be diagnosed with HSIL or invasive cancer than women with a non-smoking history (Intyre-Seltman *et al*., 2005). Strong association between smoking and penile cancer and its precursor lesions has also been reported (Maden *et al*., 1993; Daling *et al*., 2005). In women that are smoking, benzo[a]pyrene, one of major carcinogens in tobacco smoke is detected in mucus of the cervix, indicating that cervical cells of smoking women are exposed to the benzo[a]pyrene carcinogen (Melikian *et al*., 1999). Using raft cultures Alam *et al*., (2008) demonstrated that in the presence of benzo[a]pyrene a 10-fold increase of HPV-31 was observed. As a result Alam *et al*., (2008) suggested that benzo[a]pyrene in smoking women may increase HPV replication, leading to high HPV viral load and increased likelihood of developing cervical cancer. However, according to Xi *et al*., (2009) HPV viral load is not positively associated with smoking intensity and duration.

It has been reported that smoking affects the circulation of carotenoids, vitamin C and folate (Alberg *et al*., 2000; Alberg, 2002). Decreased levels of folate, carotenoids and vitamin C have been reported to lead to increased blood homocysteine which has been associated with increased risk of neoplasia (Ziegler *et al*., 2002). Free radicals and oxidants are found at a high concentration in tobacco smoke. Free radicals and oxidants are reported to damage DNA, proteins and lipids (Eiserich *et al*., 1995). That is probably why smoking is associated with increased risk of developing cervical cancer (Oh *et al*., 2008).

1.8.2. HPV type, persistent infection and viral load

Persistent infection with HR-HPV types (for example HPV 16, -18, -35 and -45) increase the likelihood of HSIL development or indeed cancer (Khan *et al*., 2005; Castellsague *et al*., 2006). Trimble *et al*., (2005) observed a 36.4% regression of LSIL in patients infected with any HPV types (except HPV 16), a 29.2% regression in patients infected with HPV-16 and other HPV types and 20.5% regression in patients infected with HPV-16 alone (Trimble *et al*., 2005). Cumulative incidence of CIN2/3 after 3 years of HPV-16 or -18 incident infection

among women was significantly higher (27%) compared to women with any HPV incident infection other than HPV-16 or -18 (Winer *et al*., 2005; Figure 1.13).

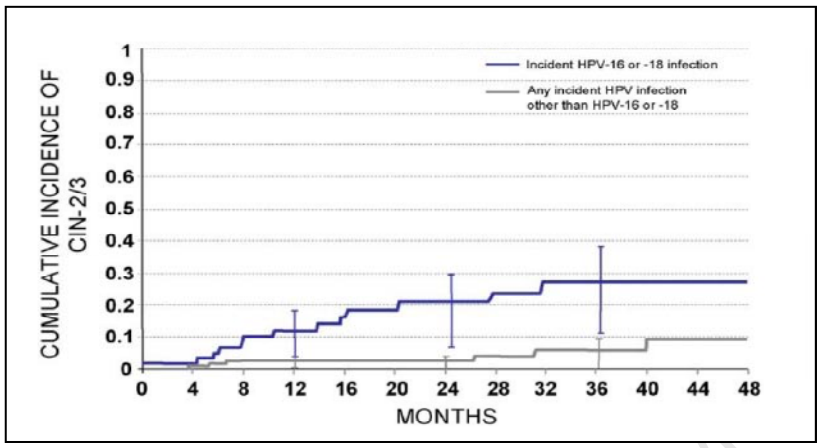

Figure 1.13. The cumulative incidence of CIN2/3 months after incident infection of HPV-16 or -18 or any HPV type other than HPV-16 or -18 (Moscicki *et al*., 2006 from Winer *et al*., 2005).

According to Nobbenhuis *et al*., (1999) the progression to and maintenance of SIL requires persistent HR-HPV infection. The same research team demonstrated that in the absence of HR-HPV, HPV infection was cleared after 6 months and no progression to SIL was observed. This indicates that infection with HR-HPV types play an important role in progression to cervical cancer. Furthermore, patients infected with HR-HPV types are less likely to have spontaneous regression than women infected with LR-HPV types (Trimble *et al*., 2005). Infection with any other HPV types concurrently is associated with longer duration of persistent HPV infection and also increased risk of disease progression (Woodman *et al*., 2001; van der Graaf *et al*., 2002).

The detection of the HPV viral load in cervical and penile cells may provide information on whether the HPV infected men or women are at risk of developing penile or cervical cancer (Moberg *et al*., 2005). High HPV viral load in cervical and penile specimens indicates a high copy number of HPV and is one of the risk factors for the development of pre-cancerous lesions and cancer in both women and men (Moberg *et al*., 2005; Daling *et al*., 2005; Bleeker *et al*., 2008). High viral load cannot be detected in women with latent HPV infection (Trottier *et al*., 2010). The presence of high HPV viral load in cervical cells of women with normal cervical cytology together with persistent HPV infection may indicate that the women are at increased risk of developing SIL and cervical cancer (Dalstein *et al*., 2003; Ho *et al*., 1998). It

is hypothesised that women or men with high viral load might be at increased risk of HPV viral integration into the human genome (Moberg *et al*., 2005). HPV integration into the human genome leads to the deregulation of the expression of viral oncogenes and disruption of important host cell genes such as Rb tumour suppressor gene (Doorbar, 2007; Mark *et al*., 1996). Disruption of tumour suppressor genes may lead to accumulation of DNA mutations in the host genome and this accumulation may play a significant role in the development of cervical cancer (Doorbar, 2007).

There are other factors which influence HPV viral load including low CD4 count. It has been reported that HPV-16 viral load increases with the decreasing CD4 count, which correlates with more severe SIL grade (Ylitalo *et al*., 2000; Lefevre *et al*., 2004). Women with cytological abnormalities (suggestive of HSIL) compared with women with normal cytology showed an 81-fold increase of HPV-16 viral load, and a significantly higher viral load was observed in women with HSIL compared to women with LSIL (Lefevre *et al*., 2004). Swan *et al*., (1999) investigated the difference in HPV DNA load between specific HPV genotypes. HPV-16 showed the highest viral load compared to all other HPV types and it was the most highly significant HPV type associated with cervical disease, while the viral load for HPV-18, -31, and -45 did not increase significantly with increasing cervical disease severity. The HPV-16 viral load median value was 60-fold higher in women with HSIL compared to women with normal cytology and 30-fold higher when compared to women with LSIL, regardless of single or multiple HPV infection (Swan *et al*., 1999).

1.8.3 Sexual transmited infections (STIs)

High prevalence of STIs is observed in young women and men between the ages of 15-25 years even though this group constitute only 25% of sexually active population (Weinstock *et al*., 2004). Infection with other micro-organisms such as human immunodeficiency virus (HIV), *Chlamydia trachomatis* and herpes simplex virus-2 (HSV-2) may possibly increase the development of cervical or penile disease (Lehtinen *et al*., 1996; Sun *et al*., 1997; Smith *et al*., 2002). HSV-2 enhances the development of cervical disease by increasing HPV replication and the integration of HPV DNA sequences into host cell chromosomes (Brink *et al*., 2002). An individual with STI is more likely to acquire or transmist STI, including HIV, compared to STI unifected individual (Weinstock *et al*., 2004).

HIV co-infection also affects the development of HPV-associated genital disease. Studies investigating the global distribution of HIV reported that three quarters of the HIV infected people world-wide live in Africa and most of them are located in sub-Saharan Africa (UNAIDS, 2010). Approximately 26 million people in Africa are living with HIV, and thousands of deaths are caused by HIV infection/ acquired immunodeficiency syndrome (AIDS) annually (UNAIDS, 2010). Infection with both HIV and HPV may lead to a serious crisis in Africa (Nosarka *et al*., 2007). HPV acquisition is found to significantly increase in women who HIV seroconverted and the risk of LSIL is also found to be increased in these women (Wang *et al*., 2011). In Zimbambean women, a 5-fold increase of multiple HPV acquisition 12-months after HIV acquisition is been observed (Nowak *et al*., 2011).

Women infected with both HIV and HPV are at increase risk of developing cervical precancerous lesions and cervical cancer compared to women infected with HPV only (La *et al.*, 1998). HPV-associated cancers in HIV-positive individuals occurred more frequently than in HIV-negative individuals. In women, the occurrence of HPV-associated cervical, vulval or vaginal cancer and anal cancer and in men, penile and anal cancers were significantly increased in HIV-positive individuals compared to HIV-negative individuals (Frisch *et al*., 2000). Viscidi *et al*., (2005) demonstrated that HIV-positive women are four times more susceptible to infection by HR-HPV types. HIV infection and its associated immune suppression increase the probability of progression to HSIL and conversely decrease the probability of regression or clearance of HPV infection (Viscidi *et al*., 2005; Sun *et al*., 1997). HIV-positive women progress to cervical cancer about 10 years earlier than HIV-negative women (Lomalisa *et al*., 2000). HIV-positive men are reported to have a higher prevalence of genital HPV DNA and a higher HPV viral load in urine than HIV-negative men (Smits *et al*., 2005). In a longitudinal study conducted by Eckert *et al*., (1999) HIV-positive women displayed an increased progression, persistence of HPV and reoccurrence of abnormal cervical cytology. In contrast, in HIV-negative women the reoccurrence of abnormal cytology was decreased.

HR-HPV positive women and men are also at increase risk of HIV acquisition and the risk if found to increase with increasing multiple HR-HPV infections (Smith-McCune *et al*., 2010; Auvert *et al*., 2011). HPV clearance of either LR-HPV or HR-HPV was significantly associated with increased risk of HIV acquisition during follow-up. While persistent HPV infection was not significantly associated with HIV acquisition (Smith-McCune *et al*., 2010). Most HPV infections are cleared by innate and adaptive immune mechanism, making it

biologically possible that the local immune response elicited by HPV infection are exposed to HIV, and that women in the process of clearing an HPV infection might be at increased risk of acquiring HIV (Smith-McCune *et al*., 2010).

It has been suggested that the high incidence of HPV infection and HPV-associated disease in HIV infected individuals could be a result of immunosuppresion caused by HIV (Palefsky *et al*., 1999; Strickler *et al*., 2005). HPV prevalence is greater in HIV infected women with ≤200/mL CD4 count and high plasma HIV RNA levels and that HIV-infected women have a deregulated anti-HPV IgA response (Palefsky *et al*., 1999; Strickler *et al*., 2005). Fewer HIV-positive women display anti-HPV IgA responses than HIV-negative women which could impact on their ability to manage HPV infection (Marais *et al*., 2000). Apart from immunosuppression there are other ways in which HIV may increase the persistence of HPV infection and development of cancer. It is reported that HIV infection increases the transcription of HPV proteins. Dolei *et al*., (1999) demonstrated that after HIV infection and in the presence of cytokines such as interleukin-6 (IL-6), the transcription of the E1 and L1 HPV proteins increases and the action is HPV dependent. Dolei *et al*., (1999) concluded that there is a direct action of HIV on HPV-18 through Tat transactivation and indirectly through IL-6 activity (Dolei *et al*., 1999). It is also reported that HIV increases the transcription of the HPV E7 protein (Arany & Tyring, 1998). Increased levels of E7 protein could possibly result in a high rate of Rb tumor suppressor protein inactivation and degradation (Seavey *et al*., 2006).

1.8.4 Contraceptives

The use of oral contraceptives by women is strongly associated with risk of cervical abnormalities due to increased risk of HPV acquisition among these women (Hildesheim *et al*., 2001; Moreno *et al*., 2002; Marks *et al*., 2011a, Marks *et al*., 2011b). The increasing years of oral contraceptive exposure is associated with increased risk of SIL and cancer (Hildesheim *et al*., 2001; Moreno *et al*., 2002). However, Vaccarella *et al*., (2006) reported that short or long term use of oral contraceptives is not associated with increased risk of HPV acquisition and persistence (Vaccarella *et al*., 2006). According to Syrjanen *et al*., (2006) the use of oral contraceptives is not an independent risk factor for HR-HPV infection, persistence or clearance and cytological abnormalities. However, the different sexual behaviours observed among women who use oral contraceptives, nonusers of oral contraceptives and those who use any

kind of contraceptive account for increased HR-HPV infection and development of cytological abnormalities. Women who use contraceptives are more likely to be those with high risk sexual behaviours (Syrjanen *et al*., 2006). Mark *et al*., (2010) conducted a study in which they treated cells with 17β-estradiol and progesterone (sex steroid hormones) and they found that these hormones suppress the inflammatory response and enhance the regulatory response to HPV-16 VLPs. These findings suggest that women on contraceptives are at increased risk of persistent HPV infection because their immune response is suppressed by these hormones. However, Shapiro *et al*., (2003) did not observe any association between injectable contraceptive; progestogen the commonly used injectable contraceptives in South Africa; with cervical cancer in South African women.

1.8.5 Parity

High parity has been reported to be associated with cervical cancer (Castellsague & Munoz, 2003). An increasing number of pregnancies are associated with an increased risk of cervical cancer (La *et al*., 1998; Hildesheim *et al*., 2001; Munoz *et al*., 2002; Moreno *et al*., 2002). It is important to note that parity is an indication of unprotected sex. Hormonal changes that occur during the pregnancy may decrease HPV clearance and increase HPV persistence thus enhancing the development of cervical abnormalities (Sethi *et al*., 1998). Therefore, parity, short and long term use of oral contraceptives might play role in progression of HPV infection to development of cervical neoplasia (Castellsague & Munoz, 2003; Vaccarella *et al*., 2006).

1.8.6 Other factors

Sastre-Garau *et al*., (2004) demonstrated that SIL regression may be influenced by age. Their study concluded that women of 30 years or younger have a 62% likelihood of SIL regression which is significantly reduced to 37% in women older than 30. The decreased proportion of SIL regression in older women can be explained by decreased immune competence in older women compared to younger women.

Ultraviolet B rays from sunlight can be carcinogenic and promote viral activation including HPV (Hrushesky *et al*., 2005). The longer the duration of sunlight exposure the greater is the likelihood of progression to cancer. This is presumably because components of sunlight repress the cellular immune response allowing HPV the opportunity to persist and result to high HPV

viral load that will enhance the development of precancerous lesion (Hrushesky *et al*., 2005). Avoiding exposure to sunlight may assist in minimising chances of SIL progression.

Socioeconomic status is considered as a secondary risk factor for HPV infection. It is measured based on education level and amount of income (Kahn *et al*., 2005). In a study by Kahn *et al*., (2005), the likelihood of HPV positive women progressing to HSIL or cancer was significantly greater in those with less than a high school education when compared with those with tertiary education. Regions with poorer socioeconomics status have increased risk of cervical cancer incidence and mortality (Müller *et al*., 2011).

Consumption of vegetables such as carrots, broccoli, cabbage or green beans by women increases their probability of clearing HPV infection more rapidly (Richardson *et al*., 2005). Consumption of fruits, vegetables, vitamins C and E, beta-carotene, alpha-carotene, lycopene, luterin and cryptoxanthin can possible protect against HPV persistent infection. Vegetables, folate, retinol, vitamins C, E and B12, beta-carotene, alpha-caroten, lycopene, luterin and cryptoxanthin also demonstrate the protective effect of cervical neoplasia (Kanetsky *et al*., 1998; Weinstein *et al*., 2001; Schiff *et al*., 2001). Tissues with folate deficiency have a high risk of DNA damage. Folate is important during DNA synthesis and DNA repair. Therefore tissues with low levels of folate are at increased risk of damage to DNA and with decreased abilities to repair DNA (Giuliano *et al*., 1998; Follen *et al*., 2001). Low levels of folate also increase blood homocysteine which has been reported to possibly increase the risk of cervical neoplasia (Alberg *et al*., 2000; Ziegler *et al*., 2002).

1.9 HPV vaccines

The development of HPV vaccines commenced in the early 90s after the discription of HPV virus-like particles (VLPs) (Kirnbauer *et al.*, 1992; Kirnbauer *et al*., 1994; Trus *et al*., 1996; Stanley *et al*., 2006). It was then demonstrated that immunization with cottontail rabbit papillomavirus (CRPV), bovine papillomavirus (BPV) and canine oral papillomavirus (COPV) L1 VLP resulted in high antibody levels and protection against viral challenge in animals (Breitburd *et al*., 1995; Suzich *et al*., 1995; Kirnbauer *et al*., 1996; Ghim *et al*., 2000). The results from animal studies showed that vaccination with HPV L1 could reduce the prevalence of genital warts, cervical precancerous lesions and cancer worldwide.

The first clinical trials were carried out using HPV-11 L1 VLPs and the study was very successful and encouraged the researchers and companies to proceed and develop vaccine based on multiple HPV types (Smith *et al*., 1995). Even though procedures involved in the development of HPV vaccine are complex, expensive research continued. Currently there are two prophylactic HPV vaccines, Gardasil® produced by Merck and Cervarix® produced by GlaxoSmithKline. Both vaccines are prepared from L1 major capsid proteins which self assemble to form an empty shell (called VLP) that resembles the HPV virion in morphology and immunogenicity for each specific type (Stanley *et al*., 2006; Arbyn & Dillner, 2007). Gardasil® protects against HPV-6, -11, -16, and -18 while Cervarix® protects against HPV-16 and -18. HPV-16 and -18 are responsible for 70% cases of cervical cancer worldwide (Clifford *et al*., 2006) and HPV-6 and -11 are associated with ~90% cases of genital warts (Giuliano, 2007). In Gardasil®, L1 VLP production is based on recombinant yeast technology and uses aluminium hydroxyphosphate sulphate as adjuvant while Cervarix® is based on recombinant baculovirus technology and uses aluminium hydroxide combined with 3-deacylated monophosphoryl lipid A. Gardasil®, is administered at month 0, 2 and 6 while Cervarix® is administered at month 0, 1 and 6. Both vaccines are injected intra-muscularly at 0.5mL. Both Gardasil® and Cervarix® HPV vaccines are approved in many countries including South Africa. In most countries it has been licensed to be used in girls and boys.

During natural HPV infection antibodies are generated by some of infected individuals however the antibody levels are low. HPV life cycle does not have a viraemic phase. As mentioned previously, HPV infection does not involve the activation of apoptosis during HPV release which assists HPV in evading the immune system. No inflammation is induced during cell death the innate immune system is neither alerted nor adaptive immunity triggered to fight HPV infection (Doorbar, 2006). The high antibody levels in individuals who received HPV vaccine compared to those with natural HPV infection are because the HPV VLPs in vaccine is delivered intra-muscularly, resulting in VLPs in blood and the production of high titres of neutralising serum antibodies. The success of the HPV vaccines in protecting against HPV infection has been attributed to the their immunogenicity and efficiency in eliciting high titres of long lasting serum neutralising antibodies (Harper *et al*., 2006; Stanley *et al*., 2006). After HPV vaccination 100% of women develop antibodies to all HPV types vaccinated against and the antibody levels are reported to remain high for more than six years after the initial dose (Stanley *et al*., 2006; Arby & Dillner, 2007; Harper *et al*., 2008). The duration of HPV vaccine protection is not yet known (Sankaranarayanan, 2009). HPV vaccines have indicated

considerable HPV type specificity apart from some cross protection shown for very phylogenetically closely related types (HPV-31 and -45; Harper *et al*., 2006; Smith *et al.*, 2008).

Assuming a 100% type specificity and vaccine efficacy of a polyvalent HPV vaccine containing L1 VLP of the eight most common HPV types could potentially prevent ~90% cases of cervical cancer (Smith *et al*., 2007). The current HPV vaccine will not only reduce genital warts and cervical cancer but will also help to reduce cancers of the anus, vulva, vagina, penis, oropharynx and mouth (Parkin & Bray 2006). HPV vaccines are very promising in reducing the cervical cancer cases and other HPV related cancers, however, there are still challenges in implementation of HPV vaccine especially in low-resource countries as HPV vaccines are expensive, delivered by intra-muscular injection of three doses over a period of 6-months and require cold chain storage. Development of a thermo-stable vaccine and a needle-free vaccine would reduce the challenges and provide more advantages (Jacob *et al*., 2005; Stanley *et al*., 2008).

The prevalence of HPV infection varies worldwide. Müller *et al*., (2010) recently reported 78% HPV prevalence in South African heterosexually active men and that HPV-6, -11, -16 and -18 were the most dominant types in men with genital warts. It is important that we determine the extent of genital HPV infection and the types of HPV in men, in addition to that of women in South Africa. The prevention of HPV infection in men is considered therefore another way of eliminating cervical, penile, anal, vaginal, oropharynx cancers and genital warts in both men and women. It is important to have studies that will be relevant in determining those HPV types associated with genital cancers in South African populations and the immune response to those types and to evaluate the possible efficacy of HPV vaccines in eliminating HPV infection.

1.10 The aims of the study

Although much is known about HPV and cervical cancer in women, data on male genital HPV infections and on heterosexually active couples is limited especially in South Africa. The overall objectives of the study were to investigate HPV natural history in South African HIV-positive, HIV-negative and HIV discordant heterosexually active couples. According to our knowledge this is the first report on HPV sharing and transmission in heterosexually active

couples that are HIV-negative, HIV-positive and HPV discordant and in which 71% of female participants have normal cervical cytology.

The aims of the study were:
i) to investigate the HPV prevalence, acquisition and clearance in South African HIV-positive and HIV-negative women and men;

ii) to investigate factors that are associated with HPV prevalence, acquisition and clearance in HIV-positive and HIV-negative women and men;

iii) to investigate factors that predict abnormal cervical cytology in women;

iv) to investigate prevalence of HPV sharing and transmission in young and older heterosexually active couples that were HIV-positive, HIV-negative, HIV-discordant where the female or the male partner was HIV-positive;

v) to investigate factors associated with HPV sharing and transmission in heterosexually active couples;

vi) to investigate *hpVIR* HPV viral load in HIV-positive and HIV-negative women and men and its influence on *hpVIR* HR-HPV sharing among sexually active couples

vii) to investigate the HPV seroprevalence of nine different types among HIV-positive and HIV-negative women and men and risk factors for HPV seropositivity; and

viii) to investigate the impact of HIV seropositivity on the prevalence, sharing, transmission and viral load of genital HPV infection in heterosexually active couples.

CHAPTER 2: HUMAN PAPILLOMAVIRUS INFECTION IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVE AND HIV-SERONEGATIVE WOMEN AND MEN

2.1 INTRODUCTION

In 2009 approximately 5.6 million people in South Africa were living with HIV (UNAIDS, 2010). HIV prevalence is reported to be higher in women compared to men and in women the peak is observed between 25-29 years of age while in men is observed between 30-34 years of age (UNAIDS, 2010). HPV-associated cancers in HIV-positive individuals occur more frequently than in HIV-negative individuals (Frisch *et al*., 2000). HIV-positive women progress to cervical cancer about 10 years earlier than HIV negative women (Lomalisa *et al*., 2000). The high prevalence of cervical cancer and HIV infection are both major public health problems in South Africa. HIV-positive men and women are reported to have a higher prevalence of genital HPV DNA, multiple HPV infection and higher HPV viral load compared to HIV-negative men and women (Palefsky *et al*., 1999; Eckert *et al*., 1999; Levi *et al*., 2002; Riva *et al*., 2007). In HIV-positive individuals HPV infection is more persistent compared to HIV-negatives (Heard *et al*., 2000; Smits *et al*., 2005). High HPV prevalence and viral load in HIV-positive individuals may indicate the reactivation of latent HPV infection and prolonged persistence of HPV due to HIV induced immune suppression as well as high risk behaviour (Critchlow *et al*., 1998; Abba *et al.,* 2003; Silverberg *et al.,* 2006). High HPV viral load is associated with increased risk of cervical abnormalities and enhanced viral transmission (Ylitalo *et al*., 2000).

HPV infection has been studied extensively in women but data on men HPV infections is limited. Men are believed to be responsible for the sustained transmission of HPV to their female partners (Castellsague *et al*., 2003). Müller *et al.,* (2010) reported a 100% HPV prevelance in South African heterosexually active men and HIV seropositivity was found to significantly influence HPV prevelance and multiple HPV infections. The risk of HPV infection is increased in women and men with multiple sexual partners (Heard *et al*., 2000; Kjaer *et al*., 2001; Winer *et al*., 2008). Women with a male partner in a polygamous relationship have a higher risk of HPV infection compared to women in a monogamous relationship (Hippelainen *et al*., 1994; Bosch *et al*., 1996). Increased lifetime number of sexual partners of both women and their male partners are associated with increased risk of cervical abnormalities (Ferreccio *et al*., 2004). The risk factors of HPV DNA detection in men and in women are reported to be similar, these include, age, age at first sexual intercourse, number of lifetime sexual partners, number of recent sexual partners, the number of lifetime sexual partners of the partner, history of STDs and non-use of condoms (Svare *et al*., 2002; Vaccarella *et al*., 2006; Giuliano *et al*., 2008).

Prevalence of HPV multiple infections in HIV-negative women is reported to range from 20% to 50% and in HIV-positive women it ranges from 50% to 80% (Franco *et al.*, 1999; Palefsky *et al*., 1999; Levi *et al*., 2002). When Chaturvedi *et al*., (2005) investigated HPV species that are commonly found in multiple HPV infections; they found that α9 HPV species (these include HPV-16, -31, -33, -35, -52 and -58) are less likely to be involved in multiple infections compared to other HPV species. The reason for these findings are not yet clear however they might be influenced by different risk factor profile and transmission pattern observed in different HPV species. The combination of HPV types in multiple infections among HPV types is reported to be formed randomly with little consideration of phytologenetically related or unrelated types (Chaturvedi *et al*., 2005). It has been reported that women who were HPV positive at baseline visit were found to be at higher risk of acquiring new HPV infection of both phylogenetically related and unrelated genotypes during follow-up visit compared to women who were HPV negative at baseline visit (Rousseau *et al*., 2001). This indicates that once women are infected with one HPV type sooner or later they will have multiple HPV infection especially those that are immunocompromised.

Male circumcision is reported to decrease the rate of HPV acquisition, HPV transmission to the sexual partner and also decrease the risk of cervical cancer in their female partners (Castellsague *et al*., 2002; Castellsague *et al*., 2003). Genital HPV prevalence in men is reported to be highest on the penile shaft with the highest proportion of multiple HPV infections followed by glans, scrotum, urethra and perianal areas; suggesting that penile shaft and glans are most important for HPV transmission during sexual intercourse (Nielson *et al*., 2007). HPV prevalence is reported to decrease with increasing age in women and then increase after menopause in some populations (Kjaer *et al*., 2000; Castle *et al*., 2005). Jacobs *et al.*, (2000) also reported a decline of HPV prevalence with age in women; however LR-HPV types were not found to decline as was reported for HR-HPV types. Along with decreasing HPV prevalence, there is a decreased prevalence of cytologic abnormalities in older women (>54 years; Kovacic *et al*., 2006). HPV prevalence is reported to increase with increasing cervical abnormalities, predominantly the HR-HPV types in which amongst them HPV-16 and -18 are associated with 70% cases of cervical cancer worldwide (Clifford *et al*., 2006). In men, HPV prevalence is reported to be higher in young men (18-20 years) and then declines, however there is no clear decreasing or increasing trend as age increase in men (Giuliano *et al*., 2008a). HPV prevalence of specific HPV type is not positively associated with age (Giuliano *et al*., 2008a; Giuliano *et al*., 2008b).

The objectives of this study were

(i) to investigate the prevalence of genital HPV infection in HIV-positive and HIV-negative men and women;

(ii) to investigate factors affecting HPV prevalence including age;

(iii) to investigate the prevalence of HPV in women with abnormal and normal cervical cytology; and

(iv) to investigate the effect of HIV status and CD4 count on abnormal cervical cytology.

2.2 MATERIALS AND METHODS

2.2.1 Study population and specimen collection

A total of 601 women and 601 men were recruited between 2006 and 2009 from the Manyanani clinic, Empilisweni centre, Gugulethu, Cape Town. There were no special eligibility criteria during recruitment except that participants had to be sexually active couples. Women and men were recruited even if their CD4 count level was low and on ARVs. When we were enrolling for the study we selected all women and men from HIV concordant and discordant partnership and then some from HIV-negative partnership. A total of 209 HIV-negative women, 277 HIV-positive women, 333 HIV-negative men and 153 HIV-positive men were enrolled for this study. The remaining 115 women and 115 men were HIV-negative. Participants were recruited by recruitment team from Gugulethu Township and the specimens were then collected at Manyanani clinic. Dr David Coetzee and Dr Mercy Kampura were responsible for the management of the clinic. The mean age of women and men participants was 35 years (range: 18-66 years) and 38 years (range: 19-67 years) respectively. Of the men in this study, 96.6% (451/467) were traditionally circumcised and 11 declined to answer the question on circumcision and the circumcision status was not noted by the clinicians during the collection of penile swabs and most of the men were Xhosa speaking where men are traditionally circumcised.

The study was approved by the Research Ethics Committee of the University of Cape Town. Cervical cells were collected using a Digene cervical sampler (Qiagen, Hilden, Germany). Samples were not collected from women if they were menstruating on the day of enrolment and if blood was visible in the cervical area. Penile cells were collected with a dry Digene swab (Qiagen, Hilden, Germany) by thorough brushing of the penile shaft and glans as well as the

foreskin in uncircumcised men. Both cervical and penile cells were stored in Digene transport medium at -80ºC until DNA was extracted.

2.2.2 HPV genotyping

The investigator (Zizipho Mbulawa) perfomed DNA extraction and HPV genotyping for 2010 WHO HPV LabNet Proficiency study to make sure that the investigator was proficient for detecting all HPV genotypes even if the viral load was low as 5IU per 5µl. The investigator was found proficient for detection of HPV using Roche Linear Array HPV genotyping test (see Appendix 1). DNA was extracted from both cervical and penile cells using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche diagnostics, Mannheim, Germany) and the automated MagNA Pure Compact machine (Roche diagnostics, Mannheim, Germany). Extracted DNA was stored at -20ºC until further use. HPV typing was performed on DNA extracted from cervical and penile cells using the Roche Linear Array HPV genotyping test (Roche diagnostics, Mannheim, Germany) according to the manufacturers' instructions. There are four major procedures involved in Linear Array HPV genotyping test, namely, specimen preparation (includes sample collection and DNA extraction), polymerase chain reaction (PCR) amplification of target DNA using HPV primers; hybridization of the amplified product to oligonucleotide probes and detection of the probe-bound amplified products by colometric detection (Roche Linear Array HPV genotyping test booklet, 2008).

HPV genotyping was performed according to manufacturer's instructions. Briefly, a total volume of 580µl master mix containing PGMY09/11 primers to amplify HPV DNA of 37 different HPV genotypes (450bp) and GH20/PC04 primers to amplify β-globin gene (268bp) was mixed with 125µl Magnesium chloride solution to give a working master mix. A total of 50µl DNA sample were added to 50µl working master mix and amplified. Positive and negative controls provided by Roche were included in each amplification procedure. The positive control consists of HPV-16 DNA and β-globin gene, and the negative control had no HPV DNA and no β-globin gene. PCR was performed under the following conditions: 50°C for 2 min to activate AmpErase, 95°C for 9 minutes, followed by 40 cycles consisting of 95°C for 30 sec, 55°C for 1 minute, 72°C for 1 minute and a final hold at 72°C for 5 minutes on an AB9700 machine (Applied Biosystems, Inc, Foster City, CA, USA). The reaction was kept at 72°C until the addition of an alkaline denaturation solution (Roche diagnostics, Mannheim, Germany) to denature the amplicons and AmpErase.

The post-amplification detection of 37 different HPV genotypes and the β-globin gene to evaluate sampling adequacy, DNA extraction and amplification efficiency was carried out using Linear Array Detection Kit (Roche diagnostics, Mannheim, Germany). Each run included a positive and a negative control to make sure that there was no contamination and the assay was working properly. HR-HPV types included HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, 73 and -82; LR-HPV types included HPV-6, -11, -40, 42, -54, -55, -61, -62, -64, -67, -69, -70, -71, -72, -81, -83, -84, -89 (HPV-CP6108) and IS39 and probable HR-HPV types included HPV-26, -53 and -66. The probable HR-HPV types were grouped with HR-HPV types. The grouping of probable HR-HPV types with HR-HPV types was suggested by the reviewers of our first paper on this study (Mbulawa *et al.*, 2009). The Linear Array HPV genotyping strips consist of cross-reactive oligonucleotide probe that hybridizes with HPV-33, -35, -52 and -58. The Roche Linear Array HPV genotyping test does not detect HPV-52 individually. The specimens that tested negative for HPV-33, -35 and -58 individually but tested positive for the HPV-52/33/35/58 group are considered to be HPV-52 positive. The specimens that tested positive for HPV-33, -35 and -58 individually and tested positive for the HPV-52/33/35/58 group are not considered to be HPV-52 positive however the co-infection of HPV-52 cannot be ruled out.

Each strip was placed into the appropriate well of the 24-well tray leaving an empty well in between strips to avoid cross contamination. A total of 75µl denatured amplicon was transferred to appropriate wells containing pre-warmed hybridization buffer. Each amplicon was hybridized to the strip at 53°C. Following hybridization, the strips were stringently washed with washing buffer containing sodium lauryl sulfate (SDS) and sodium salts to remove all unbound material. Streptavidin-horseradish peroxidase conjugate (SHPC) was added to the strip to bind to the biotin-labeled amplicon hybridised to the probe on strip. The strip was then washed to remove any unbound SHPC and a substrate solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzide (TMB) was added. The TMB substrate in the presence of hydrogen peroxide was oxidised to a blue colour by catalysis action of SHPC in the presence of hydrogen peroxide. The blue colour precipitated at probe positions where hybridization occurred. The strips were then washed with distilled water and read visually by comparing the pattern of blue lines to the Linear Array Genotyping test reference guide.

2.2.3 Statistical analysis

All comparisons of HPV prevalence levels were conducted using chi-squared tests. Factors affecting the risk of any HPV infection were assessed using multivariate logistic regression. All analyses were conducted for HR-HPV types and LR-HPV types separately, as well as for HR-HPV and LR-HPV types combined. Dr Leigh Johnson (Centre for Infectious Disease, Epidemiology and Research, University of Cape Town) conducted all statistical analyses using STATA 11.0 (StataCorp, College Station, TX, USA). X^2 test (EpiInfo Version 5 Statcalc) was also used in comparing prevalence. In all analyses P-values ≤ 0.05 were considered significant.

2.3 RESULTS

2.3.1 Genital HPV prevalence according to gender and HIV status

To ensure sample adequacy, the presence of the β-globin gene was evaluated and found to be negative in 0.4% (2/486) cervical samples and in 3.1% (15/486) penile samples. Four of these penile samples were HPV DNA positive and nine were HPV negative indicating that even if the sampling was deemed inadequate by the less sensitive β-globin gene assay. HPV DNA was still detected from the penile sample; while in cervical samples with negative β globin gene no HPV DNA was detected. These findings probably mean that some of these viruses were free virus they were not in cells or it means that few cells were collected and they were not enough to reach the required level required by the Roche Linear array HPV genotyping assay. A total of 277 HIV-positive women, 207 HIV-negative women, 149 HIV-positive men and 322 HIV-negative men had a positive β-globin band. All β-globin negative samples were excluded from the analysis. Figure 2.1 illustrates a strip with sample that was β-globin high and β-globin low positive, HPV-6, -11, 16, -18, -55, 73, and -83 positive.

Genital HPV prevalence was significantly higher in both HIV-positive women (74% 205/277) and men (81% 121/149) compared to HIV-negative women (37% 76/207) and men (49% 159/322, P<0.0001 for both men and women). HIV-negative men showed a significantly higher prevalence (49%) of genital HPV than HIV-negative women (37%, P= 0.004), while the HPV prevalence was not significantly different between HIV-positive men (81%) and women (74%; P=0.5). HIV-positive women were found to have 3.4 fold higher risk of multiple HPV

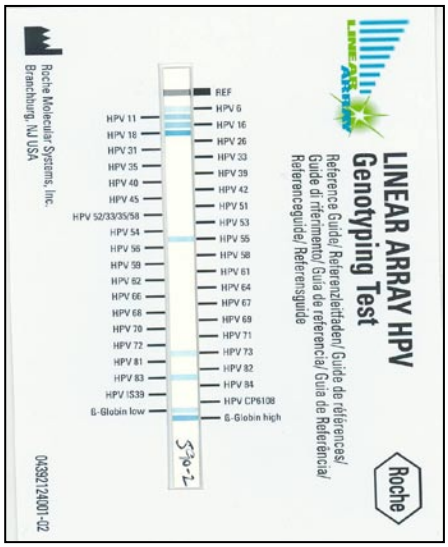

Figure 2.1. Roche linear array HPV genotyping strip demonstrating a HPV-6, -11, 16, -18, -55, 73, -83, β -globin low and high positive sample.

infection compared to HIV-negative women (50% 139/277 compared to 15% 31/207, P<0.0001). HIV-positive men were found to have 2.2 fold higher risk of multiple HPV infection (66% 99/149 compared to 30% 96/322, P<0.0001). HIV-negative men were found to have 2 fold higher risk of multiple HPV infection compared to HIV-negative women (30% 96/322 compared to 15% 31/207, P<0.0001). These results indicate that HIV-positive individuals have a significantly higher risk of having ≥2 HPV types than HIV-negative individuals regardless of gender (Figure 2.2).

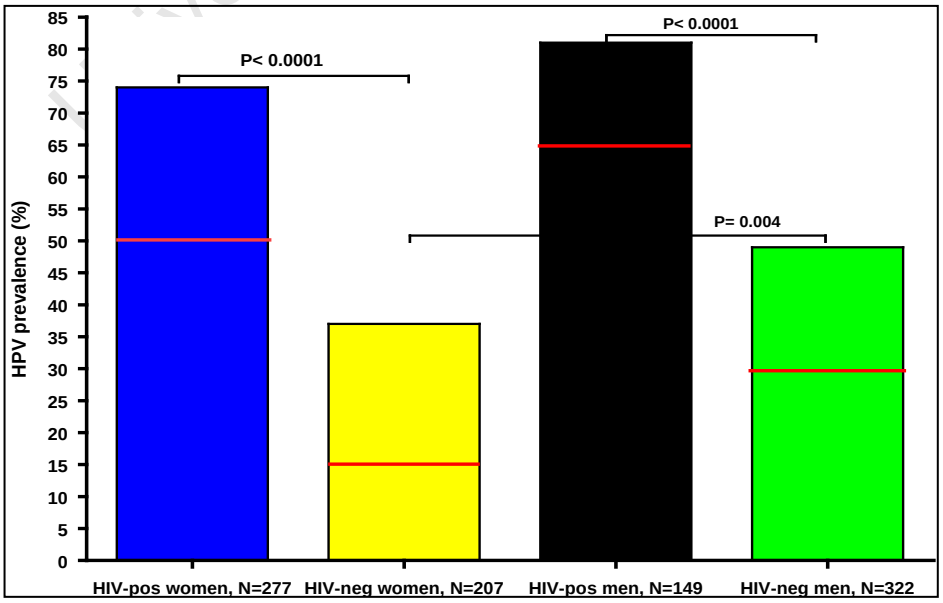

Figure 2.2. Genital HPV (HR and LR) prevalence in HIV-negative and HIV-positive women and men. Red lines indicate prevalence of multiple HPV infection. Neg: negative, Pos: positive

HIV-positive women were found to have significantly higher prevalence of HR-HPV and LR-HPV prevalence compared to HIV-negative women (HR-HPV: 52% 144/277 compared to 29% 59/207, P<0.0001; LR-HPV: 65% 181/277 compared to 28% 58/207, P<0.0001). HIV-positive men were found to have significantly higher prevalence of HR-HPV prevalence compared to HIV-negative men (67% 100/149 compared to 32% 102/322, P<0.0001) and LR-HPV (67% 101/149 compared to 39% 124/322, P<0.0001). When we group HPV prevalence according to species level HIV-positive women and men were found to have significantly higher prevalence of all HPV species (Table 2.1). HR-HPV types were found to dominate in HIV-negative women (63% 64/101); however in HIV-positive women, HIV-negative and HIV-positive men the distribution of HR-HPV and LR-HPV types were not found to differ (Table 2.1). The number of isolated HPV types varied between men and women and according to the HIV status.

Table 2.1. The prevalence of HPV species in women and men according to HIV status

	WOMEN			MEN		
Variable	**HIV+, n=277**	**HIV-, n=207**	**P-value**	**HIV+, n=149**	**HIV-, n=322**	**P-value**
HPV positive	205 (74%)	76 (37%)	**<0.0001**	121 (81%)	159 (49%)	**<0.0001**
HR-HPV	144 (52%)	59 (29%)	**<0.0001**	100 (67%)	102 (32%)	**<0.0001**
LR-HPV	181 (65%)	58 (28%)	**<0.0001**	101 (68%)	124 (39%)	**<0.0001**
α3 HPV species	217 (78%)	9 (4%)	**<0.0001**	144 (97%)	120 (37%)	**<0.0001**
α5 HPV species	46 (17%)	11 (5%)	**0.0001**	29 (20%)	30 (9%)	**0.002**
α6 HPV species	62 (22%)	26 (13%)	**<0.0001**	40 (27%)	45 (14%)	**0.0007**
α7 HPV species	84 (30%)	14 (7%)	**<0.0001**	101 (68%)	66 (21%)	**<0.0001**
α9 HPV species	127 (46%)	26 (13%)	**<0.0001**	72 (48%)	54 (17%)	**<0.0001**
α10 HPV species	25 (9%)	4 (2%)	**0.001**	43 (29%)	20 (6%)	**<0.0001**
α1/α8/α11/α13/α15 HPV species	58 (21%)	4 (2%)	**<0.0001**	51 (34%)	29 (9%)	**<0.0001**

Due to HPV multiple infection some of women are counted more than once. Bold p-values are statistically significant.
HIV+: HIV-positive. HIV-: HIV-negative. **α1** HPV species includes HPV-42. **α3** HPV species includes HPV-61, -62, -72, -81, -83, -84 and -89. **α5** HPV species includes HPV-26, -51, 69, -82 and –IS39. **α6** HPV species includes HPV-53, -56 and -66. **α7** HPV species includes HPV-18, -39, -45, -59, -68 and -70. **α8** HPV species includes HPV-40 . **α9** HPV species includes HPV-16, -31, -33, -35, -52, -58 and -67. **α10** HPV species includes HPV-6, -11 and -55. **α11** HPV species include HPV-73. **α13** HPV species includes HPV-54. **α15** HPV species includes HPV-71.

Among HIV-positive men the most frequently detected types were HPV-62 (20.8%), HPV-61 (18.8%), HPV-70 (16.8%), HPV-53 (16.1%), HPV-89 and -45 (14.8%), HPV-55, -81, -83 and -16 (13.4%). Among HIV-negative men HPV-62 (8.1%), HPV-61 (6.8%), HPV-66 (6.5%), HPV-53 (6.2%), HPV-83 (5.9%), HPV-81 (5.3%), HPV-89, -16 and -51 (5.0%) were most frequently detected. Among HIV-positive women the most frequently detected HPV types were HPV-62 (12.6%), HPV-52 (11.2%), HPV-58 (10.1%), HPV-53 (9.7%), HPV-61 and

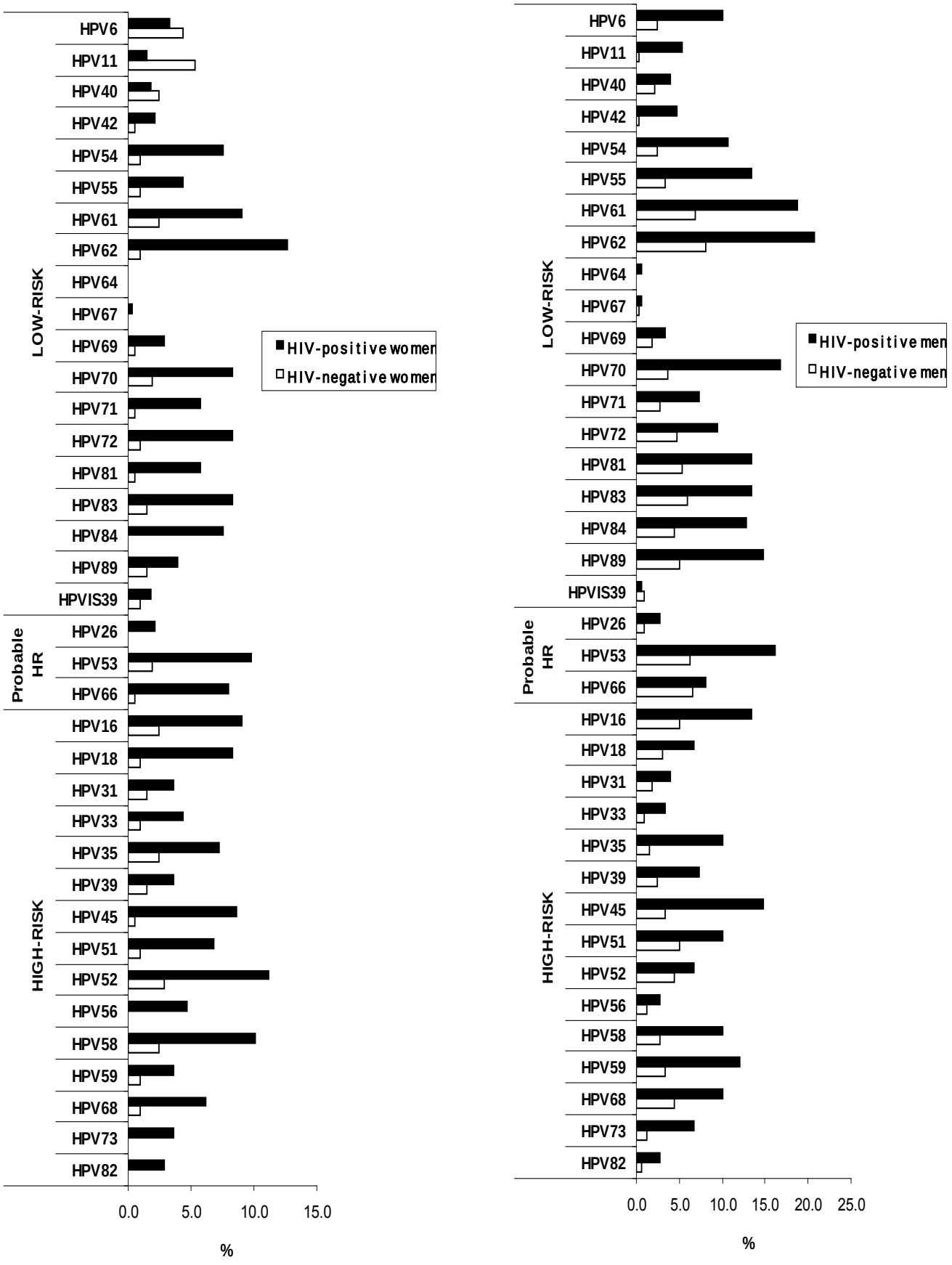

Figure 2.3. Prevalence of genital HPV types in human immunodeficiency (HIV)-positive and HIV-negative women (A) and men (B)

HPV-16 (9%), HPV-45 (8.7%), HPV-70, -72, -83 and -18 (8.3%). Among HIV-negative women HPV-11 (5.3%), HPV-6 (4.3%), HPV-52, HPV-40, -61, -16, -35 and -58 (2.4%) were frequently detected (Figure 2.3).

2.3.2 HPV prevalence in women and men and the impact of HIV and other variables

HIV-positive men with a CD4 count of <350/mL had a similar HPV prevalence when compared to HIV-positive men with CD4 counts of ≥350/mL (78% compared to 75%; odds ratio (OR), 1.19 [95% CI: 0.57-2.52]). In contrast, HIV-positive women with CD4 counts of <350/mL were found to have a higher HPV prevalence compared to HIV-positive women with CD4 counts of ≥350/mL (81% compared to 67%; OR, 2.13 [95% confidence interval (CI): 1.23-3.7]). HPV prevalence was found to decrease significantly with increasing age in both men (P=0.007) and women (P<0.001). In the unadjusted analysis, for both women and men HPV prevalence was not influenced by whether they lived with their sexual partners or not, nor the duration of the relationship nor their number of lifetime sexual partners. Similarly, in men and women young age at first sexual intercourse did not significantly influence HPV prevalence (Table 2.2).

Table 2.2. Genital HPV prevalence in men and women, and the influence of HIV infection and other variables (univariate analysis).

Variable	Men					Women				
	n	HPV%	OR	95% CI	P-value	n	HPV%	OR	95% CI	P-value
Total	471	58%				484	58%			
Age group										
<30 years	95	68%	ref		**0.007**	165	69%	ref		**<0.001**
30-39 years	185	62%	0.74	0.44-1.25		174	57%	0.59	0.38-0.92	
40-67 years	191	50%	0.47	0.28-0.78		145	46%	0.38	0.24-0.61	
HIV status										
Negative	313	49%	ref		**<0.001**	207	36%	ref		**<0.001**
Positive	158	77%	3.38	2.20-5.19		277	74%	5.01	3.39-7.40	
CD4 count (if HIV-positive)										
≥350/ml	77	75%	ref		0.64	135	67%	ref		**0.007**
<350/ml	79	78%	1.19	0.57-2.52		142	81%	2.13	1.23-3.70	
Age at first sex										
<16 years	125	60%	ref		0.927	77	56%	ref		0.079
16-18 years	247	58%	0.92	0.59-1.42		293	62%	1.28	0.77-2.12	
>18 years	94	59%	0.94	0.55-1.62		111	50%	0.78	0.43-1.39	
Lifetime number of sexual partners										
1-2	87	53%	ref		0.465	179	56%	ref		0.456
3-5	130	63%	1.52	0.88-2.64		220	57%	1.04	0.70-1.55	
6-10	128	59%	1.3	0.75-2.26		71	66%	1.55	0.87-2.75	
>10	121	56%	1.14	0.66-1.99		11	64%	1.38	0.39-4.89	

ref: Reference

2.3.3 The impact of age on HPV prevalence

In women, prevalence of any HPV, HR-HPV and LR-HPV were found to decrease with increasing age (R^2=0.79, R^2=0.93 and R^2=0.83 respectively, Figure 2.4A). When women were grouped according to HIV status HPV prevalence profile differed among HIV-positive women and HIV-negative women. HIV-positive women had a significantly higher prevalence of any HPV compared to HIV-negative women at the age of 18-25 years (86% 38/44 compared to 61% 25/41, P=0.008); 26-35 years (68% 88/129 compared to 39% 23/59, P=0.0002), at the age of 36-45 years (73% 52/71 compared to 24% 15/63, P<0.0001) and at the age of 46-66 years (82% 27/33 compared to 30% 13/44, P<0.0001). In HIV-positive women, prevalence of any HPV, HR-HPV and LR-HPV were not found to significantly decrease with increasing age and peak up in old age women (R^2=0.01, R^2=0.29 and R^2= 0.22 respectively, Figure 2.4B). Among HIV-positive women any HPV prevalence was 86% at age 18-25 years and was found to decrease at age 26-35 years to 68% and increase at age 36-45 years to 73% and at age 46-66 years to 82%. In HIV-negative women prevalence of any HPV and HR-HPV HPV were found to decrease with increasing age (R^2=0.7, R^2=0.96 respectively); while prevalence of LR-HPV was not found to significantly decrease with increasing age (R^2=0.4), Figure 2.4C). Among HIV-positive women CD4 counts were only found to be statistically different in women between the age of 46-66 years (median: 432/mL CD4 counts, range: 181-1592/mL CD4 counts) and 26-35 years (median: 335/mL CD4 counts, range: 14-1343/mL CD4 counts) and 26-35 years (median: 346/mL CD4 counts, range: 10-1762/mL CD4 counts).

In men, prevalence of any HPV, HR-HPV and LR-HPV was also found to decrease with increasing age (R^2=0.9, R^2=0.95 and R^2=0.87 respectively, Figure 2.5A). In HIV-positive men, prevalence of any HPV, HR-HPV and LR-HPV were found to decrease with increasing age (R^2=0.9, R^2=0.9 and R^2= 0.8 respectively, Figure 2.5B). In HIV-negative men prevalence of any HPV and HR-HPV HPV were also found to decrease with increasing age (R^2=0.8, R^2=0.8 respectively); while prevalence of LR-HPV was not found to significantly decrease with increasing age (R^2=0.3), Figure 2.5C). HPV prevalence was higher in HIV-positive men of all ages compared to HIV-negative men, however the difference was not significantly higher at the age of 18-25 years (88% 7/8 compared to 77% 27/35 respectively, P=0.46) and then significantly higher at the age of 26-35 years (77% 49/63 compared to 49% 46/93, P=0.0004), at the age of 36-45 years (76% 50/66 compared to 50% 51/101, P=0.001) and at the age of 46-66 years (71% 15/21 compared to 42% 35/84, P=0.01). Among HIV-positive men the level of CD4 counts was not found to significantly differ in different age groups.

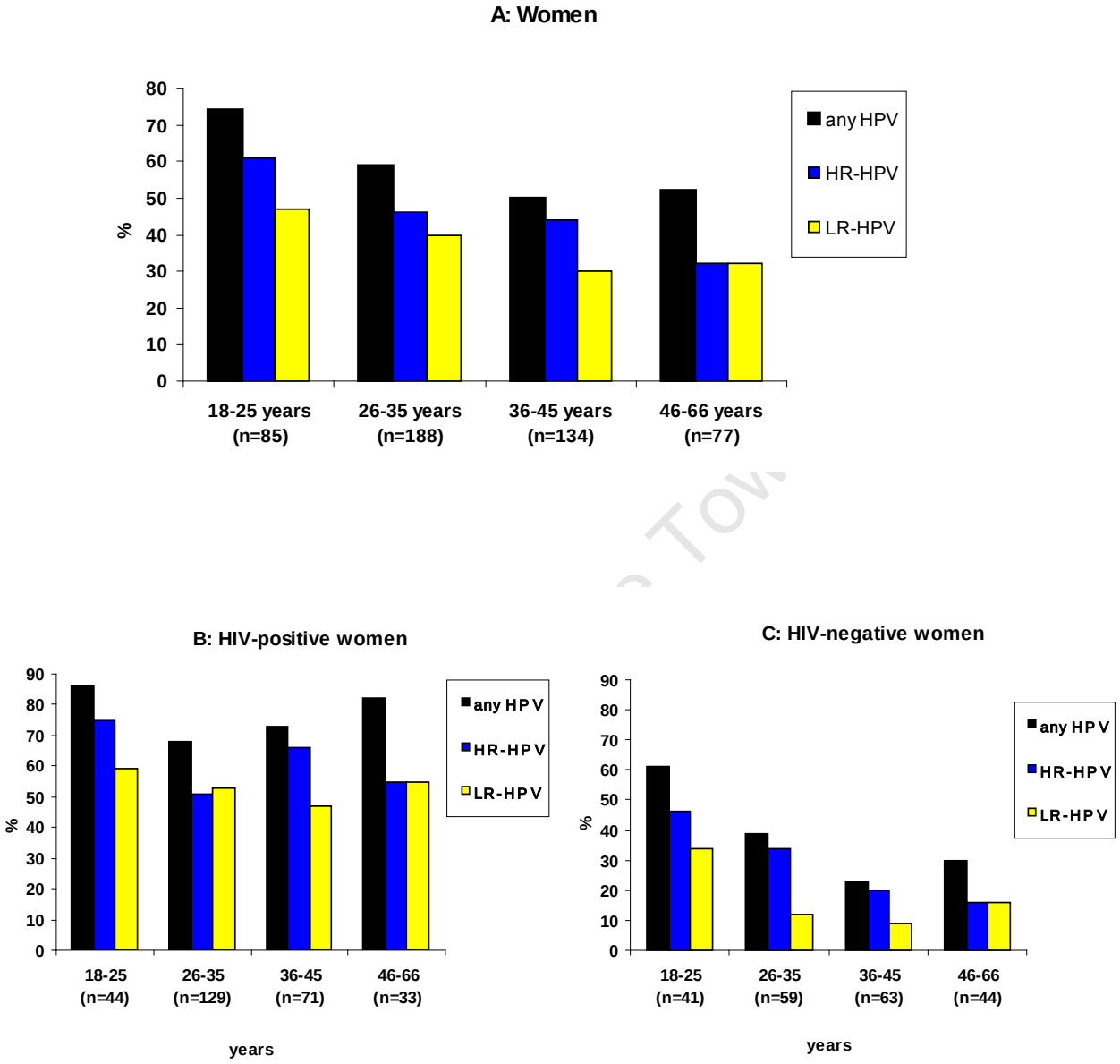

Figure 2.4. The prevalence of any HPV, HR-HPV, LR-HPV according to age in all women (A), HIV-positive women (B) and HIV-negative women (C).

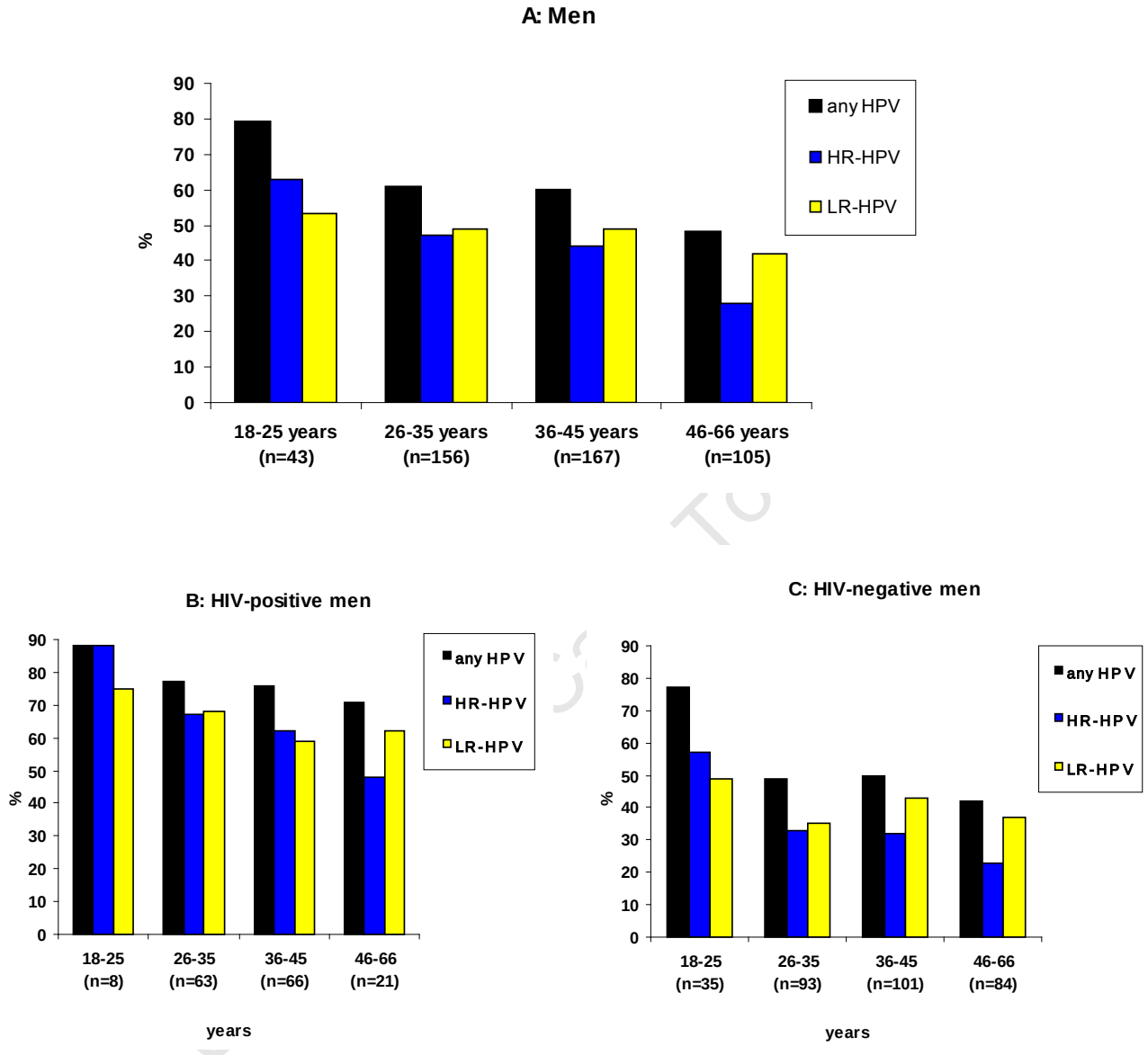

Figure 2.5. The prevalence of any HPV, HR-HPV, LR-HPV according to age in all men (A), HIV-positive men (B) and HIV-negative men (C).

2.3.4 HPV prevalence in women according to the cervical disease status.

Papanicolaou (Pap) smear results were monitored by Prof Margaret Hoffman and Dr Jennifer Moodley. Women were stratified based on Papanicolaou (Pap) smear results; 483 women had Pap smear results. A total of 67% (322/483) had normal cytology, 9% (42/483) had atypical squamous cell of undetermined significance (ASCUS), 17% (80/483) had low grade squamous intraepithelial lesion (LSIL), 2% (12/483) had high grade squamous intraepithelial lesion (HSIL) and 6% (27/483) had an inadequate sample. HPV prevalence was found to increase significantly with increasing cervical abnormalities (48% 153/322 for normal cytology; 69% 29/42 for ASCUS; 94% 75/80 for LSIL and 100% 12/12 for HSIL, R^2=0.95). HR-HPV prevalence was found to increase significantly with increasing cervical abnormalities (34% 108/322 for normal cervical cytology; 60% 25/42 for ASCUS; 85% 68/80 for LSIL and 100% 12/12 for HSIL, R^2=0.99). LR-HPV prevalence was also found to increase significantly with increasing cervical abnormalities (33% 107/322 for normal cervical cytology; 33% 14/42 for ASCUS; 59% 47/80 for LSIL and 58% 7/12 for HSIL, R^2=0.78, Figure 2.6).

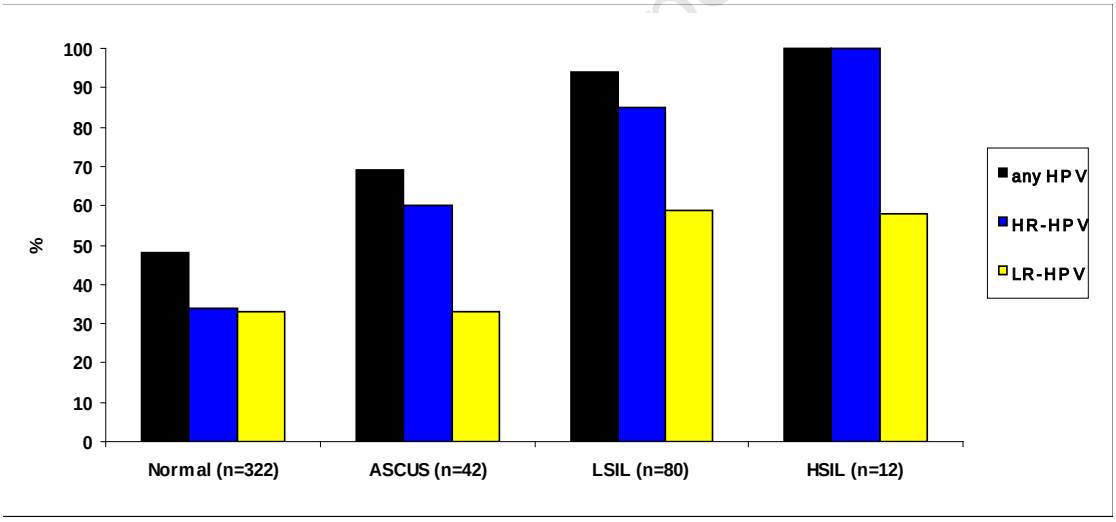

Figure 2.6. The prevalence of HPV infection in women with normal cytology, atypical squamous cell of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL).

HIV-positive women with normal cervical cytology were found to have significantly higher CD4 counts compared to HIV-positive women with abnormal (includes ASCUS, LSIL and HSIL) cervical cytology (median: 401/mL, range: 14-1762/mL compared to median: 307/mL, range: 10-1279/mL, P=0.0005, Figure 2.7).

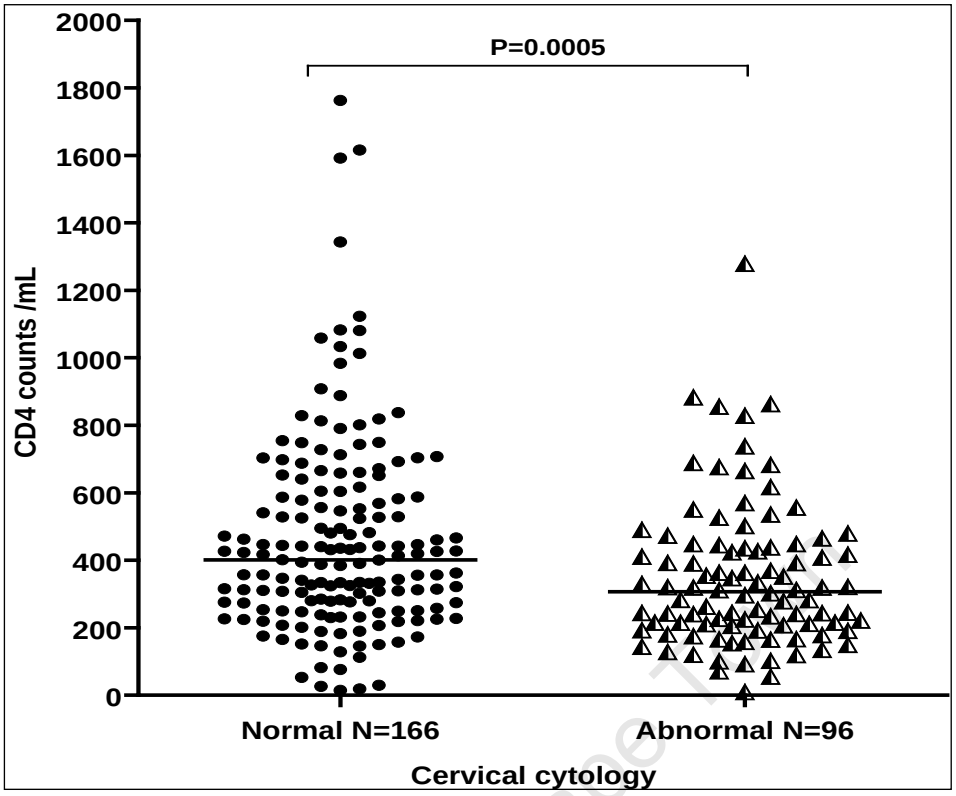

Figure 2.7. The levels of CD4 counts in HIV-positive women with normal and abnormal cytology.

HIV-positive women with normal cytology had a significantly higher prevalence of any HPV compared to HIV-negative women (66% 109/166; 28% 44/156 respectively, P<0.0001) and women with ASCUS (87% 20/23; 47% 9/19 respectively, P<0.0001). Both HIV-positive and negative women with LSIL or HSIL showed a similar prevalence of HPV. HIV-positive women with normal cytology had a significantly higher prevalence of HR-HPV prevalence compared to HIV-negative women (46% 76/166; 21% 32/156 respectively, P<0.0001) and women with ASCUS (78% 18/23; 37% 7/19 respectively, P=0.006). Both HIV-positive and negative women with LSIL or HSIL had a similar HR-HPV prevalence. HIV-positive women with normal cytology were also found to have a significantly higher prevalence of LR-HPV prevalence compared to HIV-negative women (50% 83/166; 15% 24/156 respectively, P<0.0001), women with ASCUS (48% 11/23; 16% 3/19 respectively, P=0.03) and women with LSIL (67% 42/63; 29% 5/17 respectively, P=0.006). HIV-positive and negative women with HSIL had a similar LR-HPV prevalence but the sample size was small (Table 2.3).

Women with abnormal cervical cytology were found to have a significantly higher prevalence of α3, α5, α6, α7, α9, α10 and α1/α8/α11/α13/α15 HPV species compared to women with

normal cervical cytology (Table 2.4). In women with abnormal cervical cytology the frequently detected HPV species were in descending order of prevalence, α3 (61.9% 83/135) α9 (61.2% 82/135) α7 (49.3% 66/135), α6 (29.1% 39/135) α5 (21.6% 29/135) α1/α8/α11/α13/α15 (14.9% 20/135) and then α10 (13.4% 18/135). HPV-16 and -18 were detected in 58% (7/12) of women with HSIL even though only 12 study participants had HSIL

Table 2.3. The prevalence of HPV infection in HIV-positive and HIV-negative women according to cervical cytology (normal, ASCUS, LSIL and HSIL).

		Any HPV		**P-value**	**HR-HPV**		**P-value**	**LR-HPV**		**P-value**
		%	n/t		%	n/t		%	n/t	
Normal	HIV-pos	66	109/166	**<0.0001**	46	76/166	**<0.0001**	50	83/166	**<0.0001**
	HIV-neg	28	4/156		21	32/156		15	24/156	
ASCUS	HIV-pos	87	20/23	**0.006**	78	18/23	**0.006**	48	11/23	**0.03**
	HIV-neg	47	9/19		37	7/19		16	3/19	
LSIL	HIV-pos	94	59/63	0.71	87	55/63	0.23	67	42/63	**0.006**
	HIV-neg	94	16/17		76	13/17		29	5/17	
HSIL	HIV-pos	100	10/10	na	100	10/10	na	60	6/10	0.68
	HIV-neg	100	2/2		100	2/2		50	1/2	

n/t: HPV positive samples/ total number of samples. na: not analysed

In women with normal cervical cytology the frequently detected HPV species, in descending order were α3 (25.5% 82/322) α9 (18% 58/322) α7 (18% 58/322) α1/α8/α11/α13/α15 (9.6% 31/322) α6 (8.4% 27/322) α5 (6.8% 22/322) and then α10 (3.4% 11/322). HIV-positive women with abnormal or normal cervical cytology were found to have high prevalence of all species compared to HIV-negative women with abnormal or normal cervical cytology respectively. When looking at α9 HPV species the most prevalent species in women with abnormal cervical cytology, HIV-positive women with abnormal cervical cytology were found to have significantly higher prevalence of HPV species compare to HIV-negative women with abnormal cervical cytology (72.9% 70/96 compared to 28.9% 11/35, P<0.0001). When looking at α7 HPV species the second most prevalent species in cervical cancer, HIV-positive women with abnormal cervical cytology were found to have significantly higher prevalence of HPV species compare to HIV-negative women with abnormal cervical cytology (58.3% 56/96 compared to 15.8% 6/38, P<0.0001). The high prevalence of α3 HPV species in women with abnormal cervical cytology was associated with multiple HPV infection and none of women with abnormal cervical cytology were found to have α3 HPV species as a single infection (Table 2.4). When we look at individual HPV types, women with abnormal cervical cytology were found to have a higher prevalence of HPV-58 (14.2%), HPV-16, -52 and 62 (13.4%) and

HPV-45 (10.4%) while women with normal cervical cytology were found to have a higher prevalence of HPV-62 and -82 (5.3%), HPV-61 (4.7%), HPV-58 (4.3%) and HPV-54 (4.0%).

Table 2.4 HPV prevalence in women according to cervical cytology (abnormal and normal) and HIV status

HPV type	Women with abnormal cytology						P-value*	Women with normal cytology						P-value#	P-value$
	All n=134		HIV+ n=96		HIV- n=38			All N=322		HIV+ n=166		HIV- n=156			
	n	%	n	%	n	%		N	%	n	%	n	%		
Any HPV	**116**	**86.6**	**89**	**92.7**	**27**	**71.1**	**0.0009**	**153**	**47.5**	**109**	**65.7**	**44**	**28.2**	**<0.0001**	**<0.0001**
HR-HPV	**105**	**78.4**	**83**	**86.5**	**22**	**57.9**	**0.0003**	**108**	**33.5**	**76**	**45.8**	**32**	**20.5**	**<0.0001**	**<0.0001**
LR-HPV	**68**	**50.7**	**59**	**61.5**	**9**	**23.7**	**<0.0001**	**107**	**33.2**	**83**	**50.0**	**24**	**15.4**	**<0.0001**	**0.0006**
α3	**83**	**61.9**	**77**	**80.2**	**6**	**15.8**	**<0.0001**	**82**	**25.5**	**73**	**44.0**	**8**	**5.1**	**<0.0001**	**<0.0001**
HPV61	15	11.2	13	13.5	2	5.3	0.14	15	4.7	12	7.2	2	1.3	**0.009**	**0.01**
HPV62	18	13.4	17	17.7	1	2.6	**0.02**	17	5.3	16	9.6	1	0.6	**0.0003**	**0.003**
HPV72	12	9.0	11	11.5	1	2.6	0.09	12	3.7	11	6.6	1	0.6	**0.005**	**0.02**
HPV81	13	9.7	12	12.5	1	2.6	0.07	4	1.2	4	2.4	0	0.0	**0.05**	**<0.0001**
HPV83	9	6.7	9	9.4	0	0.0	**0.04**	17	5.3	14	8.4	3	1.9	**0.009**	0.56
HPV84	12	9.0	12	12.5	0	0.0	**0.01**	9	2.8	9	5.4	0	0.0	**0.002**	**0.006**
HPV89	4	3.0	3	3.1	1	2.6	0.68	8	2.5	7	4.2	1	0.6	**0.04**	0.49
α5	**29**	**21.6**	**34**	**35.4**	**1**	**2.6**	**<0.0001**	**22**	**6.8**	**12**	**7.2**	**4**	**2.6**	**0.05**	**<0.0001**
HPV26	5	3.7	5	5.2	0	0.0	0.18	1	0.3	1	0.6	0	0.0	0.52	**0.009**
HPV51	13	9.7	13	13.5	0	0.0	**0.01**	8	2.5	6	3.6	2	1.3	0.163	**0.0009**
HPV69	1	0.7	7	7.3	0	0.0	0.09	8	2.5	1	0.6	1	0.6	0.74	0.2
HPV82	5	3.7	5	5.2	0	0.0	0.18	3	0.9	3	1.8	0	0.0	0.14	**0.05**
IS39	5	3.7	4	4.2	1	2.6	0.56	2	0.6	1	0.6	1	0.6	0.74	**0.03**
α6	**39**	**29.1**	**37**	**38.5**	**2**	**5.3**	**<0.0001**	**27**	**8.4**	**23**	**13.9**	**4**	**2.6**	**0.0003**	**<0.0001**
HPV53	17	12.7	15	15.6	2	5.3	0.09	14	4.3	11	6.6	3	1.9	**0.04**	**0.001**
HPV56	10	7.5	10	10.4	0	0.0	**0.03**	3	0.9	3	1.8	0	0.0	0.14	**0.0001**
HPV66	12	9.0	12	12.5	0	0.0	**0.01**	10	3.1	9	5.4	1	0.6	**0.01**	**0.008**
α7	**66**	**49.3**	**56**	**58.3**	**6**	**15.8**	**<0.0001**	**58**	**18.0**	**48**	**28.9**	**8**	**5.1**	**<0.0001**	**<0.0001**
HPV18	13	9.7	12	12.5	1	2.6	0.07	10	3.1	9	5.4	1	0.6	**0.01**	**0.004**
HPV39	11	8.2	6	6.3	1	2.6	0.36	8	2.5	4	2.4	2	1.3	0.37	**0.006**
HPV45	14	10.4	13	13.5	1	2.6	**0.05**	11	3.4	11	6.6	0	0.0	**0.001**	**0.003**
HPV59	6	4.5	6	6.3	0	0.0	0.13	6	1.9	4	2.4	2	1.3	0.37	0.11
HPV68	12	9.0	11	11.5	1	2.6	0.09	7	2.2	6	3.6	1	0.6	**0.07**	**0.001**
HPV70	10	7.5	8	8.3	2	5.3	0.42	16	5.0	14	8.4	2	1.3	**0.003**	0.3
α9	**82**	**61.2**	**70**	**72.9**	**11**	**28.9**	**<0.0001**	**58**	**18.0**	**46**	**27.7**	**9**	**5.8**	**<0.0001**	**<0.0001**
HPV16	18	13.4	16	16.7	2	5.3	0.08	11	3.4	9	5.4	2	1.3	**0.04**	**<0.0001**
HPV31	8	6.0	7	7.3	1	2.6	0.28	4	1.2	2	1.2	2	1.3	0.66	**0.008**
HPV33	8	6.0	8	8.3	0	0.0	0.06	7	2.2	4	2.4	2	1.3	0.37	**0.04**
HPV35	11	8.2	10	10.4	1	2.6	0.13	10	3.1	8	4.8	1	0.6	**0.02**	**0.02**
HPV52	18	13.4	15	15.6	2	5.3	0.09	11	3.4	9	5.4	1	0.6	**0.01**	**<0.0001**
HPV58	19	14.2	14	14.6	5	13.2	0.83	14	4.3	13	7.8	1	0.6	**0.002**	**0.0002**
HPV67	0	0.0	0	0.0	0	0.0	..	1	0.3	1	0.6	0	0.0	0.52	0.7
α10	**18**	**13.4**	**16**	**16.7**	**2**	**5.3**	**0.08**	**11**	**3.4**	**9**	**5.4**	**2**	**1.3**	**0.04**	**0.0001**
HPV6	8	6.0	7	7.3	1	2.6	0.28	2	0.6	2	1.2	0	0.0	0.26	**0.001**
HPV11	4	3.0	3	3.1	1	2.6	0.68	1	0.3	1	0.6	0	0.0	0.52	**0.03**
HPV55	6	4.5	6	6.3	0	0.0	0.13	8	2.5	6	3.6	2	1.3	0.18	0.2
α1/α8/α11/ α13/α15	**20**	**14.9**	**20**	**20.8**	**0**	**0.0**	**0.002**	**31**	**9.6**	**27**	**16.3**	**4**	**2.6**	**<0.0001**	0.108
HPV40	2	1.5	2	2.1	0	0.0	0.511	3	0.9	3	1.8	0	0.0	**0.09**	0.46
HPV42	4	3.0	4	4.2	0	0.0	0.26	3	0.9	2	1.2	1	0.6	0.6	0.12
HPV54	9	6.7	9	9.4	0	0.0	0.29	13	4.0	11	6.6	2	1.3	**0.015**	0.23
HPV64	0	0.0	0	0.0	0	0.0	..	0	0.0	0	0.0	0	0.0	..	..
HPV71	5	3.7	5	5.2	0	0.0	0.18	12	3.7	11	6.6	1	0.6	**0.005**	0.99

Abnormal cytology includes ASCUS, LSIL and HSIL. * comparing HIV-positive and HIV-negative women with abnormal cytology. # comparing HIV-positive and HIV-negative women with normal cytology. $ comparing all women with abnormal cytology and those with normal cytology. Due to HPV multiple infection some of women are counted more than once. **α1** HPV species includes HPV-42. **α3** HPV species includes HPV-61, -62, -72, -81, -83, -84 and -89. **α5** HPV species includes HPV-26, -51, 69, -82 and –IS39. **α6** HPV species includes HPV-53, -56 and -66. **α7** HPV species includes HPV-18, -39, -45, -59, -68 and -70. **α8** HPV species includes HPV-40 . **α9** HPV species includes HPV-16, -31, -33, -35, -52, -58 and -67. **α10** HPV species includes HPV-6, -11 and -55. **α11** HPV species include HPV-73. **α13** HPV species includes HPV-54. **α15** HPV species includes HPV-71. Bold p-values are statistically significant.

2.4 DISCUSSION

This study examined the HPV prevalence in HIV-positive and HIV-negative women and men. To our knowledge this is the first report on genital HPV in HIV-positive and HIV-negative men that were not recruited from a STI clinic but from the general population in South Africa. The impact of HIV co-infection on genital HPV prevalence was evident in the present study where we observed a higher prevalence of cervical HPV infection (74%) in HIV-positive compared to HIV-negative women (37%). A similar HPV prevalence of 70% among HIV-positive South African women has been reported else where (Dols *et al*., 2011). Tobian *et al.*, (2011) also reported a significant higher HPV prevalence between HIV-positive and HIV-negative Ugandan women (74% compared to 48%, P<0.001). However, Baay *et al*., (2004) reported a lower HPV prevalence in HIV-positive women (54%) and HIV-negative women (27%) compared to what was observed in our study.

The prevalence of HPV infection in our study was also higher in HIV-positive men (81%) compared to HIV-negative men (49%). Müller *et al*., (2010) also reported a significant higher HPV prevalence in HIV-positive South African men compared to HIV-negative South African men (90.4% compared to 65.1%, P<0.001). Müller *et al*., (2010) observed a higher HPV prevalence (65.1%) among HIV-negative men compared to our study (49%). The high prevalence of genital HPV in HIV-positive women and men was previously reported by Palefsky *et al*., (1999) and by Gomousa-Michael *et al*., (2000). A higher prevalence of cervical and penile HPV infection in HIV-positive women and men compared to HIV-negative women and men is a result of HIV induced immune suppression. HIV co-infection was found to increase genital HPV infection in both men and women (Levi *et al*., 2002; Riva *et al*., 2007)

In our study, an increase in HPV multiple infections were also observed in both HIV-positive women and men compared to HIV-negative women and men. Similar findings were reported by Levi *et al.*, (2004); Riva *et al*., (2007) and Müller *et al*., (2010). HIV-positive and HIV-negative men displayed a higher prevalence of multiple infections and LR-HPV types compared to HIV-positive and HIV-negative women and similar findings have been reported by Bleeker *et al.*, (2005b). Women and men co-infected with HIV have a higher prevalence of multiple HPV infection and a higher HPV viral load because they are more susceptible to new HPV infections and reactivation of latent HPV infection compared to HIV-negative women and men due to their HIV-induced immune suppression (Palefsky *et al*., 1999; Strickler *et al*., 2005). Immune suppression may also provide favourable conditions for the virus resulting in a

high HPV viral load in HIV-positive women and men (Levi *et al*., 2004). Interaction between HIV proteins and HIV-induced cytokines with HPV proteins is also reported to increase the rate of HPV replication resulting in high HPV viral load in HIV co-infected individuals (Dolei *et al*., 1999; Arany and Tyring *et al*., 1998). HIV-positive women had LR-HPV types that were not detected in HIV-negative women, but it remains to be determined if these LR-HPV types favour the cervix of HIV-positive women. Riva *et al*., (2007) reported a higher prevalence of HPV-62 (LR-HPV type) in HIV-positive women compared to HIV-negative women and suggested that this was probably because HPV-62 favours an immune compromised host. HPV-62 prevalence was also higher in our study in both HIV-positive and HIV-negative women.

The distribution of HPV types in women seems to be influenced by HIV co-infection and lesion type. In our study HPV-62, -52 and -58 were the three commonly detected types among HIV-positive women. However in a study reported by Marais *et al*., (2008) HPV-16, -45 and -66 were the three most common HPV types detected in Cape Town HIV-positive women. In a study reported by Firnhaber *et al*., (2009) in Johannesburg HIV-positive women HPV-16, -35, -51 were the three most common types observed. In our study among women with abnormal cytology the three most common HPV types were HPV-58, -16 and -52. In Cape Town, women with LSIL the three most common HPV types were HPV-52, -53 and -16 in women with HSIL HPV-16, 35 and -18 were the most prevalent type (Allan *et al*., 2008). While in women with HSIL from Pretoria, HPV-35, -58 and -66 were the most prevalent types (Said *et al*., 2009). The different HPV distribution in women observed in our study compared to other South African studies could be influenced by the high percentage of women with normal cytology (71%) in our study participants. According to Clifford *et al*., (2006) the prevalence of HPV-16 and -18 increases as the cervical cytology disease severity increases while all other HPV types decrease with increasing lesion severity.

The high prevalence of HPV-6 and -11 in HIV-negative women compared to HIV-positive women can be explained by the fact that only the cervix was sample. HPV-6 and -11 are more prevalent in genital warts than in cervical cancer probable if the vagina was also sampled the prevalence would differ. In HIV-positive men, HPV-6 and -11 was more prevalent compared to HIV-negative men. Müller *et al*., (2010) also reported a high prevalence of HPV-6 and 11 among HIV-positive men compared to HIV-negative men. South African men with penile

warts demonstrated a 68.5% of HPV-6/11 prevalence (Firnhaber *et al*., 2010) however in our study penile warts data was not available.

In HIV-positive men, HIV-negative men and in HIV-negative women the prevalence of HPV was found to decrease with increasing age while in HIV-positive women there was no significant change with increasing age. HPV prevalence is reported to be high in young women and men and this could be because just started participating in sexual activities and they participate frequently in sexual activities at this age (Canadas *et al*., 2004; Schiffman & Castle 2005). The risk of HPV infection was found to decrease with the increasing age in HIV-negative women and men; and this has been reported previously (Kjaer *et al*., 2000; Cuschieri *et al*., 2004), although in this study the risk of having LR-HPV types did not significantly decrease with age in men. In few studies from African countries HPV prevalence was to found to decrease with increasing age in women (Thomas *et al*., 2004; De *et al*., 2010; Hammouda *et al*., 2011). It is important to notice that HPV prevalence was very low in some of these reports, for example in Algerian women (6.3%) compared to South African women in our study (Hammouda *et al*., 2011). In previous study on South African men in Johannesburg HPV prevalence was not found to decrease with increasing age (Müller *et al*., 2010).

De Sanjosé *et al*., (2007) reviewed distribution of HPV according to age group worldwide and found that HPV prevalence decrease with increasing age and pick up in women aged 45 years in all continents except in Asia. Banura *et al*., (2008) reported a similar HPV prevalence (74.6%) among Ugandan women of 12-24 years age group as the one observed in our study (76%). It was interesting to note that HPV-prevalence decreased from age 18-25 years to 26-35 years and then increased among older HIV-positive women. The difference in effect of age on HPV between HIV-positive and HIV-negative women could be the result of the high rate of HPV reactivation due to a more suppressed immune system in older aged women as a result of hormonal changes during menopause (Palefsky *et al*., 1999; Levi *et al*., 2004; Strickler *et al*., 2005; de Sanjosé *et al*., 2007). Numerous studies report that HPV prevalence in women declines with increasing age (Kjaer *et al*., 2000). According to Kjaer *et al*., (2000) HPV prevalence declines with age even in highly sexually active women such as sex workers. It is suggested that the HPV positive women of older age fail to clear the infection they acquired at a young age or later, due to immune senescence (Giuliano *et al*., 2002'Andersson *et al*., 2005). Immune senescence in men has not been reported. The persistence of genital HPV infection

could then increase the likelihood of cervical disease progression in these women (Wallboomer *et al*., 1999).

Generally, LR-HPV types were more prevalent among HIV-negative men compared to HIV-negative women in this study. This was probably because the samples from women were only collected at the cervix and not vulva while a single swab from men sampled penile shaft, glans and foreskin (in uncircumcised men). It has been reported that more LR-types are likely to be detected in specimens from the vagina than in samples from the cervix (Jones *et al*., 2007). The difference in genital HPV types in women and men whether HIV-positive or not can be due to the different type of epithelial cells at sampled sites in women (i.e. mucosal epithelium) and men (i.e. keratinized epithelium). It is reported that mucosal epithelium is more susceptible to HPV infection and replication compared to keratinized epithelium (Thompson *et al*., 2004). Circumcised penises are protected by keratinized stratified squamous epithelium compared to the mucosal epithelium of the cervix in women (Castellsague *et al*., 2002). In our study the majority of men were traditionally circumcised and their circumcision was not medical confirmed. A high percentages of men circumcised traditionally have an incomplete circumcision with some foreskin still remaining on the glans (Morris, 2007; Bailey *et al*., 2008).

Not surprisingly the prevalence of HPV infection was found to increase with increasing cervical abnormalities confirming the role played by HPV infection in cervical abnormality and validity of the test used. All women with HSIL were found to have HPV infection, particularly HR-HPV types as reported by others (Walboomers *et al*., 1999; Lie *et al*., 2005). Among women with abnormal cytology the majority were HIV-positive. HIV-positive women with abnormal cytology were also found to have a lower CD4 count compared to those with normal cytology. Firnhaber *et al*., (2010) also reported that HIV-positive women with <200/mL CD4 count are at increased risk of cervical lesions compared to those with >500/mL CD4 counts.

Women with abnormal cytology were found to have significantly higher prevalence of α3, α5, α6, α7, α9 and α10 HPV species compared to women with normal cytology but not α1/α8/α11/α13/α15 HPV species. HIV-positive women were also found to have higher prevalence of all HPV species regardless of cytology results. The α3 HPV species were found to be the most frequently detected species followed by α9 and α7 HPV species in both women with abnormal and normal cytology. Kovacic *et al*., (2006) also reported high prevalence of α9

and α7 HPV species in women with abnormal cytology compared to women with normal cytology and that the proportion of cytologic abnormality varies by HPV type. The high prevalence of α3 HPV species types (LR-HPV) in women with abnormal cervical cytology was because of high prevalence of multiple infections in women with abnormal cervical cytology and these types were always found co-infected with HR-HPV types. Majority of women with abnormal cervical cytology were HIV-positive and the co-infection of α3 HPV species was more prevalent among HIV-positive-women. South African HIV-positive women have been reported to have higher prevalence of co-infection with LR-HPV types and this is found to increase with decreasing CD4 counts (Richter *et al*., 2008).

HPV-16 and -18 were not the dominant HPV types in this cohort, the reason of these findings is that majory of study participants had normal cervical cytology while only 2.5% (12/483) women had HSIL. Even though only 12 HSIL cases were observed in our study participants, HPV-16 and -18 were detected in 58% of women with HSIL. Firnhaber *et al*., (2010) also reported that HPV-16 and -52 were the most dorminant types in South African women with HSIL. In Ugandan women 80% of cervical carcinoma cases are associated with HPV-16 and -18 single infections (Odida *et al*., 2008). In Nigerian women 67.6% and 10.3% of invasive cervical cancer cases are associated with HPV-16 and HPV-18 respectively (Okolo *et al*., 2010). HIV-postive women from Botswana also demonstrated a 51% HPV-16 and -18 prevalence (Ramogola-Masire *et al*., 2011). These fidings indicate that proper itroduction of HPV vaccines in African coutries will be of great benefit in women.

In conclusion, data from this study showed a significant difference in HPV prevalence including multiple infections between HIV-positive women and HIV-negative women as well as HIV-positive men and HIV-negative men. We also demonstrated that among HIV-negative women HPV prevalence decreases with increasing age and peaks at age 46-66 years however in HIV-positive women the peak of HPV prevalence is observed earlier at age 36-45 years, suggesting that HIV-positive women require different cervical cytology screening strategy than the one of HIV-negative. Among men, HPV prevalence was found to decrease with increasing age regardless of HIV-status, however among HIV-negative men the LR-HPV prevalence was not found to significantly decrease with increasing age. We also demonstrate that women with abnormal cytology have high prevalence of HPV infection and the most dominant species were α9 and α7 HPV species. The α3 HPV species were more prevalent among women with

abnormal cytology that were HIV-negative not as single infection. The data from this study provides information on distribution of HPV types according to HIV status and cervical disease severity in women. The high prevalence of HPV-16, -18 and HPV related types in HIV-positive women and women with HSIL indicate that proper introduction of HPV vaccine in South Africa will be of great benefit as well as regular cervical screening in HIV-positive women.

CHAPTER 3: HUMAN PAPILLOMAVIRUS INFECTION IN HIV-SERONEGATIVE, HIV-SEROPOSITIVE AND HIV-DISCORDANT HETEROSEXUALLY ACTIVE COUPLES

3.1. INTRODUCTION

HPV is the most common sexually transmitted virus and 75% of sexually active women and men will acquire genital HPV infection at some time in their life. HPV infection can be acquired even if a sexual act is not penetrative sex (Kjaer *et al*., 2001; Schiffman & Castle, 2005; Palefsky *et al*., 2007). The highest prevalence of HPV infection and multiple HPV infections are observed in young women and men, soon after sexual debut (Kjaer *et al*., 2001; Schiffman & Castle, 2005). Women and men with a high number of current and life sexual partners and those who started sexual debut at a younger age are reported to be at increased risk of HPV infection and HPV associated disease (Kahn *et al*., 2002; Winer *et al*., 2006; Burchell *et al*., 2006, Table 3.1). The high risk sexual behavior of an individual's sexual partner is also reported to increase the risk of HPV infection and HPV associated disease (Munoz *et al*., 1996; Bosch *et al*., 1996; Winer *et al*., 2008; Trottier *et al*., 2010). A sexual partner's HPV-positive status increases the risk of HPV in women and men (Burchell *et al*., 2010a). Forming partnerships with older partners or a more sexually experience partner may increase the risk of HPV acquisition especially in young women where the cervix is still immature with an inadequate production of protective cervical mucus and increased cervical ectopy (Collins *et al*., 2005; Winer *et al*., 2008). Simultaneous infection with other STIs and immune suppression due to infection with HIV or transplantation are also associated with increased likelihood of HPV acquisition and transmission (Table 3.1, Burchell *et al*., 2006; Palefsky *et al*., 2007)

Table 3.1. Factors that increase or decrease the risk of HPV acquisition and transmission (Burchell *et al*., 2006).

	Hypothesized to affect likelihood of exposure to HPV-infected partner	Hypothesized to affect likelihood of transmission upon exposure through effects on. . .	
		Infectivity/duration	Susceptibility
Early age at sexual debut	↑		↑
Greater number of partners	↑		
Similarity or dissimilarity between individuals and their sexual partner(s)	↑/↓		
Acquisition of new partner	↑		
Concurrent/extra-dyadic partners	↑		
Short intervals between partners	↑		
Concomitant infection with other STI	↑	↑	↑
Male circumcision	↓	↓	↓
Condoms	↑/↓	↓	
Immune suppression (e.g., HIV infection, transplantation)			↑
Certain human leukocyte antigen (HLA) complex alleles and haplotypes		↑	↑
Hormonal contraceptives		↑	↑
Diet deficient in certain micronutrients		↓	
Smoking		↑	↑

Arrows indicate the direction of the association, that is, whether they increase risk via the proposed mechanism

Women with male sexual partners with HPV infection and/ or flat penile lesions are more likely to acquire HPV infection and later develop cervical disease (Heard *et al*., 2000). Men with female sexual partners with cervical or vulval HPV associated cancer are also at increased risk of becoming HPV infected and developing penile lesions (Bleeker *et al*., 2002; Bleeker *et al*., 2005b). According to Bleeker *et al*., (2002) the rate of flat penile lesion regression is lower in men with female sexual partners who are HPV positive with the same HPV type compared to those men with sexual partners who are negative, suggesting that HPV re-infection between partners may play an important role in this scenario.

The type-specific concordance in these reports varied between 2 to 64%, presumably because of the different sampling methods used. However, recent reports demonstrate a high prevalence of HPV type-specific concordance suggesting that recent, improved sampling methods afford more accurate genital HPV detection in men (Flores *et al*., 2008). There are factors that may affect HPV concordance among sexually active couples such as sexual behavior of the couple investigated, sampling method used, the sensitivity of the test used to detect DNA and the differences between acquisition rate in men and women thus resulting in different findings in different studies (Parada *et al.*, 2010).

A summary of some studies on HPV concordance and type-specific concordance among sexually active couples is presented in Table 3.2. Studies on HPV infection in sexually active couples have reported different findings with regards HPV concordance or type-specific concordance (Hippelainen *et al*., 1994; Baken *et al*., 1995; Strand *et al*., 1995; Castellsague *et al*., 1997; Rosenblatt *et al*., 2004; Bleeker *et al*., 2005a; Giovannelli *et al*., 2007). Bleeker *et al*., (2005) reported that out of all couples screened, 37% (67/181) shared at least one HPV type; however when both partners were HPV positive the HPV type-specific concordance increased to 57.8% (Bleeker *et al*., 2005a). Giovannelli *et al*., (2007) reported that all HPV positive couples evaluated in their study 64.4% (29/45) shared at least one identical HPV. Recently Parada *et al*., (2010) reported on 504 couples a 79% HPV concordance and 61.8% HPV type-specific concordance.

The aims of the study were:

(i) to investigate the HPV concordance and type-specific concordance in penile and cervical samples from heterosexually active couples that were HIV-positive, HIV-negative, HIV-

Table 3.2. Review of some studies describing HPV concordance and type-specific concordance among sexually active couples (Modified from Burchell *et al*., 2006)

Reference	Population	Sample	Age	Relationship duration	Findings
Hippelainen *et al*., 1994	Women with abnormal Pap smear and their male partners (Finland	270 couples	Women: mean 27, range 15-62. Men: mean 32, range: 17-74	Median: 18 months; mean: 41 months; range: 1-300.	6% (15/270) of couples were HPV-positive concordant for the same type.
Kyo *et al.*,1994	Women evaluated for infertility or who had CIN or cervical cancer and their male partner (Japan)	53 couples	Not reported	All married for 2+ years	17% (9/53) of couples were HPV-16 positive concordant. In couples where at least one partner had HPV (n=26), 35% were concordant. Discordance was more likely seen in female positive and male negative than female negative and male positive.
Baken *et al*., 1995	Heterosexual partners attending STD clinic (Seattle, USA)	50 couples, 45 with HPV results	Women: mean 26. Men: mean 29	Unspecified	29% (13/45) of couples were concordant for the same HPV type. In couple where at least one partner had HPV (n=41), 32% were concordant. Concordance decreased with time since last intercourse.
Castellsague *et al*., 1997	Women enrolled in case-control studies for CIN and their husbands (Spain and Columbia)	816 couples, 431 with HPV results	Men: mean 45	Excluded relationships <6 months duration	66% (286/431) of couples were HPV-positive. Of these, 2% (7/286) were HPV- type-concordant.
Franceschi *et al*., 2002	Women enrolled in case-control studies for ICC and CIS and their husbands (Spain, Columbia, Brazil, Tailand and the Philippines)	964	Men: median 45, 50, and 38 for husbands of controls women, women with ICC and women with CIS respectively	Excluded relationships <6 months duration	HPV-16 positive concordance observed in 0.02% (1/465), 4% (17/383) and 3% (4/116) of couples where the wife was control, an ICC case or a CIS case respectively
Bleeker *et al*., 2005a	Women with CIN lesion and their male partners (The Netherlands).	238 couples, 181 with HPV results	Women: mean 34.7, range: 19-55. Men: mean 37.6, range: 22-58	Mean: 10.6 years; Range: 0.6-35 years	37% (67/181) of couple have type-specific HPV concordance. In couples where HPV was present in at least one partner, 38% (67/176) were type concordant. An increasing association was seen between viral load in one partner and HPV positivity in the other.

Reference	Population	Sample	Age	Relationship duration	Findings
Giovannelli *et al*., 2007	Women with CIN and their male partners (Italy).	73 couples, 45 with HPV results	Women: mean 31, range: 21-51. Men: mean 36.7, range: 23-58	>6 months as monogamous couple.	64.4% (29/45) of couples had HPV type-specific concordance.
Benevolo *et al*., 2007	women with vulvar condylomatosis and male partner had flat aceto-whitening lesion	1 couple	33 years old women and 35 years old men	Not specified, however the relationship was a stable and monogamous	HPV type specific concordance was observed for 5 different HPV types
Hernandez *et al*., 2008	Women with their male partners (Hawaii)	38 couples, 25 with HPV results	Women: mean 26, range: 18-57 years. Men: mean 28, range: 18-59 years	Not specified	56% (14/25) of couple have HPV concordance. In couples where HPV was present in at least one partner, 79% 11/14) were type-specific concordant.
Burchell *et al*., 2009*	Women with intact uterus and no history of cervical lesions/cancer and male partners	263 couples	Women and men: age range: 18-24 years	<6 months	48% (125/263) both HPV positive. Among couples for whom at least one partner was HPV infected 64% were type-specific concordant for 1 or more types. Current partner's status was important risk factor for prevalent HPV infection.
Parada *et al*., 2010	Clinically healthy women and their male partners (Mexico)	504 couples	Women and men: age range: 18-75 years	>6 months	79% of HPV concordance. 61.8% showed type-specific concordance.

Modified from Burchell *et al*., 2006. Note * Burchell *et al*., (2010b) was reported in Burchell *et al*., (2009), however in Burchell *et al*., (2010b) more factors were investigated.

discordant couples where the female partner was HIV-positive and HIV-discordant couples where the male partner was HIV-positive;

(ii) to investigate the influence of sexual partner's HPV positive status on an individual's genital HPV and the impact of HIV configuration of the partnership;

(iii) to investigate the influence of HIV co-infection and other factors on HPV prevalence in women and men; and

(iv) to investigate the effect of women abnormal cervical cytology on the HPV prevalence of a male partner and HPV sharing between couples.

3.2 MATERIALS AND METHODS

3.2.1 Study population, specimen collection and HPV genotyping

A total of 601 black heterosexually active couples were recruited between 2006 and 2009 from the Manyanani clinic, Empilisweni centre, Gugulethu, Cape Town. For this study a total of 486 black heterosexually active couples were randomly enrolled, of these 162 were both HIV-negative, 115 were both HIV-infected, 163 were HIV-discordant in which only the female partner was HIV-positive and 46 were HIV discordant in which only the male partner was HIV-positive. The reason the remaining 115 couples were not enrolled in this study is because they were HIV-negative couples. Since there were sufficient (162 HIV-negative couples) to make statistically relevant conclusions they were not included. The mean age of women and men participants was 35 years (range: 18-66 years) and 38 years (range: 19-67 years) respectively. Samples were collected as described in chapter 2 section 2.2.1. HPV genotyping was performed as described in chapter 2 section 2.2.2.

3.2.2 Statistical analysis

Factors affecting the risk of any HPV infection were assessed using multivariate logistic regression. All analyses were conducted for HR-HPV types and LR-HPV types separately, as well as for HR-HPV and LR-HPV types combined. All statistical analyses were conducted using STATA 11.0 (StataCorp, College Station, TX, USA) by Dr Leigh Johnson (Centre for Infectious Disease, Epidemiology and Research, University of Cape Town) and X^2 test (EpiInfo Version 5 Statcalc). In all analyses P-values ≤ 0.05 were considered significant.

3.3 RESULTS

3.3.1 HPV concordance and HPV type-specific concordance in couples according to HIV status

To ensure sample adequacy, the presence of the β-globin gene was evaluated and found to be negative in 0.4% (2/486) cervical samples and in 3.1% (15/486) penile samples. All β-globin gene negative samples were excluded from the analysis resulting in analysis on samples from 469 couples. HPV concordance was defined as both partners being HPV positive with any HPV types. Type-specific HPV concordance among couples was defined as the presence of the same HPV genotypes in cervical and penile cells of a couple. Type-specific HPV concordance was also referred to as HPV sharing. In HIV-negative couples, 10% (16/155) showed type-specific HPV concordance; among these couples 15 shared 1 HPV type while 1 couple shared 2 HPV types. In HIV-positive couples 48% (54/112) showed type-specific HPV concordance; 28 couple's shared 1 HPV type and 26 shared 2-10 HPV types. In HIV-discordant couples where the female partner was HIV-positive, 30% (47/158) showed HPV type-specific concordance; 22 couple's shared 1 HPV type and 25 couples shared 2-11 HPV types. In HIV-discordant couples where the male partner was HIV-positive, 11% (5/44) showed type-specific HPV concordance; 3 couple's shared 1 HPV type and 2 couples shared only 2 HPV types (Table 3.3).

Table 3.3. Type-specific HPV concordance in HIV-negative couples, HIV-positive couples, HIV-discordant couples where the female partner was HIV-positive and HIV discordant couples where the male partner was HIV-positive.

	Both HIV+		**HIV-discordant female HIV+**		**HIV-discordant male HIV+**		**Both HIV-**	
	N=112		**N=158**		**N=44**		**N=155**	
Only female is HPV+	7	6%	38	24%	6	14%	25	16%
Only male is HPV+	17	15%	18	11%	11	25%	33	21%
HPV concordance*	77	69%	81	51%	16	36%	27	17%
Type-specific HPV concordance#	54	48%	47	30%	5	11%	16	10%
1 HPV type	28/54	52%	22/47	47%	3/5	60%	15/16	94%
2-11 HPV types	26/54	48%	25/47	53%	2/5	40%	1/16	6%

* HPV concordance is defined as both partners being HPV positive with any HPV types (different or similar).
# Type-specific HPV concordance is the number of couples who had the same HPV type(s). HPV+: HPV-positive. HIV-: HIV-negative

In the adjusted assessment, for men, HIV infection and female partner HIV-positive status were both associated with a higher risk of type-specific HPV concordance as their sexual partner, though the associations were not significant for LR-HPV types. In women, their HIV-positive status and low CD4 count were significantly associated with increased risk of type-specific HPV concordance, but their male partner's HIV-positive status and low CD4 counts were not significantly associated with type-specific HPV concordance instead male partner's HIV-positive status demonstrated a negative association (Table 3.4). The risk of type-specific HPV concordance was not significantly associated with any demographic characteristics or reported sexual behaviour, in either men or women (data not shown).

3.3.2 The influence of a sexual partner's HPV positive status on an individual's genital HPV and the impact of the HIV configuration of the partnership

HPV infection was significantly higher in men with female partners who were HPV positive compared to men with female partners who were HPV negative (72% compared to 40%; P<0.001, Table 3.5). Similarly more women had genital HPV if their male partners were HPV positive compared to women with male partners who were HPV negative (72% compared to 40%; P<0.001). However, increased HPV prevalence was influenced by HIV co- infection, the risk being higher in women and men from a partnership where both partners were HIV-positive or where only the female partner was HIV-positive and lower in partnerships where both partners were HIV-negative. In HIV-discordant couples where the male partner was HIV-positive, genital HPV was also more prevalent in women and men with HPV-positive partners compared to those whose partners were HPV-negative, although the difference was not statistically significant (Table 3.5).

3.3.3 The influence of HIV infection and other factors on HPV prevalence in men

In the adjusted assessment, for men the risk of having any HPV type and any HR-HPV was found to significantly decrease with increasing age (AOR, 0.77 [95% CI: 0.63-0.93] and AOR, 0.70 [95% CI: 0.57-0.86] respectively, per 10-year increase in age; Table 3.6). In contrast, the risk of having LR-HPV did not significantly decrease with increasing age in men (Table 3.6). HIV-positive men were found to be at higher risk of any HPV type compared to HIV negative men and the risk was found to increase with decreasing CD4 count levels (CD4 counts of ≥350/mL: AOR, 2.54 [95% CI: 1.41-4.54]; CD4 counts of <350/mL: AOR, 3.32 [95% CI:

Table 3.4. The association between type-specific HPV concordance and HIV variables in couples (multivariate analysis).

	All HPV types						Only HR-HPV types						Only LR-HPV types					
	men			women			men			women			men			women		
	AOR	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**
HIV-	ref		0.06	ref		**<0.0001**	ref		**0.023**	ref		**0.0001**	ref		0.42	ref		**<0.0001**
HIV+, CD4 ≥350	1.12	0.61-2.1		2.99	1.63-5.48		1.02	0.49-2.14		2.64	1.28-5.45		1.22	0.61-2.42		3.5	1.46-8.37	
HIV+, CD4 <350	2.02	1.12-3.6		5.82	3.1-10.1		2.23	1.21-4.13		4.24	2.19-8.23		1.72	0.77-3.85		8.4	3.46-20.4	
Partner HIV-	ref		**0.005**	ref		0.08	ref		**0.027**	ref		0.14	ref		0.1	ref		0.17
Partner HIV+, CD4 ≥350	1.81	0.98-3.4		0.61	0.34-1.1		1.84	0.9-3.74		0.64	0.32-1.28		1.69	0.64-4.47		0.58	0.28-1.18	
Partner HIV+, CD4 <350	2.76	1.51-5.1		0.55	0.33-0.97		2.6	1.29-5.22		0.55	0.29-1.02		2.71	1.02-7.22		0.58	0.29-1.17	

ref: Reference. HIV-: HIV-negative. HIV+: HIV-positive. AOR: adjusted odd ratio. Sexual behavior and demographic variables were not statistically significant and were therefore not included in the analysis.

Table 3.5. The influence of a sexual partner's HPV status on genital HPV in men and women and the impact of the HIV configuration of the partnership.

	Men		Women				
	n	**% with HPV**	**n**	**% with HPV**	**OR***	**95% CI***	**P-value**
All couples (n = 469)							
Partner HPV-negative	194	40%	194	40%	ref		
Partner HPV-positive	275	72%	275	72%	3.76	2.55-5.54	**<0.001**
HIV-negative couples (n = 155)							
Partner HPV-negative	103	32%	96	27%	ref		
Partner HPV-positive	52	50%	59	44%	2.12	1.07-4.2	**0.031**
HIV-positive couples (n = 112)							
Partner HPV-negative	28	61%	18	39%	ref		
Partner HPV-positive	84	92%	94	82%	7.12	2.41-21.0	**<0.001**
HIV-discordant couples, female HIV-positive (n = 158)							
Partner HPV-negative	40	40%	63	62%	ref		
Partner HPV-positive	118	67%	95	83%	3.04	1.45-6.37	**0.003**
HIV-discordant couples, male HIV-positive (n = 44)							
Partner HPV-negative	23	52%	17	35%	ref		
Partner HPV-positive	21	71%	27	56%	2.29	0.66-8.01	0.194

* Odds ratio for the association between male HPV and female HPV. ref- reference

1.82-6.04]); this was also the case for both HR-HPV types (CD4 counts of ≥350/mL: AOR, 2.91 [95% CI: 1.7-4.98]; CD4 counts of <350/mL: AOR, 3.1 [95% CI: 1.82-5.28]) and LR-HPV types (CD4 counts of ≥350/mL: AOR, 2.46 [95% CI: 1.45-4.17]; CD4 counts of <350/mL: AOR, 3.18 [95% CI: 1.86-5.42], Table 3.6).

None of the sexual behaviour characteristics were included in the multivariate analysis, these included total number of sexual partners, new sex partners last year, age at first sex, number of sexual acts with the study partner last month, duration of relationship with the study partner and condom use, as they did not significantly affect male HPV risk. Men with HIV-positive female partners were found to be at higher risk of any HPV, HR-HPV and LR-HPV infection compared to men with HIV-negative female partners. In men with HIV-positive female partners the risk of any HPV type was found to increase with decreasing CD4 count levels of their partner compared to men with HIV-negative female partner (CD4 counts of ≥350/mL: AOR, 2.37 [95% CI: 1.1.47-3.83]; CD4 counts of <350/mL: AOR, 3.02 [95% CI: 1.86-4.9]); this was also true for HR-HPV type (CD4 counts of ≥350/mL: AOR, 2.17 [95% CI: 1.34-3.5]; CD4 counts of <350/mL: AOR, 2.32 [95% CI 1.45-3.73]) and LR-HPV type (CD4 counts of ≥350/mL: AOR, 2.13 [95% CI: 1.33-3.41]; CD4 counts of <350/mL: AOR, 2.37 [95% CI: 1.49-3.78], Table 3.6).

Table 3.6. Risk factors for human papillomavirus **(**HPV) in men (multivariate analysis).

	All HPV types			Only HR-HPV types			Only LR-HPV types		
	AOR	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**
Age (per 10 yr increase)	0.77	0.63-0.93	**0.009**	0.70	0.57-0.86	**0.001**	0.91	0.75-1.11	0.375
HIV-negative	ref		**<0.001**	ref		**<0.001**	ref		**<0.001**
HIV-positive, CD4 ≥350/mL	2.54	1.41-4.54		2.91	1.7-4.98		2.38	1.4-4.03	
HIV-positive, CD4 <350/mL	3.32	1.82-6.04		3.1	1.82-5.28		3.09	1.81-5.27	
Partner HIV-negative	ref		**<0.001**	ref		**<0.001**	ref		**<0.001**
Partner HIV-positive, CD4 ≥350/mL	2.37	1.47-3.83		2.17	1.34-3.5		2.14	1.34-3.42	
Partner HIV-positive, CD4 <350/mL	3.02	1.86-4.9		2.32	1.45-3.73		2.54	1.59-4.04	

ref: Reference. AOR: adjusted odd ratio. Sexual behavior variables were not statistically significant and were therefore not included in the analysis.

3.3.4 The influence of HIV infection and other factors on HPV prevalence in women

In the adjusted assessment for women the risk of having any HPV type, was found to significantly decrease with increasing age, and the same relationship was observed for HR-HPV and LR-HPV (Table 3.7). HIV-positive women were found to be at higher risk of any HPV type compared to HIV-negative women and the risk was found to increase with decreasing CD4 count levels among HIV-positive women (CD4 counts of ≥350/mL: AOR, 3.26 [95% CI: 2.04-5.2]; CD4 counts of <350/mL: AOR, 6.86 [95% CI: 4.08-11.54]); this was also the case for HR-HPV type (CD4 counts of ≥350/mL CD4: AOR, 2.89[95% CI: 1.81-4.6]; CD4 counts of <350/mL: AOR, 4.22 [95% CI: 2.64-6.76]) and LR-HPV type (CD4 counts of ≥350/mL CD4: AOR, 4.72 [95% CI: 2.82-7.92]; CD4 counts of <350/mL CD4: AOR, 6.31 [95% CI: 3.78-10.52]) (Table 3.7). The risk of HPV was not found to differ between women with a HIV-positive male partner and women with a HIV-negative male partner, either for HR or LR types. Neither the HIV-positive status nor the level of the CD4 count of the male partner influenced HPV prevalence in women (Table 3.7).

3.3.5 The effect of female abnormal cervical cytology on the HPV prevalence of a male partner and HPV sharing between couples

Men with a female partner with abnormal cervical cytology were found to have a higher prevalence of any HPV, HR-HPV and LR-HPV types (67% 88/131; 54% 71/131 and 50% 66/131), but the prevalence was statistically significant for any HPV and HR-HPV types not LR-HPV types, compared to men with a female partner with normal cervical cytology (57% 178/315; 40% 126/315 and 46% 146/315 respectively, P=0.04 for any HPV type, P=0.006 for HR-HPV types and P=0.44 for LR-HPV types; Figure 3.1). The impact of abnormal cervical cytology on HPV type-specific concordance was investigated. To do this, couples were stratified by HIV status and whether the women had normal or abnormal cervical cytology. The degree of HPV type-specific concordance in HIV discordant, HIV-negative and HIV-positive couples was found to increase where female partners showed abnormal cervical cytology (ASCUS, LSIL and HSIL) compared to those where the female partner had normal cervical cytology (68% 89/131 compared to 34% 108/314, P<0.0001, Figure 3.1). However, the high HPV viral load in women with abnormal cervical cytology must be considered in these observations.

Table 3.7. Risk factors for human papillomavirus **(**HPV) in women (multivariate analysis).

	All HPV types			**Only HR-HPV types**			**Only LR-HPV types**		
	AOR	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**
Age (per 10 yr increase)	0.75	0.61-0.92	**0.006**	0.74	0.61-0.91	**0.005**	0.73	**0.59-0.92**	**0.008**
HIV-negative	ref		**<0.001**	ref		**<0.001**	ref		**<0.001**
HIV-positive, CD4 ≥350/mL	3.26	2.04-5.2		2.89	1.81-4.6		4.85	2.89-8.13	
HIV-positive, CD4 <350/mL	6.86	4.08-11.54		4.22	2.64-6.76		6.28	3.77-10.49	
Partner HIV-negative	ref		0.298	ref		0.232	ref		0.779
Partner HIV-positive, CD4≥350/mL	1.58	0.88-2.84		1.54	0.9-2.64		1.06	0.61-1.84	
Partner HIV-positive, CD4 <350/mL	1.13	0.65-1.98		0.92	0.54-1.56		1.22	0.71-2.1	

ref: Reference. AOR: adjusted odd ratio. Sexual behavior variables were not statistically significant and were therefore not included in the analysis.

A significantly higher genital HPV type-specific concordance was observed in HIV-positive couples where the female partners had abnormal cervical cytology compared to those where female partners had normal cervical cytology (87% 33/38 compared to 59% 41/70, P=0.003). A significantly higher genital HPV type-specific concordance was observed in couples that were HIV-discordant were the female partner was HIV-positive and had abnormal cervical cytology compared to those with female partners with normal cervical cytology (65% 37/57 compared to 43% 40/93, P=0.009). A significantly higher genital HPV type-specific concordance was observed in couples that were both HIV-negative with female partners with abnormal cervical cytology compared to those with female partners with normal cervical cytology (34% 11/32 compared to 13% 15/112, P=0.007, Figure 3.2). HPV type-specific concordance was not statistically different in couples that were HIV-discordant where the male partner was HIV-positive with female partners with abnormal cervical cytology compared with those with female partners with normal cervical cytology (75% 3/4 compared to 31% 12/39, P=0.11). The small number of women with normal cervical cytology in HIV-discordant relationships where the male partner was HIV-positive possibly skewed these results.

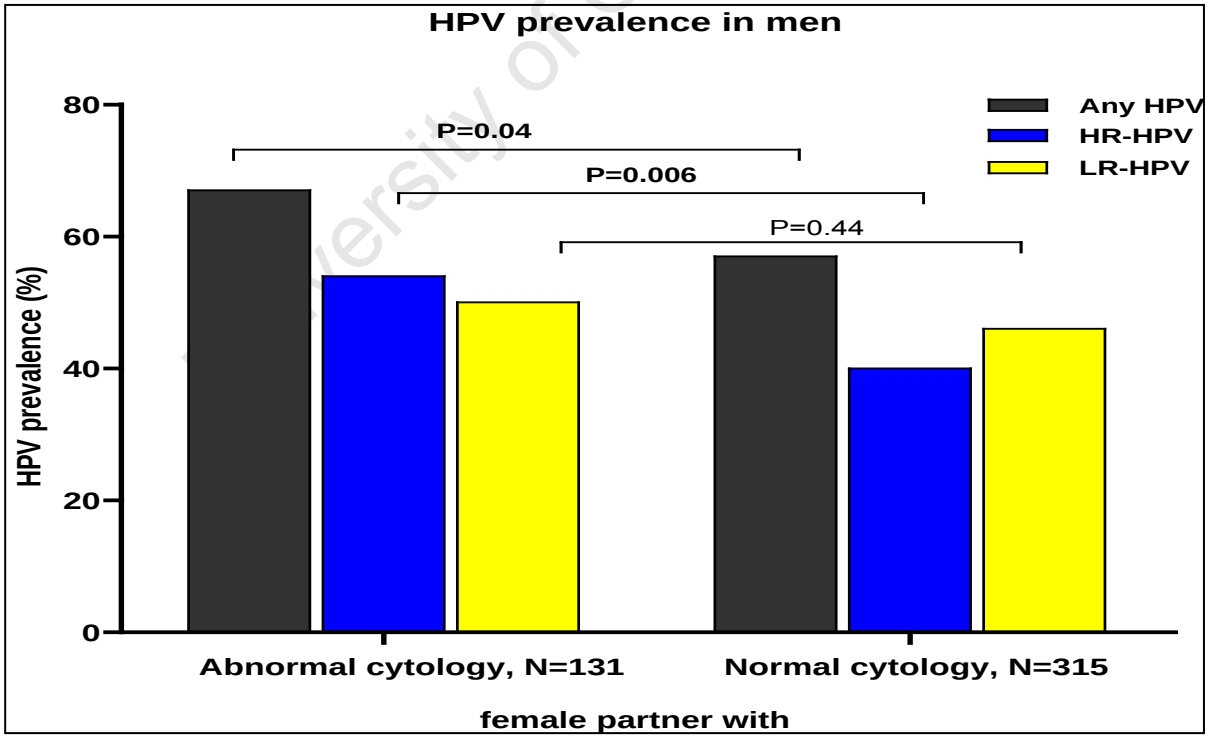

Figure 3.1. The effect of abnormal cytology of the female partner on HPV prevalence in men

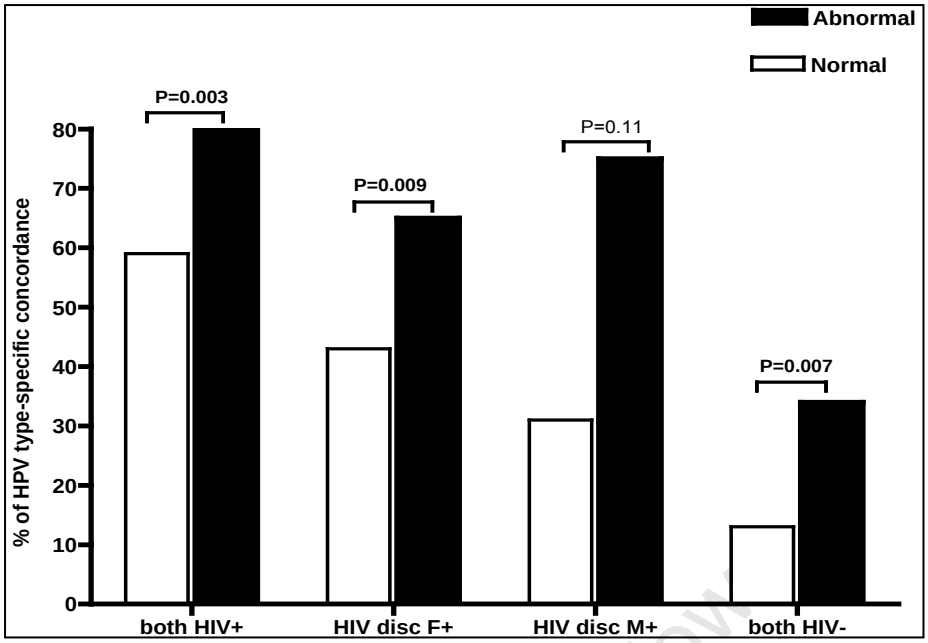

Figure 3.2. Genital HPV type-specific concordance in couples stratified for female cervical cytology, normal or abnormal (ASCUS, LSIL and HSIL). HIV disc F+: HIV discordant couples where the female partner is HIV-positive. HIV disc M+: HIV discordant couples where the male partner is HIV-positive. HIV-: HIV-negative. HIV+: HIV-positive

3.4 DISCUSSION

This study examined, in detail, the relationship between HIV and HPV prevalence among sexual couples, and demonstrates the influence of a partner's HIV positive status on HPV prevalence in women and men from HIV-concordant and HIV-discordant relationships. An individual's HIV infection was associated with increased genital HPV in both men and women, with a stronger association by decreasing CD4 count in women than men as has been earlier reported by others (Riva *et al*., 2007). Men with HIV-positive female partners were also found to have a higher risk of any HPV, HR-HPV and LR-HPV infection compared to men with HIV-negative female partners. However, women with HIV-positive male partners were not found to have a higher risk of any HR, HR-HPV or LR-HPV infection compared to women with HIV-negative male partners. These findings suggest that women's HPV risk is influenced only by their own HIV status, while the HPV prevalence in men is determined by their own HIV-positive status and that of their female partner. HIV-positive women are reported to have a higher cervical HPV viral load compared to HIV-negative women and this has been associated with low CD4 count levels suggesting that HIV co-infection may increase genital HPV viral load in women (Lefevre *et al*., 2004). The finding that a women's HPV infection

risk was not influenced by her male partner's HIV status may be explained by the low rate of male to female HPV transmission compared to female to male HPV transmission, possibly due to less virus available for transmission in men (Hernandez *et al*., 2008). The finding that men's HPV infection risk was influenced by his female partner's HIV status may also be explained by the high rate of female to male HPV transmission compared to female to male HPV transmission (Hernandez *et al*., 2008).

We demonstrated that a partner's HPV positive status increases the risk of HPV in women and men; similar observations have been reported elsewhere (Burchell *et al*., 2010a). Parada *et al*., (2010) also reported that detection of genital HPV DNA in women and men is associated with the detection of HPV in their sexual partner. HIV-negative couples were found to have a significantly lower prevalence of type-specific HPV concordance compared to couples in which both partners were HIV-positive and HIV-discordant couples in which the female partner was HIV-positive. HIV-positive and HIV-discordant couples where the female partner was HIV-positive were more likely to share multiple HPV types while HIV-negative couples were more likely to share single HPV types. The higher prevalence of type-specific HPV concordance in couples that were both HIV-positive and HIV-discordant where female partner was HIV-positive could be due to the higher prevalence of HPV observed in HIV-positive women and men. It was interesting to note that type-specific HPV sharing did not differ between HIV-negative couples and HIV-discordant couples where the male partner was HIV-positive and the number of HPV types shared was similar.

Hippelainen *et al*. (1994) showed a lower level of HPV infection in both partners (24.4%) compared with our study and the HPV type-specific concordance was only 22.7% despite the women in their study having abnormal cervical cytology (Hippelainen *et al*., 1994). Castellsague *et al*., (2002) described in a Columbian study, a high risk cervical cancer area and a Spanish study, a low risk cervical cancer area, that the prevalence of HPV concordance of any type in both partners was 9% in Columbia and 4.9% in Spain (Castellsague *et al*., 1997). The Columbian findings were similar to our findings for HIV-negative couples and the prevalence was far higher in our HIV-positive and discordant couples. Bleeker *et al*., (2005a) reported that out of all couples screened, 37% (67/181) shared at least one HPV type; however when both partners were HPV positive the HPV type-specific concordance increased to 57.8%

(Bleeker *et al*., 2005a). The HPV concordance in Bleeker *et al*., (2005a) study was not higher than the one observed in our study even though the women partners had cervical intraepithelial neoplasia while in our study only 29% of women had abnormal cytology (includes ASCUS, LSIL and HSIL). It was also found that among men type-specific HPV concordance was influenced by both their own HIV status and their female partner's HIV-positive status, while in women HPV type-specific concordance was associated with their own HIV status and low CD4 count but not their male partner's HIV-positive status. The higher frequency of type-specific HPV concordance in couples that were HIV-positive or HIV-discordant, specifically where the female partner was HIV-positive, could be the result of higher HPV-viral load seen in HIV-positive individuals due to immune suppression. It has been demonstrated that women and men with high HPV viral load more frequently shared HPV compared to those with a lower HPV viral load (Bleeker *et al*., 2005a).

We observed that men with female partners with abnormal cervical cytology were found to have a higher prevalence of HPV and HPV sharing compared to men with female partners with normal cervical cytology regardless of HIV status. Castellsague *et al*., (1997) also reported that men with female partners with cervical neoplasia had a higher HPV prevalence compared to men with female partners with normal cytology. The observed increased HR-HPV prevalence in men with female partner with abnormal cervical cytology compared to those with normal cervical cytology but not the LR-HPV prevalence can be explained by the high association of HR-HPV types with abnormal cervical cytology compared to LR-HPV types and high HPV viral load in women with abnormal cervical cytology. Women with abnormal cervical cytology were found to significantly share more HPV types compared to women with normal cervical cytology. These findings could be explained by high HPV prevalence and HPV viral load found in cervical samples from women with abnormal cervical cytology compared to normal cervical cytology. Therefore the high viral load in women with abnormal cervical cytology might increase the risk of HPV sharing with the partner and transmission (Bleeker *et al*., 2005a).

In conclusion, results from this study demonstrated that men's HIV status did not influence the prevalence of genital HPV (any type), HR-HPV or LR-HPV in their female partner whereas women's HIV-positive status increased the HPV risk of their male partner. We also demonstrated that, in men, type-specific HPV concordance with their female partner is influenced by their own HIV-status and that of their female partner. However in women type-

specific HPV concordance is influenced by their own HIV-status and not that of their male partner. Abnormal cervical cytology was found to influence HPV prevalence of their male partners and type-specific HPV concordance in the partnership. Irrespective of sexual behavior, HIV-positive status in women as well as abnormal cervical cytology status influences HPV type-specific concordance within the partnership. The data from this study substantially increases the very limited data reported on type-specific genital HPV concordance between sexually active couples and especially HIV-positive and HIV-discordant couples

CHAPTER 4: *hpVIR* HIGH-RISK HUMAN PAPILLOMAVIRUS VIRAL LOAD IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVE AND HIV-SERONEGATIVE WOMEN AND MEN

4.1 INTRODUCTION

Cervical or penile infection with HR-HPV and its persistence may leads to the development of cervical or penile lesions (Castellsague *et al*., 2006; Munoz *et al*., 2006). Women who are found to harbour HR-HPV but have normal cervical cytology are reported to have a higher risk of developing HSIL compared to women with normal cytology and are HR-HPV negative (Munoz *et al*., 2006). Elevated HPV viral load predicts the progression of precancerous lesions and cancer in both women and men (Moberg *et al*., 2005). The HPV viral load significantly increase with increasing cervical cytology abnormalities and women with high HPV viral load were found to have significantly increased risk of disease progression after 2 years compared to women with low HPV viral load (Swan *et al*., 1999; Lefevre *et al*., 2004; Moberg *et al*., 2005; Carcopino *et al*., 2011; Lowe *et al*., 2011). Ho *et al*., (2006) followed women with LSIL and observed that women with increased viral load by real-time PCR had 7-fold higher risk of developing HSIL compared to women with LSIL but without increased HPV viral load.

It has been suggested that high HPV viral load would increase the risk of HPV persistence over time and the risk of disease progression (van Duin *et al*., 2002; Wu *et al*., 2006; Zhang *et al*., 2010). Persistent HR-HPV infection is the risk factor for cervical disease progression (Fontaine *et al*., 2008). A significant clustering of HPV types and species is reported in women with abnormal cervical cytology compared to women with normal cervical cytology. It is also reported that women with multiple HPV infection have increased risk of HPV persistence and cervical disease progression (Ho *et al*., 1998; Fife *et al*., 2001; Trottier *et al*., 2006; Trottier *et al*., 2008, Spinillo *et al*., 2009). The frequency of HPV DNA integration is also reported to increase with increasing HPV viral load (Peitsaro *et al.*, 2002). Women with male sexual partners with HPV infection are more likely to acquire HPV infection and develop cervical disease (Heard *et al*., 2000; Burchell *et al*., 2010b). It was previously reported that men with a high HPV viral load more frequently share HPV types with their female partner compared to men with low viral load, suggesting that high viral load may enhance viral transmission between partners (Bleeker *et al*., 2005a).

HIV co-infected women progress to cervical cancer about 10 years earlier than HIV-negative women (Lomalisa *et al*., 2000). Even though data on their sexual behaviour including when they started sexual activity was not available, however, they find that HIV-positive women presented with invasive cervical cancer 10 years earlier than the HIV-negative women (Lomalisa *et al*., 2000). Immune competence plays an important role in cervical disease

progression. Women with low CD4 cell counts are reported to have a higher viral load and a greater risk of HPV persistent infection (Fontaine *et al*., 2008; Denny *et al*., 2008; Luchters *et al*., 2010). That is why after controlling for age and CD4 cell counts, HIV-positive women with a HPV-16 viral load of $<10^7$ copies/µg were found to have 13.5 month median duration of HPV persistent infection compared to women with HPV-16 viral load of $\geq 10^7$ copies/µg who had 21.3 month median HPV duration (Fontaine *et al*., 2008). Women with a stable HPV viral load were less likely to show progressive cervical disease compared to those with HPV viral load which increased by 2 logs (Fontaine *et al*., 2008). Women with low CD4 cell count are at increased risk of HPV acquisition and persistence infection making them at risk of progressing to cervical cancer. Cervical cancer rate in HIV-positive women has not gone down despite the use of ARTs because the use of ARTs only will not reduce the risk of cervical cancer, however, the use of ARTs will make HIV-positive women to live longer (Atashili *et al*., 2011). Atashili *et al*., (2011) suggested that cervical cancer cases will drop in South Africa if HIV-positive women on ARTs get cervical cancer screening even if once in their lifetime

Delmas *et al*., (2000) reported a 23% cumulative incidence of LSIL after 2 years in HIV-positive women with normal cervical cytology and HPV DNA negative results at baseline (Delmas *et al*., 2000). Whereas Harris *et al*., (2005) reported a 9% cumulative incidence of LSIL in HIV-positive women with a CD4 count <200/µl, 9% also in women with CD4 count 200-500/µl and 4% in women with CD4 count >500/µl. However participants in the Delmas *et al*., (2000) study were younger than those in the Harris *et al*., (2005) study. It has been reported that young age is a risk factor for HPV infection and LSIL, therefore the young age of the women in the Delmas *et al*., (2000) study could account for the increased cumulative incidence of HPV observed, compared to women in the Harris *et al*., (2005) study.

In the present study we detected and quantified genital HPV viral load in men and women using the in-house *hpVIR* assay which detects and quantifies 12 HR-HPV (includes HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 and -59) types associated with 93.1% of cervical cancer cases worldwide (Moberg *et al*., 2003; Clifford *et al*., 2006; Gustavsson *et al*., 2009). The HR-HPV types detected by *hpVIR* assay were then referred to as *hpVIR* HR-HPV types because we previously reported on 15 HR-HPV types detected by Roche assay.

The objectives of the study were:

(i) to investigate the respective *hpVIR* HR-HPV viral loads in HIV-positive and HIV-negative women and men;

(ii) to investigate the effect of CD4 counts and HIV viral load on *hpVIR* HR-HPV viral load;

(iii) to investigate the association of *hpVIR* HR-HPV viral load with cervical abnormality;

(iv) to investigate factors associated with cervical abnormalities; and

(v) to investigate the association between cervical or penile *hpVIR* HR-HPV viral load and HPV sharing between partners.

4.2 MATERIALS AND METHODS

4.2.1 Study population and specimen collection

Study participants were recruited from the Manyanani clinic, Empilisweni Centre, Cape Town, South Africa. The Research Ethics Committee of the University of Cape Town approved all aspects of the investigation. All available samples underwent HPV viral load analyses even if they were HR-HPV negative by linear array HPV genotyping. A total of 292 HIV-negative women, 258 HIV-positive women, 412 HIV-negative men and 153 HIV-positive men were enrolled. However when the participants were grouped with their partners there we 542 couples enrolled, of these 246 were both HIV-negative, 103 were both HIV-infected, 155 were HIV-discordant where the female was HIV-positive and 41 were HIV discordant where the male was HIV-positive. The mean age of the women and men were 34 years (range, 18-66 years) and 38 years (range, 19-78 years) respectively. Of the men enrolled in the study 94% were circumcised. Samples were collected as described in chapter 2 section 2.2.1.

4.2.2 Detection and quantification of HPV DNA

DNA was extracted from both cervical and penile cells using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany) and automated MagNA Pure Compact machine (Roche Diagnostics, Mannheim, Germany). DNA was stored at -20°C and shipped to University of Uppsala, Sweden for detection and quantification of HPV DNA. Since the assay that detects and quantifies HPV DNA by real-time (RT) PCR was not set-up in Prof A.L Williamson's laboratory it was arranged for me to go to Prof Ulf Gyllensten's laboratory in Sweden. Inger Gustavsson trained me to perform the assay. HPV was detected and quantified by a RT-PCR based assay described by Gustavsson *et al*., (2009) and is called *hpVIR*. Even though *hpVIR* is an "in-house method", it has been reported to have similar sensitivity and specificity to Hybrid Capture 2 (HC2), a Food and Drug Administration (FDA)

approved assay (Gustavsson *et al*., 2009). The *hpVIR* assay detects HR-HPV types individually or in groups and includes their viral load, while HC2 identifies 13 HR-HPV types as a group (Gustavsson *et al*., 2009).

The *hpVIR* assay is based on four parallel real-time PCRs from each DNA sample, one reaction to quantify the amount of a human single copy gene (house keeping gene) (HMBS, Homo sapiens hydroxymethylbilane synthase; GenBank accession no. M95623.1) and the three other reactions to detect and quantify HR-HPVs (includes HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59. A housekeeping gene must not change with changing conditions of the cells. HMBS is reported to be one of the best housekeeping gene (Cicinnati *et al*., 2008). There are difficulties in finding a good housekeeping gene to be used as normalization control for example the level of other genes may vary in cells from different cytological cervical lesions and normal cytology (Cicinnati *et al*., 2008). Use of a second housekeeping gene in our study would improve or confirm the findings.

The results are presented as individual types, except for HPV18 and - 45 which are detected as a phylogenetically related group and HPV33, -52, and -58 which are similarly detected. The real-time PCR assay was carried out in a final volume of 25 µl, containing 3 µl template DNA from cervical cells or 6 µl from penile cells, Taqman® Universal PCR master mix with no AmpErase® UNG (Applied Biosystems, Inc, Foster City, CA, USA), 3.1 µg of bovine serum albumin (Sigma, St Louis, MO, USA), 200 nM of each primer (Thermo Hybaid, Waltham, MA, USA) and probe (Applied Biosystems, Inc, Foster City, CA, USA). Amplification and detection steps were performed using 7900 HT Sequence Detection System (Applied Biosystems, Inc, Foster City, CA, USA). The primers and probes sequences are according to Moberg *et al*., (2003) and Gustavsson *et al*., (2009). The amplification ramp includes an initial hold program of 10 minutes at 95°C followed by a two-step cycle consisting of 95°C for 15 seconds and 57°C for 1 minute that was repeated 40 times. The sensitivity of the HPV assay was determined using plasmids containing the full genome of different HPV types. Standard curves ranging from 10^2 to 10^5 copies were established for each HPV type or group of HPV types to be detected. A highly significant linear relationship was seen between HPV copy number and threshold cycle (C_t) for all HPV types detected by the system. The threshold for a positive HPV type was set at 10 copies per PCR. Similarly, a linear relationship was seen between copy number of the human HMBS gene and threshold cycle, and as threshold for

inclusion in the study a copy number of 10 genomic equivalents were used. The RT-PCR data was analysed with the applying software SDS version 2.2. and the HPV copy number and viral load (HPV copy number per human genome equivalent) was calculated using Microsoft excel. Copies per cell were calculated by dividing HPV copies per sample by copies of house-keeping gene HMBS.

4.2.3 Statistical analyses

Statistical analyses were performed using X^2 test (EpiInfo Version 5 Statcalc) and the Mann-Whitney test (GraphPad Prism® 5) when comparing HPV viral load between groups. Kruskal-Wallis test was used when analysing the effect of CD4 counts on *hpVIR* HR-HPV viral load. Univariate analyses were conducted using STATA 11.0 (StataCorp, College Station, TX, USA). Advanced statistics were performed by Dr Leigh Johnson (Centre for Infectious Disease Epidemiology and Research, University of Cape Town). In all analyses P-values ≤0.05 were considered significant.

4.3 RESULTS

4.3.1 hpVIR HR-HPV prevalence in women and men according to HIV status

To determine if the genital sampling was adequate, a house keeping gene known as HMBS copies was quantified in each sample. HMBS copies were found to be <10 in 3/550 (0.5%) cervical cells and in 64/565 (11%) penile cells. The samples with <10 HMBS copies were not included in the analysis. Both HIV-positive women and men had a significantly higher prevalence of *hpVIR* HR-HPV (51% 131/257; 59% 85/143 respectively) compared to HIV-negative women and men (21% 61/290; 32% 113/358 respectively, P<0.0001 for both women and men, Figure 4.1).

HIV-positive men were found to have a higher *hpVIR* HR-HPV prevalence compared to HIV-positive women however the difference was not statistically significant (59% 85/143 compared to 51% 131/257, P=0.1). HIV-negative men were found to have a significantly higher *hpVIR* HR-HPV prevalence compared to HIV-negative women (32% 113/358 compared to 21% 61/290, P=0.003). Among HPV-positive participants, both women and men were found to have a significantly higher prevalence of multiple *hpVIR* HR-HPV infections compared to HIV-negative women and men (49% 65/131 compared to 28% 17/61, P=0.007 for women and 60% 51/85 compared 33% 31/113, P=0.0002 for men). HIV-positive women and men were found to

have a higher prevalence of all the *hpVIR* HR-HPV genotypes detected compared to HIV-negative women and men respectively (Figure 4.2). HIV-positive men and HIV-negative men

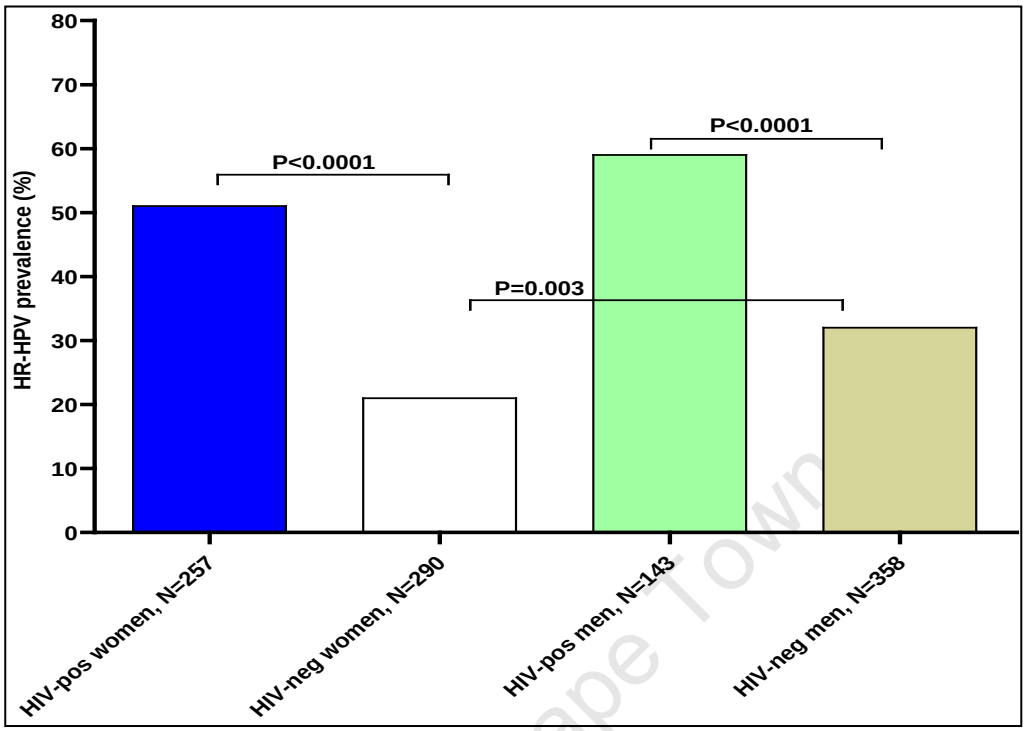

Figure 4.1. *hpVIR* HR-HPV prevalence in women and men according to HIV status.

were found to have a higher prevalence of all *hpVIR* HR-HPV genotypes detected compared to HIV-positive women and HIV-negative women respectively except for HPV-31 in HIV-positive men and women and HPV-35 in HIV-negative men and women. The HPV33/52/58 phylogentic group was the most prevalent (Figure 4.2).

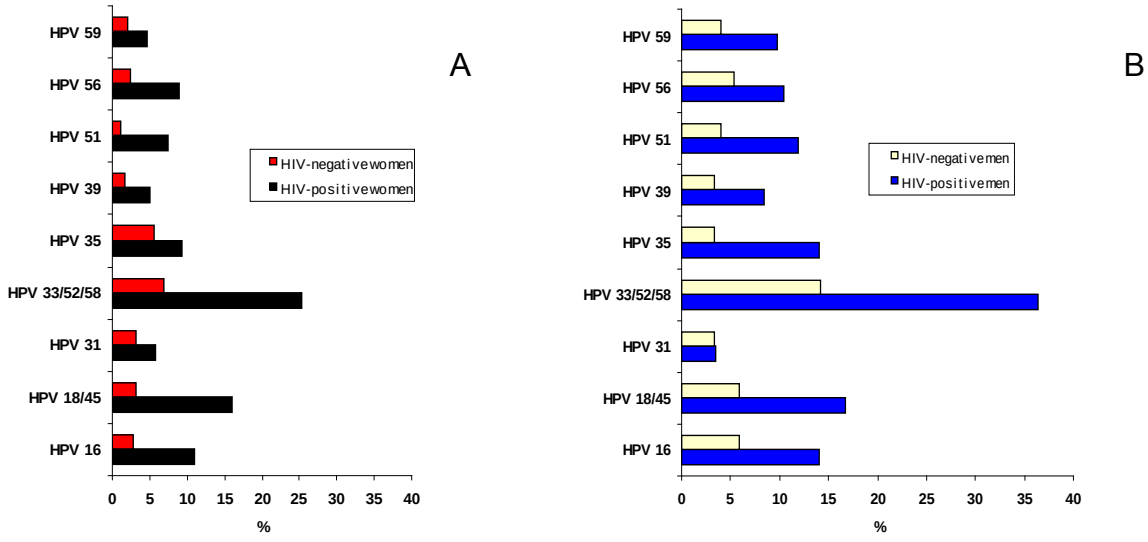

Figure 4.2. *hpVIR* HR-HPV genotypes in HIV-positive and HIV-negative women (A) and men (B).

The agreement between *hpVIR* and Roche HPV genotyping assay was investigated using *kappa* analysis. In women the agreement of detecting HPV-16 was *k*=0.91 and in men it was *k*=0.87. This indicates good agreement between *hpVIR* assay in this chapter and Roche HPV genotyping assay reported in chapter 2 and 3. Even though *hpVIR* assay uses 3µl of DNA template it detects HPV types similar with Roche assay that uses 50µl of DNA template.

4.3.2 hpVIR HR-HPV viral load in men and women

Genital *hpVIR* HR-HPV viral load was compared between men and women for each of the HPV types studied. Women were found to have a higher number of HPV-16, -18/45, -33/52/58, -39, -51 copies per sample compared to men and also for combined *hpVIR* HR-HPV groups (Table 4.1). When we look at *hpVIR* HR-HPV copies per cell, men were found to have higher *hpVIR* HR-HPV copies per cell compared to women for HPV-16 (median: 5.6 range: 0-7353 compared to: 1.2 range: 0-97.2 respectively, P=0.07), HPV-51 (median: 6.8 range: 0-2785 compared to: 2.02 range: 0-782.2 respectively, P=0.13) and for all *hpVIR* HR-HPV (median: 2.0 range: 0-23530 compared to: 1.0 range: 0-1866 respectively, P=0.04).

The higher *hpVIR* HR-HPV viral load we observed in men could be the results of fewer cells sampled at penile sites compared to cervical sites, the different cell types sampled at the different sites from women (mucosal epithelium) and men (keratinised epithelium) could also played a role in this. When participants were stratified according to their HIV status and gender; in general HIV-positive women were found to have a higher median *hpVIR* HR-HPV viral load per cell for all types, except HPV-59, compared to HIV-negative women however this was only statistically significant for α9 HPV species (HPV-16, -31, -33, -35, -52, -58; P=0.022, Table 4.2). HIV-positive men and were found to have a significantly higher number of copies per cell for HPV-39 compared to HIV-negative men (P=0.024, Table 4.3). In men, HPV viral load of all other types was not found to differ significantly between HIV-positive and HIV-negative men.

Table 4.1. *hpVIR* HR-HPV types in women and men and their HPV viral load expressed as number of copies per cell and sample.

Subjects, viral load	Women	Men	P-value
HPV-16 positive subjects	n= 36	n= 41	
No of copies/sample	10860 (29 - 1457000)	1711 (13,2 - 7618000)	**0.04**
No of copies/cell	1,2 (0 - 97,2)	5,6 (0 - 7353)	0.07
HPV-18/45 positive subjects	n= 50	n= 45	
No of copies/sample	7919 (9 - 4511000)	1947 (13,5 - 987100)	**0.02**
No of copies/cell	3,5 (0 - 411,0)	3,9 (0 - 1921)	0.65
HPV-31 positive subjects	n= 24	n = 17	
No of copies/sample	2591 (25 - 2268000)	552 (16,9 - 1925000)	0.21
No of copies/cell	0,5 (0 - 256)	1,02 (0 - 66,9)	0.19
HPV-33/52/58 positive subjects	n= 85	n= 102	
No of copies/sample	28290 (12 - 25090000)	636,1 (11 - 44070000)	**<0,0001**
No of copies/cell	2,8 (0 - 1414)	1,7 (0 - 23530)	0.47
HPV-35 positive subjects	n= 40	n= 32	
No of copies/sample	1710 (26 - 2960000)	5562 (3,1 - 19930000)	0.12
No of copies/cell	2,8 (0 - 7105)	0,61 (0 - 1866)	**0.02**
HPV-39 positive subjects	n= 18	n= 24	
No of copies/sample	7192 (2,3 - 5152000)	240,8 (6,1 - 134700)	**0.03**
No of copies/cell	0,63 (0 - 1234)	0,85 (0 - 293)	0.92
HPV-51 positive subjects	n= 22	n= 31	
No of copies/sample	32900 (94,8 - 5251000)	3423 (13,3 - 954100)	**0.0007**
No of copies/cell	2,02 (0 - 782,2)	6,8 (0 - 2785)	0.13
HPV-56 positive subjects	n= 30	n= 34	
No of copies/sample	1636 (0,6- 16110000)	287,6 (10,2 - 104600)	0.07
No of copies/cell	0,13 (0 - 384,3)	3,8 (0 - 3655)	0.1
HPV-59 positive subjects	n= 18	n= 28	
No of copies/sample	467 (13,8 - 701600)	253 (13,7 - 1056000)	0.42
No of copies/cell	0,09 (0 - 305)	0,81 (0 - 707)	0.2
HPV-16, -18/45, -31 positive subjects	n= 110	n= 103	
No of copies/sample	5645 (9 - 4511000)	1611 (13 - 7618000)	**0.002**
No of copies/cell	2 (0 - 411)	3 (0 - 7353)	0.23
HPV-33/52/58, -35, -39, -51, -56, -59 positive subjects	n= 213	n= 251	
No of copies/sample	8162 (0,6 - 25090000)	608 (6 - 44070000)	**<0,0001**
No of copies/cell	1 (0 - 1866)	2 (0 - 23530)	0.06
All HPV positive subjects	n= 323	n= 354	
No of copies/sample	7013 (0,6 - 25090000)	735 (6 - 44070000)	**<0,0001**
No of copies/cell	1 (0 - 1866)	2 (0 - 23530)	**0.04**

Note: Data are no. (%) of subjects or median (range) and the P-values were calculated by Mann-Whitney test (two-tailed), significantly P-values are in bold writing.

Table 4.2. *hpVIR* HR-HPV viral load per sample in women according to HIV-status.

HPV type	HIV-negative women		HIV-positive women		P-value
	n	Median	n	Median	
HPV-16	8	0.43	29	2.35	0.238
HPV-18/45	9	0.27	41	1.33	0.398
HPV-31	9	0.25	15	0.7	0.698
HPV-33/52/58	22	1.02	67	3.18	0.220
HPV-35	16	0.51	24	1.14	0.456
HPV-39	7	0.1	14	0.36	0.709
HPV-51	3	1.15	19	2.17	0.363
HPV-56	9	0.024	25	0.091	0.339
HPV-59	8	0.089	14	0.009	0.838
α5 HPV species	3	1.15	19	2.17	0.363
α6 HPV species	9	0.024	25	0.091	0.339
α7 HPV species	24	0.15	58	0.65	0.245
α9 HPV species	47	0.63	100	3.9	**0.022**

α5 and α6 HPV species include HPV-51 and -56 respectively; **α7** HPV species includes HPV-18, -39, -45 and -59; **α9** HPV species includes HPV-16, -31, -33, -35, -52 and -58. P-values were calculated by Mann-Whitney test (two-tailed). Significant P-values are in bold.

4.3.3 The effect of CD4 counts on hpVIR HPV viral load in HIV-positive women and men

HIV-positive women with CD4 counts >350/mL had significantly lower α7 HPV species viral loads (median 0.12 copies per cell) than HIV-positive women with CD4 ≤350/mL (median 1.52 copies per cell, P= 0.008), but none of the median HPV viral loads for other HPV types/species were found to be significantly lower in HIV-positive women with CD4 counts >350/mL (results not shown). Unexpectedly, HIV-positive men with CD4 counts >350/mL had significantly higher HPV-39 viral loads (median 4.29 copies per cell) than HIV-positive men with CD4 ≤350/mL (median 0.026 copies per cell, P= 0.017), however HIV-positive men with CD4 counts >350/mL and infected with HPV-39 were few (n=3). None of the median HPV CD4 counts >350/mL and infected with HPV-39 were few (n=3). None of the median HPV viral loads for other HPV types/species were found to be significantly different in HIV-positive men according to CD4 level.

Table 4.3. *hpVIR* HR-HPV viral load per sample in men according to HIV-status.

HPV type	HIV-negative women		HIV-positive women		P-value
	n	Median	n	Median	
HPV-16	21	3.55	24	0.92	0.666
HPV-18/45	23	3.94	24	3.05	0.670
HPV-31	10	0.91	7	1.02	0.845
HPV-33/52/58	67	0.42	67	1.01	0.193
HPV-35	17	0.4	27	1.48	0.952
HPV-39	14	1.28	17	0.04	**0.024**
HPV-51	15	8.22	22	1.88	0.370
HPV-56	30	0.24	23	0.06	0.566
HPV-59	16	1.01	19	0.11	0.059
α5 HPV species	15	8.22	22	1.88	0.370
α6 HPV species	30	0.24	23	0.06	0.566
α7 HPV species	46	3.24	45	0.77	0.132
α9 HPV species	96	1.01	89	1.48	0.228

α5 and α6 HPV species include HPV-51 and -56 respectively; **α7** HPV species includes HPV-18, -39, -45 and -59; **α9** HPV species includes HPV-16, -31, -33, -35, -52 and -58. P-values were calculated by Mann-Whitney test (two-tailed). Significant P-values are in bold.

4.2.4 Prevalence of hpVIR HR-HPV and viral load in women with normal and abnormal cervical cytology

When women with valid cervical *hpVIR* HR-HPV data were stratified according to cervical cytology, a total of 374 had normal cervical cytology, 40 had ASCUS, 77 had LSIL, and 11 had HSIL. Women with normal cervical cytology were on average 35 years of age (range: 18-66 years), women with ASCUS 34 years (range: 21-61 years), women with LSIL 34 years (range: 18-49 years) and women with HSIL 39 years (range: 32-47 years). In women with normal cervical cytology 22% (82/374) were found to have *hpVIR* HR-HPV infection, in women with ASCUS 46% (22/48) were found to have *hpVIR* HR-HPV infection, in women with LSIL 79% (61/77) were found to have *hpVIR* HR-HPV and women with HSIL 100% (11/11) were found to have *hpVIR* HR-HPV infection. The prevalence of *hpVIR* HR-HPV infection was found to increase with increasing cervical disease and thus was positively associated with cervical disease (R^2=0.993, Figure 4.3).

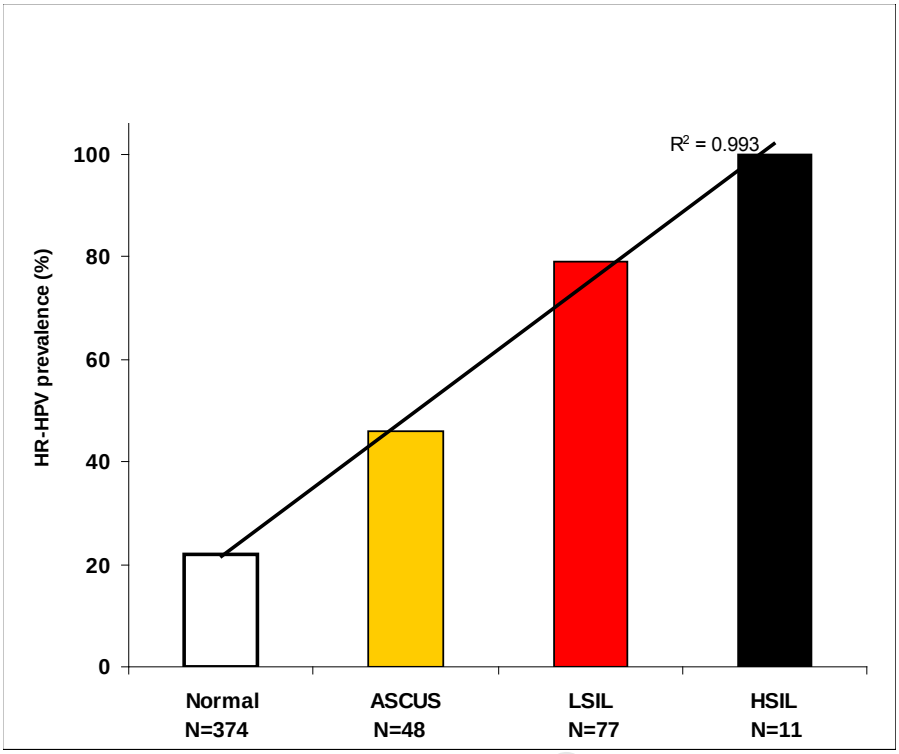

Figure 4.3. Prevalence of *hpVIR* HR-HPV in women with normal cervical cytology (N=374), atypical squamous cell of undetermined significance (ASCUS, N=48), low-grade squamous intraepithelial lesion (LSIL, N=77), and high-grade squamous intraepithelial lesion (HSIL, N=11).

hpVIR HR-HPV prevalence was then further stratified according to age and cervical cytology. Women who were ≥30 years were found to have a higher *hpVIR* HR-HPV prevalence compared to women <30 years of age (normal cervical cytology: 25% 20/59 compared to 17% 23/137, P=0.08; ASCUS: 47% 14/30 compared to 44% 8/18, P=0.88 and LSIL: 66% 21/32 compared to 89% 39/44, P=0.015) however the difference was only statistically significant for women with LSIL. Women with HSIL were all above the age of 30 years (Figure 4.4). Women with ASCUS, LSIL and HSIL were found to have significantly higher *hpVIR* HR-HPV copies per sample and copies per cell compared to women with normal cervical cytology (Table 4.4), while a comparison between women with different grades of cervical disease showed no significant difference. Women with ASCUS, LSIL and HSIL were found to have significantly higher α9 HPV copies per sample and copies per cell compared to women with normal cervical cytology, for α7 HPV species viral load was statistically different between women with normal cervical cytology and ASCUS and women with LSIL while for α5/6 HPV species viral load was statistically different between women with normal cervical cytology and LSIL not ASCUS (Table 4.4). Women with abnormal cervical cytology were found to have a higher *hpVIR* HR-HPV viral load compared to women with normal cervical cytology.

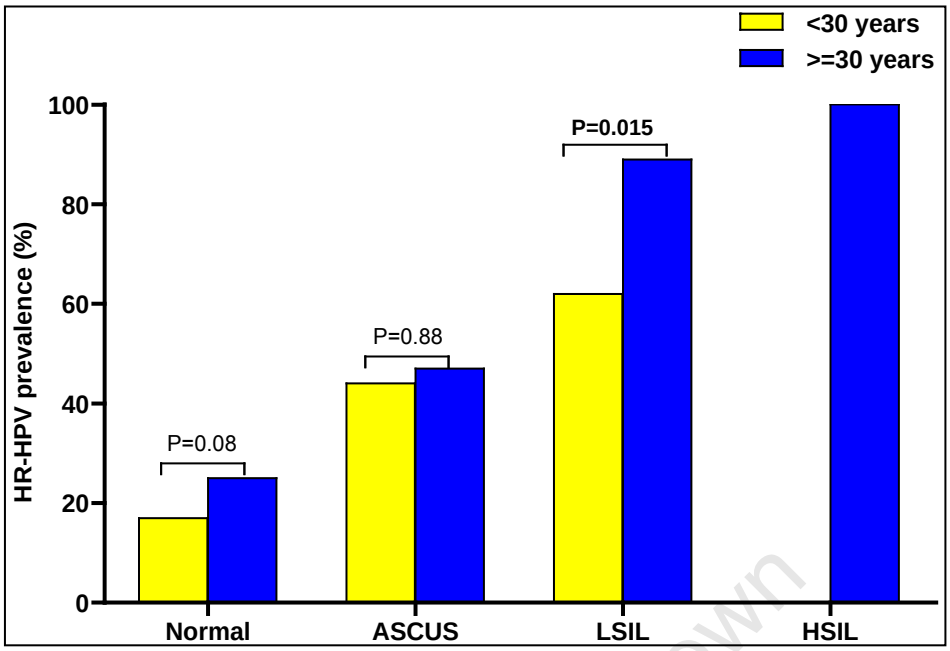

Figure 4.4. Prevalence of *hpVIR* HR-HPV in women with normal cytology, atypical squamous cell of undetermined significance (ASCUS) low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) according to age.

Table 4.4. *hpVIR* HR-HPV viral load in women with normal and abnormal cervical cytology according to species level.

HPV Species	Cytology	No of copies/samples	P-value	No of copies/cell	P-value
α5/6					
	Normal	978 (0-320800)	ref	0.1 (0-75)	ref
	ASCUS	338200 (2-5251000)	0.19	71 (0-782)	0.09
	LSIL	31840 (20-16110000)	**0.009**	2 (0-384)	**0.01**
	HSIL	…	…	…	…
α7					
	Normal	941 (2-5152000)	ref	0.01 (0-485)	ref
	ASCUS	18670 (73-861500)	**0.008**	3 (0-411)	**0.008**
	LSIL	16930 (1-4511000)	**0.03**	2 (0-1234)	**0.05**
	HSIL	…	…	…	…
α9					
	Normal	2424 (3-25090000)	ref	0.2 (0-1414)	ref
	ASCUS	31610 (6-3328000)	**0.05**	1.8 (0-519)	**0.02**
	LSIL	76269 (12-19930000)	**<0.0001**	9 (0-1866)	**<0.0001**
	HSIL	73290 (29-10970000)	**0.02**	18 (0-870)	**0.003**
All					
	Normal	2123 (0.6-25090000)	ref	0.12 (0-1414)	ref
	ASCUS	25140 (2-5251000)	**0.0009**	2.2 (0-782)	**0.0002**
	LSIL	41140 (1-19930000)	**<0.0001**	4 (0-1860)	**<0.0001**
	HSIL	60280 (29-10970000)	**0.002**	14 (0-870)	**0.002**

Note: Data are median (range) and the P-values were calculated by Mann-Whitney test (two-tailed), significantly P-values are in bold writing. Ref- reference. **α5/6** HPV species includes HPV-51 and -56; **α7** HPV species includes HPV-18, -39, -45 and -59; **α9** HPV species includes HPV-16, -31, -33, -35, -52 and -58.

Factors that are associated with abnormal cervical cytology are presented in Table 4.5. Predictors of abnormal cervical cytology were found to be HIV-positive status, <5 years of relationship with the study partner, infected with α5, α6, α7 or α9 HPV species. Women infected with α7 HPV species with ≥0.6 HPV copies per cell were at greater risk of having abnormal cervical cytology (odds ratio (OR), 7.08 [95% confidence interval (CI): 3.51-14.31]) followed by women with <0.6 HPV copies per cell (OR, 3.01 [95% CI: 1.52-5.97]) when compared with women with no α7 HPV infection. Women infected with α9 HPV species with ≥2.5 HPV copies per cell were at greater risk of having abnormal cervical cytology (OR, 15.80 [95% CI: 8.56-29.14]) followed by women with <2.5 HPV copies per cell (OR, 3.27 [95% CI: 1.89-5.75]) when compared with women with no α9 HPV infection (Table 4.5). However, in multivariate analysis HIV status and duration of relationship with the study partners were not significant predictors of abnormal cytology only high α α6, α7 and α9 HPV species viral loads remain to be predictors of abnormal cytology (Table 4.5).

4.3.5 The relationship between genital hpVIR HR-HPV viral load and type-specific HPV concordance in couples

Type-specific HPV concordance among couples was defined as the presence of the same HPV genotypes in cervical and penile cells of a couple. To investigate the association of *hpVIR* HR-HPV viral load on type-specific *hpVIR* HR-HPV concordance study participants were grouped according to couples. Samples from couples that were *hpVIR* HR-HPV positive by *hpVIR* were analysed to determine the relationship between cervical HPV viral load and type-specific HPV concordance with male partners. Women infected with *hpVIR* HR-HPV and type-specific HPV concordance with their male partner were found to have significantly higher copies of *hpVIR* HR-HPV per cell compared to women that were not having type-specific HPV concordance with their male partner (6 median value, range: 0-1866 compared to 0.4 median, range: 0-1234, P<0.0001). Women with type-specific HPV concordance with their male partner were also found to have significantly higher copies of HPV-16/18/45/31 (four most common *hpVIR* HR-HPV types) and HPV-33/52/58/35/39/51/56/59 per cell compared to women that were not having type-specific HPV concordance with their male partners (P=0.003 and P=0.0002 respectively, Table 4.6).

Women infected with *hpVIR* HR-HPV and type-specific HPV concordance with their partner were found to have a higher *hpVIR* HR-HPV viral load compared to women that were not having type-specific HPV concordance with their male partners, suggesting that a high HPV

Table 4.5. The predictors of abnormal cervical cytology in women, (univariate analysis)

Variable	n	HPV%	OR	95% CI	P-value
Age group					
<30 years	196	27%	ref		0.07
30-39 years	187	33%	1.31	0.84-2.03	
≥40 years	151	21%	0.73	0.44-1.2	
HIV status					
Negative	279	19%	ref		**<0.0001**
Positive	255	36%	2.35	1.59-3.48	
CD4 count (if HIV-positive)					
≥350/mL	128	32%	ref		0.18
<350/mL	127	40%	1.42	0.85-2.38	
HIV viral load (if HIV-positive)					
<4 log	86	34%	ref		0.36
≥4 log	138	40%	1.3	0.74-2.29	
Living together with study partner					
No	202	28%	ref		0.7
Yes	330	27%	0.93	0.63-1.37	
Duration of relationship with study partner					
<5 years	112	29%	ref		**0.04**
≥5 years	84	17%	0.48	0.24-0.97	
Age at first sex					
<18 years	266	24%	ref		0.09
≥18 years	265	31%	1.39	0.95-2.04	
Lifetime number of sexual partners					
1-2	231	30%	ref		0.50
3-5	227	25%	0.79	0.52-1.19	
>5	73	26%	0.83	0.46-1.5	
Number of sex acts with study partner in last month					
<5	367	26%	ref		0.36
≥5	160	30%	1.21	0.8-1.82	
Ever used a condom with current study partner					
No	166	25%	ref		0.34
Yes	359	29%	1.23	0.81-1.87	
Experienced genital discharge in last 12 months					
No	443	27%	ref		0.73
Yes	90	29%	1.09	0.66-1.81	
Experienced genital ulcer in last 12 months					
No	502	27%	ref		0.31
Yes	31	35%	1.5	0.7-3.2	
α5 HPV infection					
No	510	26%	ref		**0.006**
Yes	22	55%	3.4	1.44-8.06	
α6 HPV infection					
No	498	25%	ref		**<0.0001**
Yes	34	65%	5.59	2.69-11.62	
α7 HPV infection					
None	456	22%	ref		**<0.0001**
HPV VL <0.6 copies/cell	37	46%	3.01	1.52-5.97	
HPV VL ≥0.6 copies/cell	39	67%	7.08	3.51-14.31	
α9 HPV infection					
None	396	17%	ref		**<0.0001**
HPV VL <2.5 copies/cell	68	43%	3.27	1.89-5.75	
HPV VL ≥2.5 copies/cell	68	72%	15.80	8.56-29.14	

Note: VL: viral load per cell, **α5** HPV species is HPV-51; **α6** HPV species is HPV-56; **α7** HPV species are HPV-18, -39, -45 and -59; **α9** HPV species are HPV-16, -31, -33, -35, -52 and -58.

viral load in women may play role in HPV transmission to their male partner. Men that were positive for any *hpVIR* HR-HPV type were then selected and analysed for the relationship between penile *hpVIR* HR-HPV viral load and type-specific HPV concordance with their female partner. There was no significant difference in viral load between men that were type-specific HPV concordance with their female partner and those that were not having type-specific HPV concordance with their female partner. Therefore no clear association was observed between penile *hpVIR* HR-HPV viral load and type-specific HPV concordance in couples (Table 4.7).

4.4 DISCUSSION

In this study we investigated the *hpVIR* HR-HPV viral load in HIV-positive and HIV-negative women and men. HIV-positive women and men were found to have higher *hpVIR* HR-HPV prevalence, more total *hpVIR* HR-HPV and a greater *hpVIR* HR-HPV viral load compared to HIV-negative women and men. Similar findings were previously reported elsewhere (Palefsky *et al*., 1999; Gomousa-Michael *et al*., 2000; Viscidi *et al*., 2003; Strickler *et al*., 2005). HIV infection was found to be the predictor of abnormal cytology. The high *hpVIR* HR-HPV prevalence and viral load in HIV-positive women and men could be due to suppressed immune system caused by HIV infection that could also result to reactivation of latent infection and high susceptibility to HPV acquisition. The role played by immune system on HPV viral load can also be explained by the increasing *hpVIR* HR-HPV viral load with decreasing CD4 count among HIV-positive women and men. High *hpVIR* HR-HPV viral load in HIV-positive individuals seem to be influenced by the CD4 count level as the *hpVIR* HR-HPV viral load was found to significantly increase with decreasing CD4 counts, similar observations have been reported elsewhere (Swan *et al*., 1999; Fontaine *et al*., 2008; Denny *et al*., 2008). When comparing women and men, we found that *hpVIR* HR-HPV viral load was higher in women compared to men regardless of HIV-status. However comparing HPV viral load between women and men may not be accurate because fewer cells were collected in penile samples from men compared to the cervical samples from women and would account for the low *hpVIR* HR-HPV viral load/sample in men compared to women. Also, the different cervical cells present in women may provide a different environment for HPV replication; the cervical cells are more favourable for producing high HPV viral loads compared to penile cells (Bleeker *et al*., 2005a; Flores *et al*., 2006).

Table 4.6. The relationship between cervical *hpVIR* HR-HPV viral load and type-specific HPV concordance with a male partner

Viral load for female partner	type-specific HPV concordance with male partner	not type-specific HPV concordance with male partner	P-value
HPV-16 positive subjects	n=14	n= 18	
No of copies/sample	21270 (188 - 630400)	10830 (129 - 1093000)	0.86
No of copies/cell	5.2 (0 - 97)	0.8 (0 - 31)	0.48
HPV-18/45 positive subjects	n= 16	n= 30	
No of copies/sample	99140 (17 - 2955000)	5645 (19 - 4511000)	**0.03**
No of copies/cell	19 (0 - 411)	0.2 (0 - 152)	**0.004**
HPV-31 positive subjects	n= 8	n= 13	
No of copies/sample	8084 (31 - 2268000)	2600 (25 - 514500)	0.32
No of copies/cell	1.3 (0 - 225)	0.2 (0 - 256)	0.25
HPV-33/52/58 positive subjects	n=46	n= 28	
No of copies/sample	82780 (155 - 25090000)	8816 (16 - 6591000)	0.06
No of copies/cell	9.2 (0- 1414)	0.87 (0 - 255)	**0.04**
HPV-35 positive subjects	n= 11	n= 24	
No of copies/sample	44020 (72 - 19930000)	5629 (13 - 4344000)	0.36
No of copies/cell	4.4 (0 - 1866)	0.6 (0 - 802)	0.29
HPV-39 positive subjects	n= 3	n= 12	
No of copies/sample	8900 (133 - 161700)	11210 (17 - 5152000)	0.9
No of copies/cell	15 (0 - 70)	1.3 (0 - 1234)	0.83
HPV-51 positive subjects	n= 8	n= 12	
No of copies/sample	289800 (6465 - 2060000)	8894 (95 - 5251000)	**0.03**
No of copies/cell	10 (1 - 193)	0.4 (0 - 782)	**0.04**
HPV-56 positive subjects	n= 15	n= 12	
No of copies/sample	1242 (28 - 16110000)	1636 (20 - 2387000)	0.51
No of copies/cell	0.2 (0 - 384)	0.08 (0 - 283)	0.29
HPV-59 positive subjects	n= 4	n= 12	
No of copies/sample	117500 (6209 - 701600)	195 (14 - 604200)	**0.02**
No of copies/cell	30 (1 - 305)	0.02 (0 - 31)	**0.02**
HPV-16, -18/45, -31 positive subjects	n= 38	n= 61	
No of copies/sample	34930 (17 - 2955000)	4145 (19 - 4511000)	**0.03**
No of copies/cell	7 (0 - 411)	0,5 (0 - 256)	**0.003**
HPV-33/52/58, -35, -39, -51, -56, -59 positive subjects	n= 87	n= 100	
No of copies/sample	45570 (28 - 25090000)	3752 (13 - 6591000)	**0.0004**
No of copies/cell	6 (0 - 1866)	0,3 (0 - 1234)	**0.0002**
All HPV positive subjects	n= 125	n= 161	
No of copies/sample	37970 (17 - 25090000)	3983 (13 - 6591000)	**<0,0001**
No of copies/cell	6 (0 - 1866)	0,4 (0 - 1234)	**<0,0001**

Note: Data are no. (%) of subjects or median (range) and the P-values were calculated by Mann-Whitney test (two-tailed

Table 4.7. The relationship between penile *hpVIR* HR-HPV viral load and type-specific concordance with female partner

Viral load for male partner	type-specific concordance with female partner	not type-specific concordance with female partner	P-value
HPV-16 positive subjects	n= 12	n= 25	
No of copies/sample	3866 (157 – 174200)	1711 (13 - 923200)	0.47
No of copies/cell	13 (0.17 - 188)	4 (0 - 7353)	0.34
HPV-18/45 positive subjects	n=16	n= 26	
No of copies/sample	1798 (30 – 142300)	2985 (14 - 987100)	0.77
No of copies/cell	4.4 (0 - 238)	3.3 (0 - 1921)	0.92
HPV-31 positive subjects	n= 10	n= 7	
No of copies/sample	301 (17 - 10930)	779 (189 - 1925000)	0.23
No of copies/cell	0.6 (0 - 20)	1.6 (0 - 67)	0.47
HPV-33/52/58 positive subjects	n= 40	n= 55	
No of copies/sample	850 (16 - 804800)	397 (11 - 1196000)	0.33
No of copies/cell	2.3 (0 - 334)	1.4 (0 - 23530)	0.9
HPV-35 positive subjects	n= 11	n= 17	
No of copies/sample	2464 (47 – 2960000)	464 (26 - 435900)	0.45
No of copies/cell	3.4 (0.2 - 5305)	2.7 (0 - 7105)	0.4
HPV-39 positive subjects	n= 3	n= 19	
No of copies/sample	12970 (332 - 134700)	198 (13 - 72200)	0.06
No of copies/cell	10 (6 - 19)	0.6 (0 - 29)	0.08
HPV-51 positive subjects	n= 9	n= 18	
No of copies/sample	31960 (66 – 954100)	1472 (38 - 22660)	0.11
No of copies/cell	13 (0 - 2785)	6 (0 - 105)	0.37
HPV-56 positive subjects	n= 14	n= 19	
No of copies/sample	380 (10 - 31570)	235 (19 - 104600)	0.99
No of copies/cell	5.5 (0 - 92)	2 (0 - 3655)	0.9
HPV-59 positive subjects	n= 4	n= 23	
No of copies/sample	176 (42 - 359600)	541 (14 - 1056000)	0.97
No of copies/cell	1 (0 - 153)	0.8 (0 - 707)	0.81
HPV-16, -18/45, -31 positive subjects	n= 38	n= 58	
No of copies/sample	1630 (17 – 174200)	1661 (13 - 1925000)	0.73
No of copies/cell	4 (0 - 238)	2,5 (0 - 7353)	0.99
HPV-33/52/58, -35, -39, -51, -56, -59 positive subjects	n= 81	n= 151	
No of copies/sample	855 (10 – 2960000)	400 (11 - 1196000)	**0.05**
No of copies/cell	3 (0 - 5305)	1,5 (0 - 23530)	0.14
All HPV positive subjects	n= 119	n= 209	
No of copies/sample	1611 (10 – 2960000)	694 (11 - 1925000)	0.09
No of copies/cell	3 (0 - 5305)	2 (0 - 23530)	0.22

Note: Data are median value (range) and the P-values were calculated by Mann-Whitney test (two-tailed).

Cervical *hpVIR* HR-HPV infection (R^2=0.993) and HPV viral load were positively associated with cervical abnormality and all women with HSIL were found to have *hpVIR* HR-HPV infection at their cervix, similar observation we observed when HPV types were grouped

according to species. HPV viral load is predicted as a marker for HPV persistent infection and risk of developing precancerous lesions and cancer (Sun *et al*., 2002; Moberg *et al*., 2005; Gravitt *et al*., 2007; Guo *et al*., 2010). *hpVIR* HR-HPV prevalence was significantly increasing with age among women with LSIL while in women with normal cervical cytology and ASCUS it did not. Peak prevalence of precancerous lesion is been reported in women older than 30 years compared to young age women (Schiffman & Castle, 2005). The positive association between *hpVIR* HR-HPV and cervical precancerous lesions has been reported in numerous studies which concluded that persistence HPV infection is necessary for the development of cervical cancer (Andersson *et al*., 2005). According to Fontaine *et al*., (2008) women who did not develop cervical abnormalities during follow up were those with stable HPV viral load while those who developed cervical disease were more likely to be those with increased HPV viral load. Women with ASCUS or LSIL or HSIL had a higher viral load of α7 and α9 HPV species compared to women with normal cervical cytology. Clustering of different HPV species was more likely to be observed in women with abnormal cervical cytology compared to women with normal cervical cytology. A high prevalence of HPV types and species clustering in women with abnormal cervical cytology has been previously reported and the clustering of different HPV species in women is associated with increased risk of HPV persistence and cervical disease progression (Ho *et al*., 1998; Trottier *et al*., 2008; Spinillo *et al*., 2009).

The high HPV viral load in women with abnormal cervical cytology and the high prevalence of HIV infection among women with abnormal cervical cytology might increase the risk of HPV sharing with the partner and transmission. We observed that women have higher HPV viral load per sample compared to men. Bleeker *et al*., (2005a) also reported that female cervical scrape specimens have a higher HPV viral load compared to male penile scrape specimens. However, comparing HPV viral load between women and men may not be accurate because the number of cells obtained in female cervical samples is higher than those obtained in male penile samples (Bleeker *et al*., 2005a; Flores *et al*., 2006). As previously mentioned, the different type of genital cell epithelium observed in women and men may provide a different environment for HPV replication and cervical specimen has more than epithelial cells (Flores *et al*., 2006). We reported that women with high HPV viral load frequently shared HPV compared to those with lower HPV viral load, similar observations were reported elsewhere (Bleeker *et al*., 2005a). The present study provides biological support that immune suppressed conditions increase *hpVIR* HR-HPV prevalence as well as viral load and among sexually active partners increased HPV viral load could increase the probability of HPV sharing and

transmission among partners. Measuring HPV viral load may identify HPV persistence and also identify those women and men at risk of developing intraepithelial neoplasia.

In conclusion, HIV co-infection significantly influenced HR-HPV prevalence in both men and women; and HR-HPV viral load in women. High HR-HPV viral load were found to be the predictors of cervical abnormal cytology. Measuring HPV viral load may identify HPV persistence and also identify those women and men at risk of developing intraepithelial neoplasia. Data from this study will assist policy-makers in management of cervical abnormalities in South African HIV-positive women.

CHAPTER 5: HUMAN PAPILLOMAVIRUS SERUM ANTIBODIES TO NINE HPV TYPES IN HIV-SEROPOSITIVE AND HIV-SERONEGATIVE WOMEN AND MEN

5.1 INTRODUCTION

HPV serology is an important tool in sero-epidemiology studies. Its specificity is determined by the type of assay used. HPV antibody detection can be very useful in assessing present and past HPV infection in both women and men (Stanley, 2006). Sero-epidemiology has been successfully used as the basis of the design of HPV vaccination programmes (Ryding *et al*., 2008). HPV DNA detection at a particular site provides information about HPV at that specific site while HPV serology determines HPV past and present HPV exposure at any anatomic sites. HPV antibodies can be detected in individuals who are not HPV infected or infected with HPV type other than the antibody detected, demonstrating past infection or possible cross reactivity between types (Touze *et al*., 1998; Dillner, 1999; Stanley 2006). After HPV infection only a certain proportion of women will seroconvert and seroconversion can take 7 months or longer to be detected (Carter *et al*., 2000; Stanley, 2006). Among women who were HPV-16 infected, 56.7% seroconverted within 8.3 months for IgG and 37.0% for IgA within 14 months (Ho *et al*., 2004). HPV DNA detection is not the optimum test to determine new infections especial in sexual experienced individuals; because the presence of new HPV DNA during a follow up visit does not always demonstrate new infections. It can demonstrate reactivation of latent HPV infection or sampling error of previous sampling (Carter *et al*., 1996; Thompson *et al*., 2004). The combination of serology and DNA detection could provide the maximum information on HPV infection. HPV serology is not a best tool to detect HPV infection because it determines HPV past and present HPV exposure at any anatomic sites. HPV antibodies can be detected in individuals who are not HPV infected or infected with HPV type other than the antibody detected, demonstrating past infection or possible cross reactivity between types. HPV serology is different from serology for other STDs, for example HSV serology can be used to management of patients with first incident of genital herpes infection (Page *et al*., 2003).

In the early 90s, an HPV virus-like particles (VLP) serological assay was established (Kirnbauer *et al*., 1992; Kirnbauer *et al*., 1994). In order to produce HPV virions, xenografts of human tissue in mice were initially used, however, the technique was very time consuming and lab-intensive and produced few HPV virions. VLPs are non-infectious and resemble the HPV virion with regards to the morphology and immunogenicity of each specific type and also resemble the authentic virions (Kirnbauer *et al*., 1992; Roden *et al*., 1996). VLPs can be produced in different cell culture systems including mammalian, insect, plant and yeast cells (Kirnbauer *et al*., 1994). The production of VLPs in insect cells using recombinant baculovirus to yield substantial amounts of VLPs allowing the development of VLP-based enzyme-linked

immunosorbent assay (ELISA) in which HPV VLPs were used as antigens (Kirnbauer *et al*., 1994). Antibody results from ELISA were consistent with HPV DNA results by PCR. This was a breakthrough in humoral immune response studies as detecting antibody responses using bacterially expressed fusion proteins or synthetic peptides as an antigen in ELISA was complex (Kirnbauer *et al*., 1994). Preparation of high quality VLPs is an important factor in the VLP based ELISA to limit cross reactivity between HPV types (Wang *et al*., 2003).

More recently, pseudovirions were produced which allowed the development of a pseudovirion-based papillomavirus neutralisation assay. High yields of HPV pseudovirions consisting of capsid protein and reporter gene can be produced using 293TT cells (Pastrana *et al*., 2004; Buck *et al*., 2005). During HPV pseudovirons production L1 and L2 capsid proteins are co-transfected together with a reporter gene into 293TT cells (Buck *et al*., 2004). In pseudovirion-based papillomavirus neutralisation assay the presence of HPV neutralising antibodies in a sample are determined by the intensity of the secreted alkaline phosphatase in cell supernatant (Pastrana *et al*., 2004; Buck *et al*., 2005). According to Pastrana *et al*., (2004) the pseudovirus-based neutralisation assay is more sensitive and more genotype-specific than the VLP-based ELISA. Cervical and serum HPV-16 neutralising antibodies detected using pseudovirion-based neutralisation assay are associated with HPV-16 infection but not with cervical disease (Mbulawa *et al*., 2008). HPV ELISA detects both binding and neutralising antibodies while pseudovirus-based neutralisation assay detects only neutralising antibodies (Pastrana *et al*., 2004).

HPV multiplex serology technique permits the detection of more than 100 different HPV types simultaneous (Opalka *et al*., 2003; Waterboer *et al*., 2005). Multiplex serology allows data generation of large epidemiologic studies in a few days as 1000 samples per day can be tested. a low volume of sample is required (2µl) and risk of cross contamination between samples is reduced in multiplex serology technique. The HPV multiplex serology has been used extensively in monitoring HPV vaccines. HPV multiplex serology testing for vaccine efficacy is needed even though there are only 2 or 4 HPV types in current HPV vaccines so that immunity to non-vaccine HPV types can be studied. There are different multiplex serology assays. The multiplex serology used in the current study is the same as the one described by Waterboer *et al*., (2005) and is based on glutathione S-transferase fusion-L1 protein not VLPs. High concordance between glutathione S-transferase (GST) capture ELISA and multiplex serology was observed and samples that were only positive by multiplex serology and not by

ELISA demonstrated very low titres. HPV seropositivity by multiplex serology is not found to correlate very well with HPV DNA at both genital and oral sites but associated with age at onset of sexual activity, number of sexual partners and history of genital warts (Syrjanen *et al*., 2009).

HPV IgA and IgG antibodies have been detected in cervical mucus; IgA is detected in cervical mucus even in the absence of serum HPV IgA while cervical HPV IgG is frequently detected in those women with high titres of serum HPV IgG (Lorincz *et al*., 1992, Marais *et al*., 2000). It is possible that through transudation, the IgG antibodies from serum can enter mucosal surfaces (Bontkes *et al*., 1999). HPV persistence and HSIL were associated with serum HPV IgG responses while HPV clearance and persistence were observed in women with Th1 responses (Carter *et al*., 1996; de Gruijl *et al*., 1999). Antibody titres produced in natural infection are of lower titre and probably do not always reach the level required to provide protection. The high antibody titres observed in vaccinated candidates are associated with complete protection against HPV infection (Harper *et al*., 2006; Villa *et al*., 2006). However, the minimum level of HPV antibody correlating with protection against infection is unknown.

When comparing serum antibody responses between women and men, it was found that antibodies to HR-HPV types in women are initially detected after puberty indicating initiation of sexual activity, and increase between 25-34 years. In men, a significant increase between 25-34 years was not observed (Michael *et al*., 2008). The antibody increase and peak in young women has been reported elsewhere (Marais *et al*., 1997; Stone *et al*., 2002; Shin *et al*., 2003). Sasagawa *et al*., (2003) observed that women between 40-49 years of age had a high prevalence of HPV IgG responses against HPV-16, -18, -31 or -45 compared to women between 25-29 years of age even though cervical HPV DNA prevalence was found to decrease with increasing age. Cumulative exposure to HPV in older women could result in a high prevalence of HPV IgG responses in older women (Wang *et al*., 2000; Onda *et al*., 2003). HIV-positive women are reported to have a higher prevalence of HPV IgG antibodies compared to HIV- negative women (Viscidi *et al*., 2003). According to Marais *et al*., (2009), after HIV seroconversion in women, the prevalence of serum HPV-16 IgA, cervico-vaginal IgA and IgG declined while the prevalence of serum HPV-16 IgG was found to increase. IgG is the most abundant isotype in serum followed by IgA, however IgA at mucosal sites is reported to play a very significant role in protection against pathologic agents (Mestecky & Fultz 1999). Antibody responses to HPV infection are determined as a key of protective immunity (Stanley

et al., 2006). According to Ho *et al*., (2004), the ability of a host to develop a strong humoral immunity may be detected by the presence of IgA antibodies.

The aims of the study were:

i) to determine the distribution of HPV antibodies in South African women and men;

ii) to investigate factors that are associated with HPV seropositivity such as genital HPV DNA positivity, HIV status, CD4 counts, use of antiretroviral (ARV) therapy, age, cervical abnormalities, marital status, smoking, number of sexual partners, age at first sexual intercourse and genital ulcers; and

iii) to investigate HPV seroconversion over a 12-month period in women and men and predictors of HPV seroconversion.

5.2 METHODS AND METHODS

5.2.1 Study population and specimen collection

Study participants were recruited from the Manyanani clinic, Empilisweni Centre, Cape Town, South Africa. The Research Ethics Committee of the University of Cape Town approved all aspects of the investigation. Study participants were 171 HIV-negative women, 265 HIV-positive women, 277 HIV-negative men and 159 HIV-positive men. The mean ages were 35 years (range, 18-65 years) and 38 years (range, 19-67 years) for women and men respectively. Of the men enrolled in the study 94% (404/428) were circumcised and 7 men had missing data on circumcision. Blood specimens were taken from all participants at both baseline and 12-months for measurement of serum antibody responses to HPV-11, -16, -18, -31, -33, -35, -45, -52, and -58. After collection, the blood samples were centrifuged at 1500rpm for 5 minutes to separate serum. The sera samples were aliquoted and stored at -80°C. Genital samples were collected and stores as described in section 2.2.1.

5.2.2 HPV serology

Serum was stored at -80°C and shipped on dry ice to German Cancer Research Centre (DFKZ), Heidelberg, Germany for detection of antibodies by multiplex serology. Since the multiplex serology technology was not available in South Africa it was arranged for the investigator (ZZA Mbulawa) to travel to DFKZ laboratory to train. HPV serology data was performed with the assistance of Dr Tim Waterboer and Dr Michael Pawlita of DFKZ. Detection of serum antibodies to the L1 major capsid protein of HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52 and HPV-58 was performed using multiplex serology based on

glutathione S-transferase fusion-L1 protein capture on fluorescent beads as described by Watertboer *et al*., (2005 and 2006) and serum was tested at 1:100 dilution. The secondary antibody used in this assay recognizes IgA, IgG and IgM together. Cut-off for positivity was determined using serum obtained from Korean female students who were HPV DNA negative (by Pap smears) and self-reported virgins and the cut-off was 400 median fluorescence intensity (MFI) for the L1 antigen of the 9 individual HPV types. Each sample was duplicated and averaged. Seroconverters were define as participants whose serum samples had a ≤400MFI at baseline and who at the 12-month visit showed a 2-fold increased MFI over the baseline visit which was also >400MFI (Syrjanen *et al*., 2009).

5.2.3 HPV genotyping

Samples were collected as described in chapter 2 section 2.2.1. DNA was extracted from both cervical and penile cells using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany) and automated MagNA Pure Compact machine (Roche Diagnostics, Mannheim, Germany). HPV typing was performed on DNA extracted from cervical and penile cells as described in section 2.2.2.

5.2.4 Statistical analyses

Statistics analyses were performed using X^2 test (EpiInfo Version 5 Statcalc) and Mann-Whitney test (GraphPad Prism® 5) when comparing HPV antibody magnitudes. Advanced statistics was performed by Dr Henri Carrara (Department of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town) using STATA 11.0 (StataCorp, College Station, TX, USA). HPV prevalence data were tabulated by key demographic and behavioural variables. Univariate analyses to estimate Odds Ratios and 95% confidence intervals were performed using logistic regression. All univariate analyses were all adjusted for age as a continuous variable because age is such an important confounder. Multivariate model building included age as a continuous variable and factors that demonstrated significant trend in the univariate analyses.

5.3 RESULTS

5.3.1 Serum HPV antibody prevalence in women and men

More women were found to have antibody responses to all of the 9 HPV types tested compared to men (53% 232/435 compared to 31% 137/436, P<0.0001). In women seroprevalence for

HPV-11 (34%) was the highest followed by HPV-31 (30%), HPV-16 (24%), HPV-18 (24%), HPV-45 (22%), HPV-35 (18%), HPV-52 (14%), HPV-58 (11%) and antibodies to HPV-33 (8%) were less prevalent. In men seroprevalence for HPV-11 (14%) was the highest followed by HPV-31 (11%), HPV-35 (10%), HPV-16 (7%), HPV-52 (7%), HPV-58 (7%), HPV-18 (5%), HPV-45 (5%) and antibodies to HPV-33 (3%) were less prevalent (Figure 5.1). Multiple seroprevalence was defined as antibody reactivity to more than one HPV type. Women were found to have a 2.8-fold significantly higher seroprevalence of multiple types (2-9) compared to men (34%, 150/435 compared to 12%, 54/436, P<0.0001, Figure 5.1).

Women were found to have a 2-fold to 4-fold higher seroprevalence compared to men and similar findings were observed when stratified according to the age (Figure 5.2). HPV seroprevalence was found to increase in both women and men with increasing age, from age 18-29 years to 30-40 years, however seroprevalence decreased amongst those aged 41-67 years [women: 30-40 years (58% 93/161) compared to 18-29 years (48% 76/160, P=0.07) and compared to 41-67 years (49% 56/114); men: 30-40 years (32% 62/191) compared to 18-29 years (19% 16/84, P=0.02) and compared to 41-67 years (22% 35/161, P=0.02) Figure 5.2]. Multiple seroprevalence in women was also found to increase with increasing age but decreased at 41-67 years; however, in men aged 41-67 no decrease was observed (Figure 5.2).

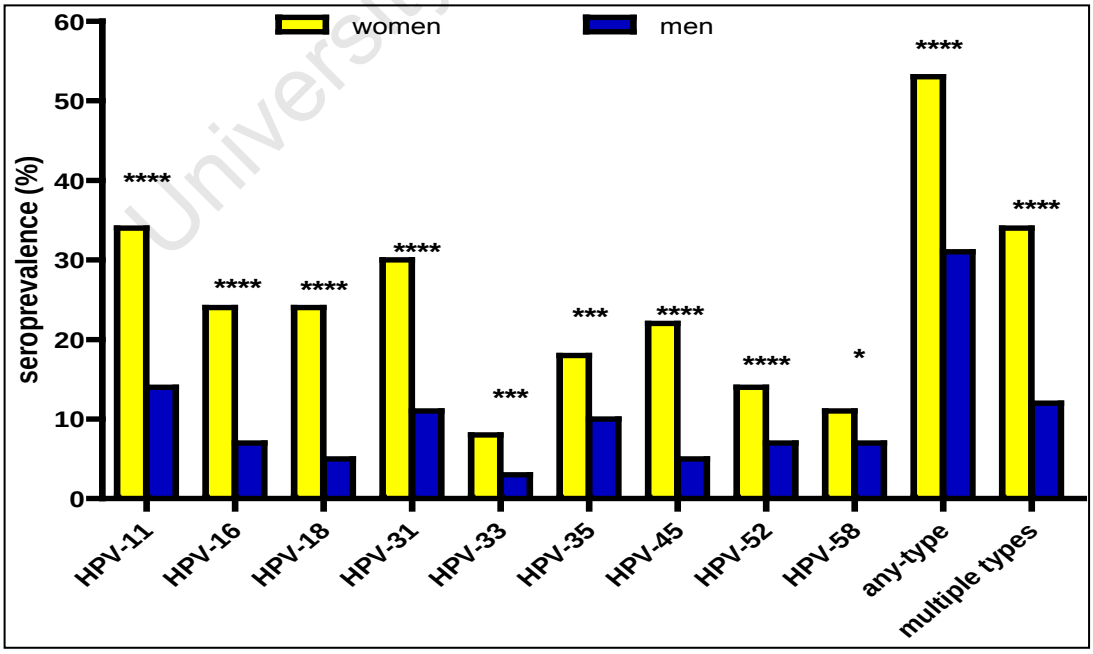

Figure 5.1. Human papillomavirus (HPV) serum antibody prevalence in women and men. Significantly more women had antibodies to all 9 types tested and more women had antibodies to multiple types compared to men. Note: * p<0.05, ***p=0.001, ****p<0.0001.

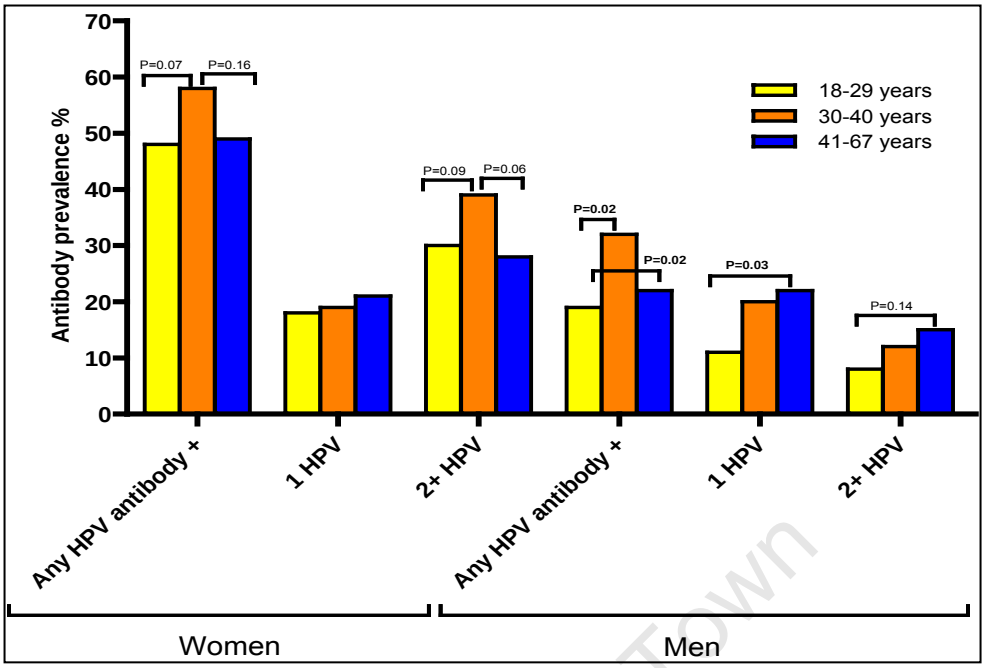

Figure 5.2. Human papillomavirus (HPV) serum antibody prevalence and multiple seroprevalence in women and men according to age.

5.3.2 HPV type-specific seroprevalence according to genital HPV DNA infection in women and men

HPV type-specific seroprevalence according to genital HPV DNA infection in women is demonstrated in Table 5.1. HPV-11 seropositivity was 40% in HPV DNA negative women, 29.6% in women HPV DNA positive for types other than HPV-11 and 60% in women HPV-11 DNA positive. Women that were HPV-11 DNA positive were found to have a 2.3 risk (95% CI: 0.4-14.4) of having HPV-11 antibodies compared to HPV DNA negative women. HPV-16 seropositivity was 25.6% in HPV DNA negative women, 22.6% in women HPV DNA positive for types other than HPV-16 and 25% in women HPV-16 DNA positive. Women that were HPV-16 DNA positive were not more likely to have HPV-16 antibodies compared to HPV DNA negative women. HPV-18 seropositivity was 25.6% in HPV DNA negative women, 20.5% in women HPV DNA positive for types other than HPV-18 and 35.7% in women HPV-18 DNA positive. Women that were HPV-18 DNA positive had 1.6 risk (95% CI: 0.7-3.8) of having HPV-18 antibodies compared to HPV DNA negative women.

HPV α7 species seropositivity was 27.3% in HPV DNA negative women, 22.4% in women HPV DNA positive for types other than α7 HPV species and 36.5% in women α7 HPV species DNA positive. The risk of having α7 HPV species antibodies in women that were α7 HPV

species positive was 1.5 times (95% CI: 0.8-2.9) greater compared to HPV DNA negative women. α9 HPV species seropositivity was 42.4% in HPV DNA negative women, 44.2% in women HPV DNA positive for types other than α9 HPV species and 39.1% in women α9 HPV species DNA positive. Antibody responses were not found to differ between women that were α9 HPV species positive compared to HPV DNA negative women (OR: 0.9, 95% CI: 0.5-1.4; Table 5.1).

Table 5.1. HPV type-specific seroprevalence as related to genital HPV DNA at the baseline visit in women

Cervical DNA status	n seroposositive/ n total (% seropositive)	OR (95% CI)	P-value
	HPV-11		
HPV DNA negative	67/172 (40.0)	ref	
HPV pos but HPV-11 negative	76/257 (29.6)	0.7 (0.4-1.0)	**0.04**
HPV-11 positive	3/5 (60.0)	2.3 (0.4-14.4)	0.36
	HPV-16		
HPV DNA negative	44/172 (25.6)	ref	
HPV pos but HPV-16 negative	51/226 (22.6)	0.8 (0.5-1.3)	0.49
HPV-16 positive	9/36 (25)	1.0 (0.4-2.2)	0.94
	HPV-18		
HPV DNA negative	44/172 (25.6)	ref	
HPV pos but HPV-18 negative	48/234 (20.5)	0.8 (0.5-1.2)	0.23
HPV-18 positive	10/28 (35.7)	1.6 (0.7-3.8)	0.27
	α7 HPV species		
HPV DNA negative	47/172 (27.3)	ref	
HPV pos but α7 HPV negative	47/210 (22.4)	0.8 (0.5-1.2)	0.27
α7 HPV positive	19/52 (36.5)	1.5 (0.8-2.9)	0.2
	α9 HPV species		
HPV DNA negative	73/172 (42.4)	ref	
α9 HPV pos but HPV negative	57/129 (44.2)	1.1 (0.7-1.7)	0.76
α9 HPV positive	52/133 (39.1)	0.9 (0.5-1.4)	0.56

ref: reference**. α7 HPV species** includes HPV-18 and -45. **α9 HPV species** includes HPV-16, -31, -33, -35, -52 and -58.

HPV type-specific seroprevalence according to genital HPV DNA infection in men is demonstrated in Table 5.2. HPV-11 seropositivity was 12.6% in HPV DNA negative men, 13.8% in men HPV DNA positive for other types but not HPV-11 and 40% in men HPV-11 DNA positive. Men that were HPV-11 DNA positive had 1.6 risk (95% CI: 1.2-17.7) of having HPV-11 antibodies compared to HPV DNA negative men. HPV-16 seropositivity was 5.7% in HPV DNA negative men, 9% in men HPV DNA positive for types other than HPV-16 and 0% in men HPV-16 DNA positive. HPV-18 seropositivity was 3.4% in HPV DNA negative men, 5.5% in men HPV DNA positive for types other than HPV-18 and 4.6% in men HPV-18 DNA positive. Men that were HPV-18 DNA positive had 1.3 risk (95% CI: 0.3-11.7) of having HPV-18 antibodies compared to HPV DNA negative men. α7 HPV species seropositivity was

5.1% in HPV DNA negative men, 7.7% in men HPV DNA positive for other types but not α7 HPV species and 4.1% in men α7 HPV species DNA positive. Men that were α7 HPV species positive were more likely to have antibody responses compared to α7 HPV DNA negative men. α9 HPV species seropositivity was 22.3% in HPV DNA negative women, 23.8% in women HPV DNA positive for types other than α9 HPV species and 23.7% in women α9 HPV species DNA positive. Women that were α9 HPV species positive were not more likely to have antibody responses compared to HPV DNA negative women (OR: 1.1, 95% CI: 0.6-2.0; Table 5.2).

Table 5.2. HPV type-specific seroprevalence as related to genital HPV DNA at the baseline visit in men

Penile DNA status	n seroposositive/ n total (% seropositive)	OR (95% CI)	P-value
	HPV-11		
HPV DNA negative	22/175 (12.6)	ref	
HPV pos but HPV-11 negative	34/247 (13.8)	1.1 (0.6-2.0)	0.72
HPV-11 positive	4/10 (40)	1.6 (1.2-17.7)	**0.03**
	HPV-16		
HPV DNA negative	10/175 (5.7)	ref	
HPV pos but HPV-16 negative	20/223 (9.0)	1.6 (0.7-3.6)	0.23
HPV-16 positive	0/34 (0.0)	..	..
	HPV-18		
HPV DNA negative	6/175 (3.4)	ref	
HPV pos but HPV-18 negative	13/235 (5.5)	1.6 (0.6-4.4)	0.32
HPV-18 positive	1/22 (4.6)	1.3 (0.2-11.7)	0.79
	α7 HPV species		
HPV DNA negative	9/175 (5.1)	ref	
HPV pos but α7 HPV negative	16/208 (7.7)	1.5 (0.7-3.6)	0.32
α7 HPV positive	2/49 (4.1)	0.8 (0.2-3.8)	0.76
	α9 HPV species		
HPV DNA negative	39/175 (22.3)	ref	
HPV pos but α9 HPV negative	38/160 (23.8)	1.1 (0.7-1.8)	0.75
α9 HPV positive	23/97 (23.7)	1.1 (0.6-2.0)	0.79

ref: reference. Neg: negative, pos: positive

5.3.3 HPV antibody response according to cervical cytology

HPV seroprevalence was found to be similar in women with normal cytology, ASCUS, LSIL and those with HSIL (43% 121/284; 39% 15/38; 40% 30/75 and 42% 5/12 respectively). Among women that were HPV seropositive, multiple seroprevalence was higher in women with abnormal cervical cytology (includes ASCUS, LSIL and HSIL) compared to women with normal cervical cytology (64% 32/50; 47% 57/121, P=0.04). Multiple seroprevalence was found to increase as cervical cytology abnormalities increased in severity and then decrease in women with HSIL (normal cervical cytology: 47% 57/121, ASCUS: 67% 10/15, LSIL: 70% 21/30 and HSIL: 20% 1/5).

Antibody titres were defined as the level of MFI. Women with abnormal cervical cytology were found to have higher antibody titres compared to women with normal cervical cytology and this was found to be significant for HPV-16 (median: 77 MFI, range: 0- 5366 MFI compared to median: 46 MFI, range: 0-4290 MFI, P=0.04), HPV-45 (median: 43 MFI, range: 0- 6230 MFI compared to median: 27 MFI, range: 0- 3098 MFI, P=0.035, Table 5.3) and all HPV types (median: 53 MFI, range: 0- 7145 MFI compared to median: 42 MFI, range: 0- 5430 MFI, P=0.0002). There was no difference in seroprevalence to all HPV types tested between women with normal cervical cytology with those with abnormal cervical cytology.

Table 5.3. HPV seroprevalence and antibody titres (MFI) for women with normal and abnormal cervical cytology

	Normal cytology N=284		**Abnormal cytology N=125**			
Antibodies	**n (%)**	**MFI median (range)**	**n (%)**	**MFI median (range)**	**P-value[a]**	**P-value[b]**
HPV-11	71 (25)	122 (0-4997)	29 (23)	137 (0-3161)	0.7	0.74
HPV-16	40 (14)	46 (0-4290)	20 (16)	77 (0-5366)	0.61	**0.04**
HPV-18	35 (12)	37 (0-4290)	22 (18)	51 (0-5291)	0.16	0.2
HPV-31	55 (19)	59 (0-5430)	25 (20)	77 (0-7145)	0.88	0.06
HPV-33	12 (4)	19 (0-2232)	11 (9)	29 (0-2034)	0.06	0.07
HPV-35	30 (11)	45 (0-2626)	21 (17)	54 (0-5527)	0.08	0.31
HPV-45	37 (13)	27 (0-3098)	19 (15)	43 (0-6230)	0.56	**0.035**
HPV-52	29 (10)	41 (0-3924)	14 (11)	52 (0-3855)	0.76	0.12
HPV-58	21 (7)	30 (0-5068)	12 (10)	37 (0-3084)	0.45	0.18
All HPV types	121 (43)	42 (0-5430)	50 (40)	53 (0-7145)	0.55	**0.0002**

[a] compare seroprevalence, [b] compare median antibody titre between women with normal and abnormal cytology.

5.3.4 HPV antibody response according to HIV status

HIV-positive women were found to have a significantly higher HPV-16 antibody titre compared to HIV-negative women (median: 135 MFI, range: 0- 5987 MFI compared to median: 88 MFI, range: 0- 5366 MFI; P=0.03, Figure 5.3a). The titres of antibody to HPV-11, -18, -31, -33, -35, -45, -52 and -58 were not found to differ significantly between HIV-negative women and HIV-positive women (Table 5.4). HIV-positive women were found to have higher antibody titre of combined HPV compared to HIV-negative women (median: 100 MFI, range: 0- 5987 MFI compared to median: 87 MFI, range: 0- 8024 MFI; P=0.07, Table 5.4), however it was not found to be significantly higher. HIV-positive men were found to have significantly

higher HPV-45 antibody titres compared to HIV-negative men (median: 16 MFI, range: 0-2173 MFI compared to median: 8 MFI, range: 0- 3804 MFI; P=0.003, Figure 5.3b).

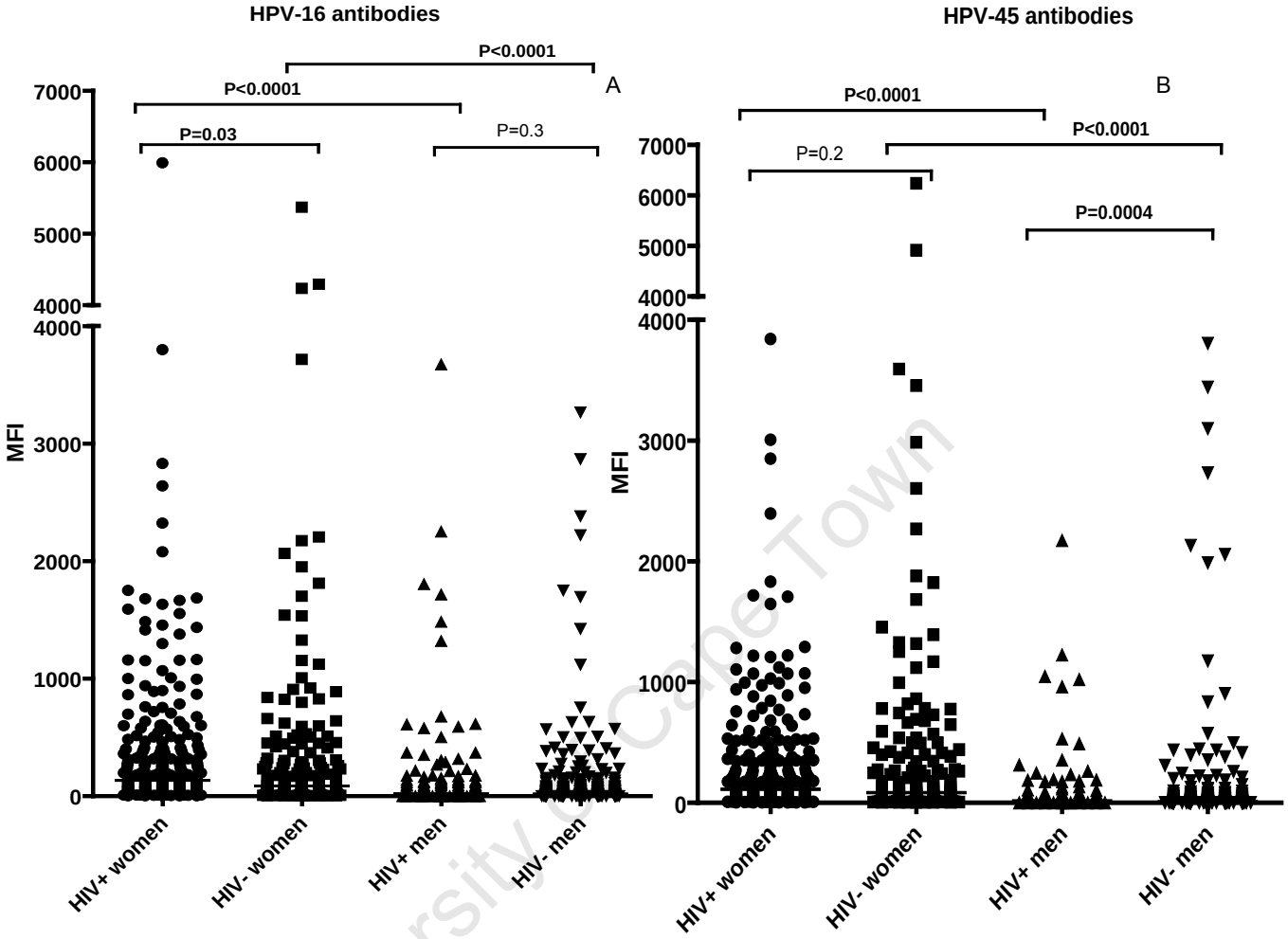

Figure 5.3. HPV-16 (A) and HIV-45 (B) antibody titres in HIV-positive and HIV-negative women and men. HIV+: HIV-positive, HIV-: HIV-negative.

The antibody titres to HPV-11, -16, -18, -31, -33, -35, -52 and -58 were not found to differ significantly between HIV-negative men and HIV-positive men (Table 5.5). However, for all HPV types HIV-positive men demonstrated higher antibody titres compared to HIV-negative men (median: 24 MFI, range: 0- 12510 MFI compared to median: 20 MFI, range: 0- 5455 MFI; P=0.02, Table 5.5). HPV antibody titres for all types were found to be significantly higher in women compared to men regardless of HIV-status. When women and men were grouped according to HIV status, HIV-positive women were found to have significantly higher antibodies titres for all HPV types investigated compared to HIV-positive men. HIV-negative women were also found to have significantly higher antibodies titres for all HPV types investigated compared to HIV-negative men (Table 5.5).

Table 5.4. HPV antibody titres in HIV-positive and HIV-negative women

	Women				
	HIV-positive (n=265)		HIV-negative (n=170)		P-value
Antibody	median	range	median	range	
HPV-11	200	0-3007	289	1-8024	0.07
HPV-16	135	0-5987	88	0-5366	**0.03**
HPV-18	116	0-3294	92	0-5291	0.21
HPV-31	140	1-4060	158	0-7145	0.97
HPV-33	41	0-1535	31	0-2034	0.06
HPV-35	76	0-3650	79	0-5527	0.79
HPV-45	112	0-3850	76	0-6230	0.2
HPV-52	81	0-2566	66	0-3855	0.26
HPV-58	56	1-1542	17	0-6758	0.70
All HPV types	100	0-5987	86	0-8024	0.07

Table 5.5. HPV antibody titres in HIV-positive and HIV-negative men.

	Men				
	HIV-positive (n=160)		HIV-negative (n=277)		P-value
Antibody	median	range	median	range	
HPV-11	79	0-2231	70	0-5455	0.65
HPV-16	20	0-3764	18	0-3265	0.31
HPV-18	16	0-1666	14	0-3859	0.18
HPV-31	28	0-5430	27	0-4420	0.94
HPV-33	9	0-2232	9	0-1773	0.95
HPV-35	34	0-12510	23	0-2603	0.26
HPV-45	16	0-2173	8	0-3804	**0.004**
HPV-52	25	0-3934	8	0-3376	0.21
HPV-58	21	0-5086	17	0-3363	0.40
All HPV types	24	0-12510	20	0-5455	**0.02**

5.3.5 Factors associated with HPV seropositivity in women

Investigation of factors associated with HPV seropositivity was only restricted to HPV-11, -16, -18, α7 and α9 HPV species in both women and men. Table 5.6 demonstrates the determinants of HPV-11, -16 and -18 seropositivity at baseline in women (univariate analysis). HPV-11

seropositivity was associated with the use of ARVs among the HIV-positive women (OR: 0.2, 95% CI: 0.1-0.6, P=0.004); being unmarried (OR: 2.0, 95% CI: 1.1-3.6, P=0.02); having developed a genital ulcer 1+ month ago (OR: 1.9, 95% CI: 0.5-7.7, P=0.38) but not significantly; current smoking (OR: 1.6, 95% CI: 1.0-2.6, P=0.04) and previous smoking (OR: 1.9, 95% CI: 0.9-3.9, P=0.08). In women, HPV-16 seropositivity was also associated with being unmarried (OR: 2.7, 95% CI: 1.3-5.7, P=0.007); having 3-4 lifetime sexual partners (OR: 1.9, 95% CI: 1.1-3.2, P=0.015), having developed a genital ulcer 1+ month ago (OR: 6.3, 95% CI: 1.1-38.9, P=0.04); and previous smoking (OR: 2.1, 95% CI: 1.0-4.5, P=0.08) but not significantly. In HIV-positive women, HPV-18 seropositivity was associated with increased CD4 counts (OR: 3.1, 95% CI: 1.1-8.3, P=0.03); being unmarried (OR: 2.1, 95% CI: 1.0-4.1, P=0.04) having developed a genital ulcer 1+ month ago (OR: 3.7, 95% CI: 0.6-22.5, P=0.15) but not significantly; and current smoking (OR: 1.7, 95% CI: 1.1-2.9, P=0.03). Factors such as HIV status, age at first sexual intercourse, number of sexual partners over the previous year, number of new sexual partners over the previous year, sexual frequency over the last month, genital ulcer and abnormal cytology were not significantly associated with HPV-11, -16 or -18 seropositivity in women (Table 5.6).

In women, α9 HPV (HPV-16, -31, -33, -35,-52 and/ or -58) seropositivity was significantly associated with being unmarried (OR: 1.9, 95% CI: 1.1-3.2, P=0.02) and having developed a genital ulcer ≥1 month ago (OR: 5.3, 95% CI: 1.3-21.7, P=0.02). α7 HPV (HPV-18 and/ or HPV-45) seropositivity was associated with CD4 counts ≥500/mL among HIV-positive women (OR: 2.7, 95% CI: 1.1-7.1, P=0.04) and having developed a genital ulcer ≥1 month ago (OR: 3.5, 95% CI: 0.7-17.1, P=0.13) however the latter association was not significant. α9 HPV seropositivity was not significantly associated with any factor assessed in women.

Table 5.6. The HPV-11, -16 and -18 antibodies in women and factors associated with seropositivity, adjusted for age as a continuous variable

		Women: HPV-11			Women: HPV-16			Women: HPV-18		
Variable	**n**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**
Age										
<30 years	144	31.3	1.0		1.0	1.0		1.0	1.0	
30-39 years	162	38.3	1.4 (0.8 - 2.2)	0.12	28.4	1.2 (0.8-1.8)	0.34	29.0	**1.7 1.0-2.9)**	**0.05**
40-49 years	97	33.0	1.1 (0.6 - 1.9)	0.78	20.6	0.9 (0.5-1.4)	0.59	23.7	1.3 (0.7-2.4)	0.43
50+ years	31	22.6	0.6 (0.26 - 1.6)	0.34	12.9	0.5 (0.2-1.4)	0.22	12.9	0.6 (0.2-1.9)	0.40
HIV										
negative	169	38.5	1.0		23.8	1.0		24.3	1.0	
positive	265	30.6	0.7 (0.5 - 1.0)	0.07	24.2	1.0 (0.6-1.6)	0.97	23.0	0.9 (0.6-1.5)	0.73
CD4 count*										
<200/mL	45	40.0	1.0		20.0	1.0		13.3	1.0	
200 – 349/mL	84	22.6	**0.4 (0.2 - 1.0)**	**0.04**	27.4	1.5 (0.6-3.6)	0.36	22.6	1.9 (0.7-5.1)	0.21
350 – 499/mL	63	31.8	0.7 (0.32 - 1.6)	0.42	20.6	1.1 (0.4-2.8)	0.9	19.1	1.6 (0.5-4.5)	0.42
500+/mL	72	21.9	0.7 (0.33 - 1.6)	0.41	25.0	1.4 (0.5-3.3)	0.51	31.9	**3.1 (1.1-8.3)**	**0.03**
ARV status*										
yes	15	66.8	1.0		40.0	1.0		33.3	1.0	
no	206	29.0	**0.2 (0.1 - 0.6)**	**0.004**	25.2	0.5 (0.2-1.5)	0.21	23.8	0.6 (0.2-1.9)	0.39
Married										1.00
yes	77	22.1	1.0		11.7	1.0		14.3	1.0	
no	356	36.2	**2.0 (1.1-3.6)**	**0.02**	26.7	**2.7 (1.3-5.7)**	**0.007**	25.6	**2.1 (1.0-4.1)**	**0.04**
Age at first sex										
<15 years	26	38.5	1.0		23.0	1.0		23.1	1.0	
15+	407	33.4	0.8 (0.4-1.9)	0.63	24.1	1.1 (0.4-2.8)	0.87	23.6	1.0 (0.4-2.7)	0.94
Number of sex partners										
1- 2	163	38.0	1.0		19.6	1.0		21.5	1.0	
3 - 4	153	34.0	0.8 (0.5 - 1.3)	0.47	31.4	**1.9 (1.1-3.2)**	**0.015**	28.8	1.5 (0.9-2.5)	0.13
5 - 9	89	29.2	0.7 (0.4 - 1.2)	0.18	19.1	1.0 (0.5-1.9)	0.98	20.2	0.9 (0.5-1.8)	0.83
10+	28	21.4	0.5 (0.2 - 1.2)	0.1	25.0	1.4 (0.6-3.6)	0.469	17.9	0.8 (0.3-2.3)	0.68
Sex partners previous year										
0 or 1	379	32.7	1.0		23.2	1.0		23.5	1.0	
3+	52	38.5	1.3 (0.7 - 2.3)	0.44	28.9	1.3 (0.7-2.5)	0.42	25.0	1.1 (0.6-2.1)	0.83

		Women: HPV-11			Women: HPV-16			Women: HPV-18		
Variable	**n**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**
New sex partners previous year										
0 or 1	378	31.5	1.0		24.1	1.0		23.0	1.0	
3+	17	41.2	1.5 (0.55 - 4.0)	0.43	11.8	0.4 (0.1-1.8)	0.23	23.5	1.0 (0.3-3.2)	0.97
Sex frequency previous month										
0	37	35.1	1.0		29.7	1.0		29.7	1.0	
1	121	33.1	0.9 (0.5 - 1.9)	0.77	26.5	0.8 (0.4-1.8)	0.62	21.5	0.6 (0.3-1.5)	0.29
2	135	31.9	0.8 (0.4 - 1.8)	0.64	19.3	0.5 (0.2-1.2)	0.13	21.5	0.6 (0.3-1.4)	0.28
3	57	36.8	1.0 (0.4 - 2.5)	0.92	22.8	0.7 (0.3-1.7)	0.39	28.1	0.9 (0.4-2.2)	0.83
4	80	32.5	0.8 (0.4 - 1.9)	0.69	26.3	0.8 (0.3-1.8)	0.56	25.0	0.8 (0.3-1.8)	0.55
Genital ulcer										
no	387	34.9	1.0		24.0	1.0		24.6	1.0	
yes	47	23.4	0.6 (0.3-1.1)	0.11	23.4	0.9 (0.5-1.9)	0.86	14.9	0.5 (0.2-1.2)	0.14
Genital ulcer recency										
<1 month	25	20.0	1.0		8.0	1.0		8.0	1.0	
≥1 month	19	31.6	1.9 (0.5-7.7)	0.38	36.8	**6.3 (1.1-38.9)**	**0.04**	26.3	3.7 (0.6-22.5)	0.15
Cervical cytology										
normal	284	34.5	1.0		23.6	1.0		25.0	1.0	
ASCUS	38	36.8	1.1 (0.5 - 2.2)	0.78	18.4	0.7 (0.3-1.7)	0.48	29.0	1.2 (0.6-2.6)	0.60
LSIL	75	26.7	0.7 (0.37 - 1.18)	0.16	25.3	1.0 (0.6-1.9)	0.88	16.0	0.6 (0.3-1.1)	0.09
HSIL	12	25.0	0.7 (0.2 - 2.5)	0.53	33.3	1.7 (0.5-5.8)	0.4	16.7	0.6 (0.1-2.9)	0.53
Smoking										
never	277	30.0	1.0		21.7	1.0		20.2	1.0	
previously	35	42.9	1.9 (0.9-3.9)	0.08	34.3	2.1 (1.0-4.5)	0.06	28.6	1.7 (0.7-3.7)	0.21
current	122	39.3	**1.6 (1.0-2.6)**	**0.04**	26.2	1.4 (0.8-2.4)	0.18	29.5	**1.7 (1.1-2.9)**	**0.03**

Numbers will not always add up to 437 because of some of the participants did not provide all required information, n: number, Ab%: percentage of women with positive antibody response. Ab: antibodies. *: HIV-positive. Age adjusted as a continuous variable.

Multivariate analysis included variables such as age, HIV status, CD4 count, ARVs, marital status, total number of sex partners and smoking habit. Table 5.7 demonstrates the determinants of HPV-11, -16 and -18 seropositivity at baseline in women (multivariate analysis). HIV-positive women were more likely to have antibodies to HPV-11 (OR: 4.7, 95% CI: 1.6-16.7, P=0.02) and HPV-16 (OR: 1.8, 95% CI: 0.5-6.2, P=0.36) compared to HIV-negative women but not for HPV-18, α7 and α9 HPV species. HIV-positive women with ≥500/mL CD4 counts were more likely to have antibodies to HPV-18 (OR: 3.5, 95% CI: 1.1-10.9, P=0.03) and α7 HPV species (OR: 3.0, 95% CI: 1.0-8.8, P=0.05) compared to HIV-positive women with <200/mL CD4 counts but not for HPV-11, HPV-16 and α9 HPV species. Women that were HIV-positive and not using ARVs were found to be significantly less likely to develop antibodies to HPV-11 (OR: 0.2, 95% CI: 0.1-0.7, P=0.01) compared to those using ARVs but not for HPV-16, HPV-18, α7 and α9 HPV species. Women that were married were found to be more likely to have detectable antibodies compared to married women and this was significant for HPV-16 (OR: 2.7, 95% CI: 1.2-6.1, P=0.01) and α9 HPV species (OR: 1.9, 95% CI: 1.1-3.5, P=0.02). Women that were previously smoking were more likely to have HPV-11 antibodies compared to women who never smoked (OR: 2.6, 95% CI: 1.1-539, P=0.03, Table 5.7).

Surprisingly the level of antibodies was found to decrease with increasing number of total sex partners for HPV-11, -16 and -18 (Table 5.7). When the number of sex partners or new sex partners in previous year was used in multivariate analysis instead of total number of sex partners, HPV-11 seropositivity was found to be influenced by HIV-positive status in women (OR: 3.1, 95% CI: 0.8-12.0; and OR: 3.6, 95% CI: 1.0-12.9 respectively, data not shown). Factors that were found to have an association with HPV seropositivity for HPV-11, -16, -18, α7 and α9 HPV species in multivariate analysis when total number of sex partners was included in the analysis were also found to have an association when the number of sex partners or new sex partners in previous year were used instead of total number of sex partners.

Table 5.7. The HPV-11, -16 and -18 antibodies in women and factors associated with seropositivity in multivariate analysis

		WOMEN: HPV-11			WOMEN: HPV-16			WOMEN: HPV-18		
Variable	**n**	**%**	**OR (95% CI)**	**P-value**	**%**	**OR (95% CI)**	**P-value**	**%**	**OR (95% CI)**	**P-value**
Age*	434	33.6	1.0 (0.9-1.0)	0.08	24.0	1.0 (0.2-1.0)	0.17	23.5	1.0 (0.9-1.0)	0.29
HIV										
negative	169	38.5	1.0		23.8	1.0		24.3	1.0	
positive	265	30.6	**4.7 (1.3-16.7)**	**0.02**	24.2	1.8 (0.5-6.2)	0.36	23	0.9 (0.2-3.5)	0.86
CD4 count[+]										
<200/mL	45	40.0	1.0		20.0	1.0		13.3	1.0	
200 - 349/mL	84	22.6	0.4 (0.2-1.0)	0.05	27.4	1.4 (0.5-4.0)	0.48	22.6	2.0 (0.6-6.5)	0.23
350 - 499/mL	63	31.8	0.7 (0.3-1.8)	0.44	20.6	1.2 (0.4-3.4)	0.78	19.1	1.9 (0.3-6.2)	0.31
500+/mL	72	21.9	0.7 (0.5-1.8)	0.49	25.0	1.5 (0.5-4.1)	0.46	31.9	**3.5 (1.1-10.9)**	**0.03**
ARV status[+]										
yes	15	66.8	1.0		40.0	1.0		33.3	1.0	
no	206	29.0	**0.2 (0.1-0.7)**	**0.01**	25.2	0.5 (0.1-1.4)	0.18	23.8	0.5 (0.1-1.6)	0.24
Married										
yes	77	22.1	1.0		11.7	1.0		14.3	1.0	
no	356	36.2	1.8 (1.0-3.5)	0.07	26.7	**2.7 (1.2-6.1)**	**0.01**	25.6	1.8 (0.9-3.8)	0.12
Total number of sex partners										
1- 2	163	38.0	1.0		19.6	1.0		21.5	1.0	
3 - 4	153	34.0	0.9 (0.5-1.5)	0.61	31.4	1.6 (0.9-2.8)	0.09	28.8	1.5 (0.9-2.7)	0.15
5 - 9	89	29.2	0.7 (0.4-1.3)	0.27	19.1	0.8 (0.4-1.7)	0.60	20.2	1.0 (0.5-2.0)	0.97
10+	28	21.4	0.3 (0.1-1.0)	**0.05**	25.0	0.8 (0.3-2.4)	0.68	17.9	0.6 (0.2-2.0)	0.44
Smoking										
never	277	30.0	1.0		21.7	1.0		20.2	1.0	
previously	35	42.9	**2.6 (1.1-5.9)**	**0.03**	34.3	1.8 (0.8-4.4)	0.18	28.6	1.9 (0.8-4.6)	0.17
current	122	39.3	1.5 (0.9-2.5)	0.13	26.2	1.3 (0.8-2.3)	0.30	29.5	1.5 (0.9-2.7)	0.12

* Age was included as a continuous variable. Only variables that appear on this table were included in multivariate analysis. [+]:HIV-positive

5.3.6 Factors associated with HPV seropositivity in men

Table 5.8 shows the determinants of HPV-11, -16 and -18 seropositivity at baseline in men (univariate analysis). In men, HPV-11 seropositivity was associated with the use of ARVs among the HIV-positive men (OR: 0.2, 95% CI: 0.1-0.8, P=0.03); but not significantly associated with the number of new sexual partners over the previous year (OR: 2.2, 95% CI: 0.6-7.9, P=0.21); or having developed a genital ulcer ≥1 month ago (OR: 2.0, 95% CI: 0.2-20.7, P=0.56). In men, HPV-16 seropositivity was not significantly associated with any factors assessed, (Table 5.8). In men, HPV-18 seropositivity was significantly associated with the use of ARVs among the HIV-positive men (OR: 0.1, 95% CI: 0.0-1.0, P=0.05) and the number of new sexual partners over the previous year (OR: 6.8, 95% CI: 1.2-37.3, P=0.03). The number of sexual partners over the previous year did not demonstrated a significant association with HPV-18 seropositivity (OR: 3.5, 95% CI: 0.7-17.1, P=0.12).

Factors such as HIV status, age at first sexual intercourse, number of sexual partners, sexual frequency over the last month, and smoking were not significantly associated with HPV-11, -16 or -18 seropositivity in men (Table 5.8). In men α7 HPV seropositivity was associated with number of new sexual partners over the previous year (OR: 7.2, 95% CI: 1.4-35.5, P=0.02); genital ulcer (OR: 2.6, 95% CI: 1.0-6.9, P=0.05) and number of sexual partners over the previous year (OR: 2.8, 95% CI: 0.7-11.6, P=0.16) but the latter not significantly. In men, α9 HPV seropositivity was not significantly associated with any factor assessed. In determining predictors of HPV seropositivity some of the possible confounding factors were excluded including oral and injectable contraceptives because few participants used contraceptives (data not shown).

Multivariate analysis for men included; variables such as age, HIV status, CD4 count, ARVs, marital status, total number of sex partners and smoking habits. Table 5.9 demonstrates the determinants of HPV-11, -16 and -18 seropositivity at baseline in men (multivariate analysis). HIV-positive men were found to be more likely to have antibodies to HPV-11 (OR: 4.2, 95% CI: 0.8-23.3, P=0.10), HPV-18 (OR: 6.3, 95% CI: 0.5-82.3, P=0.16) and α7 HPV species (OR: 5.5, 95% CI: 0.6-47.2, P=0.12) compared to HIV-negative men but the associations were not significant. In men HIV-positive status was found not to show any association with α9 HPV species (OR: 1.2, 95% CI: 0.3-6.1, P=0.79). HIV-positive men with ≥500/mL CD4 counts were also found to be more likely to have antibodies compared to HIV+ women with <200/mL CD4

Table 5.8. The HPV-11, -16 and -18 antibodies in men and factors associated with seropositivity, adjusted for age as a continuous variable

		Men: HPV-11			Men: HPV-16			Men: HPV-18		
Variable	**n**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**
Age										
<30 years	84	11.9	1.0		4.8	1.0		6.0	1.0	
30-39 years	177	13.6	1.1 (0.5 - 2.4)	0.73	6.2	1.3 (0.4-4.0)	0.64	4.0	0.7 (0.2-2.0)	0.46
40-49 years	106	17.9	1.5 (0.7 - 3.2)	0.3	9.4	2.0 (0.6-6.1)	0.23	4.7	0.8 (0.3-2.5)	0.69
50+ years	65	10.8	0.9 (0.34 - 2.4)	0.84	7.7	1.1 (0.6-5.8)		4.6	0.8 (0.2-3.0)	0.71
HIV										
negative	277	15.2	1.0		6.5	1.0		5.1	1.0	
positive	155	11.6	0.7 (0.4 - 1.3)	0.3	7.7	1.3 (0.59-2.8)	0.53	3.9	0.7 (0.3-2.0)	0.56
CD4 count*										
<200/mL	31	9.7	1.0		3.2	1.0		3.2	1.0	
200 – 349/mL	48	12.5	1.3 (0.31 - 5.8)	0.7	6.3	2.2 (0.2-23.2)	0.51	4.2	1.3 (0.1-15.0)	0.83
350 – 499/mL	35	8.6	0.8 (0.1 - 4.4)	0.8	5.7	2.3 (0.2-28.2)	0.51	0.0		
500+/mL	38	15.8	1.7 (0.4 - 7.7)	0.46	15.8	6.1 (0.7-56.1)	0.11	7.9	2.6 (0.3-26.0)	0.42
ARV status*										
yes	12	33.3	1.0		16.7	1.0		16.7	1.0	
no	111	9.9	**0.2 (0.1 - 0.8)**	**0.03**	6.3	0.4 (0.1-2.3)	0.3	2.7	**0.1 (0.0-1.0)**	**0.05**
Married										
yes	77	11.7	1.0		6.5	1.0		3.9	1.0	1.00
no	354	14.4	1.3 (0.6 - 2.7)	0.54	7.1	1.1 (0.4-3.1)	0.8	4.8	1.2 (0.3-4.3)	0.76
Age at first sex										
<15 years	53	20.8	1.0		3.8	1.0		3.8	1.0	
15+	377	13.0	0.6 (0.3 - 1.2)	0.13	7.2	1.9 (0.4-8.3)	0.38	4.8	1.3 (0.3-5.8)	0.73
Number of sex partners										
1- 2	82	14.6	1.0		6.1	1.0		6.1	1.0	
3 - 4	71	16.9	1.2 (0.49 - 2.8)	0.72	9.9	1.8 (0.5-6.0)	0.33	8.5	1.4 (0.4-4.8)	0.60
5 - 9	128	10.9	0.7 (0.3 - 1.6)	0.43	8.6	1.5 (0.5-4.4)	0.47	2.3	0.4 (0.1-1.6)	0.18
10+	148	14.9	1 .1 (0.5 - 2.2)	0.95	4.1	0.6 (0.2-2.1)	0.44	4.1	0.7 (0.2-2.2)	0.51

		Men: HPV-11			Men: HPV-16			Men: HPV-18		
Variable	**n**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**	**Ab %**	**OR (95% CI)**	**P-value**
Sex partners previous year										
0 or 1	137	11.7	1.0		3.7	1.0		2.2	1.0	
3+	49	18.4	1.6 (0.7 - 4)	0.29	10.2	2.9 (0.8-10.7)	0.1	8,2	3.5 (0.7-17.1)	0.12
New sex partners previous year										
0 or 1	171	12.3	1.0		4.7	1.0		2.3	1.0	
3+	15	26.7	2.2 (0.6 - 7.9)	0.21	13.3	3.0 (0.54-16.0)	0.21	20.0	**6.8 (1.2-37.3)**	**0.03**
Sex frequency previous month										
0	33	15.2	1.0		9.1	1.0		9.1	1.0	
1	93	18.3	1.2 (0.4 - 3.7)	0.69	3.2	0.3 (0.1-1.8)	0.21	5.4	0.5 (0.1-2.4)	0.43
2	106	9.4	0.6 (0.18 - 1.8)	0.35	7.6	0.9 (0.2-3.5)	0.85	2.8	0.3 (0.1-2.5)	0.13
3	76	7.9	0.5 (0.13 - 1.7)	0.25	9.2	1.1 (0.3-4.5)	0.92	5.3	0.5 (0.1-2.5)	0.43
4	119	18.5	1.3 (0.43 - 3.65)	0.67	6.7	0.8 (0.2-3.2)	0.75	4.2	0.4 (0.1-1.8)	0.25
Genital ulcer										
no	383	14.4	1.0		6.3	1.0		4.4	1.0	
yes	46	10.9	0.7 (0.3-1.9)	0.52	13.0	2.4 (0.9-6.2)	0.08	6.5	1.5 (0.4-5.3)	0.54
Genital ulcer recency										
<1 month	15	6.7	1.0		6.7	1.0		0.0		
≥1 month	30	13.3	2.0 (0.2-20.7)	0.56	16.7	3.7 (0.4-38.3)	0.28	10.0	..	..
Smoking										
never	70	18.6	1.0		5.7	1.0		8.6	1.0	
previously	62	11.3	0.6 (0.2 - 1.5)	0.25	9.7	1.5 (0.4-5.7)	0.56	3.2	0.4 (0.1-1.9)	0.24
current	297	13.1	0.7 (0.3 - 1.3)	0.25	6.7	1.1 (0.4-3.3)	0.88	4.0	0.5 (0.2-1.2)	0.14

Numbers will not always add up to 437 because of missing information, n: number, Ab%: percentage of women with positive antibody response. Ab: antibodies. *: HIV-positive. Age adjusted as a continuous variable.

counts for HPV-11 (OR: 2.9, 95% CI: 0.5-13.1, P=0.29), HPV-18 (OR: 5.1, 95% CI: 0.3-95.1, P=0.28), α7 HPv species (OR: 1.9, 95% CI: 0.2-14.5, P=0.52) and α9 HPV species (OR: 2.2, 95% CI: 0.6-8.4, P=0.24) but the association was not statistically significant. HIV-positive men not on ARVs treatment were significantly more likely to have HPV-11 (OR: 0.1, 95% CI: 0.0-5.8, P=0.01) and HPV-18 (OR: 0.1, 95% CI: 0.0-0.7, P=0.03) antibodies but not α7 and α9 HPV species compared to those on ARVs. Men that were not married showed an increased likelihood of α9 HPV species antibodies compared to married men (OR: 2.4, 95% CI: 1.1-5.1, P=0.02) but not for HPV-11, -18 and α7 HPV species. Smoking in men was found not to influence HPV antibodies response (Table 5.9).

Table 5.9. The HPV-11, -16 and -18 antibodies in men and factors associated with seropositivity in multivariate analysis

		MEN: HPV-11			MEN: HPV-18		
Variable	**n**	**%**	**OR (95% CI)**	**P-value**	**%**	**OR (95% CI)**	**P-value**
Age	432	13.9	1.0 (1.0-1.0)	0.98	4.63	1.0 (0.9-1.0)	0.74
HIV							
negative	277	15.2	1.0		5.1	1.0	
positive	155	11.6	4.2 (0.8-23.6)	0.10	3.9	6.3 (0.5-82.3)	0.16
CD4 count[+]							
<200/mL	31	9.7	1.0		3.2	1.0	
200 - 349/mL	48	12.5	0.7 (0.1-3.7)	0.67	4.2	1.0 (0.1-14.2)	0.99
350 - 499/mL	35	8.6	0.9 (0.1-5.4)	0.91	0.0		
≥500/mL	38	15.8	2.5 (0.5-13.1)	0.29	7.9	5.1 (0.3-95.1)	0.28
ARV status[+]							
yes	12	33.3	1.0		16.7	1.0	
no	111	9.9	**0.1 (0.0-5.8)**	**0.01**	2.7	0.1 (0.0-0.7)	**0.03**
Married							
yes	77	11.7	1.0		3.9	1.0	
no	354	14.4	1.2 (0.5-2.7)	0.66	4.8	1.2 (0.3-4.5)	0.82
Total number of sex partners							
1- 2	82	14.6	1.0		6.1	1.0	
3 - 4	71	16.9	0.8 (0.3-2.0)	0.60	8.5	0.9 (0.2-3.5)	0.88
5 - 9	128	10.9	0.6 (0.3-1.5)	0.28	2.3	0.4 (0.1-1.9)	0.25
≥10	148	14.9	1.0 (0.5-2.3)	0.91	4.1	0.7 (0.2-2.6)	0.64
Smoking							
never	70	18.6	1.0		8.6	1.0	
previously	62	11.3	0.5 (0.2-1.5)	0.24	3.2	0.3 (0.1-2.0)	0.23
current	297	13.1	0.6 (0.3-1.3)	0.20	4.0	0.4 (0.1-1.3)	0.14

* Age was included as a continuous variable. Only variables that appear on this table were included in multivariate analysis [+]: HIV-positive.

5.3.7 HPV seroconversion at 12 months period in women and men

HPV seroconversion (over 12 months) was analysed in those women and men (201 and 217 respectively) that were HPV antibody positive for less than 9 HPV antibodies at the baseline visit. Women had a significantly higher seroconversion rate than men (24.8% 52/201, 14.7% 32/217 respectively, P=0.009, Table 5.10). In women who seroconverted, antibodies to HPV-11 (7.1% 9/127) were the most common, followed by HPV-31 (6.5% 9/138), HPV-18 (4.7% 7/149), HPV-16 (4.6% 7/151), HPV-45 (4% 6/150), HPV-52 (2.9% 5/173), HPV-58 (2.2% 4/182), HPV-35 (1.8% 3/168) and HPV-33 (1.1% 2/188). In men who seroconverted, -11 (4.3% 8/186) was also the most common antibody type, followed by HPV-16 (2.1% 4/195), HPV-31 (2.1% 4/190), HPV-18 (2.0% 4/203), HPV-35 (2.0% 4-196), HPV-52 (2.0 % 4/199), HPV-58 (1% 2/196), HPV-45 (0.5% 1/199), and HPV-33 (0.5% 1/206; Table 5.10).

HPV seroconversion was observed in 65 participants, of which 74% (48/65) seroconverted to one HPV type while 26% (17/65, P<0.0001) seroconverted to multiple (2-6) HPV types. Participants in whom HPV seroconversion was observed were not always those with genital HPV DNA at the baseline, 6-month or 12-month visits. This observation probably indicates an infection at another site. Factors associated with HPV seroconversion was also investigated, however, due to small number of seroconverters conclusion on factors associated with HPV seroconversion was not made.

Table 5.10. Human papillomavirus (HPV) seroconversion in women and men after 12-months

Variable	Women		Men		P-value
	n/t	%	n/t	%	
HPV-11	9/127	7.1	8/186	4.3	0.29
HPV-16	7/151	4.6	4/195	2.1	0.15
HPV-18	7/149	4.7	4/203	2.0	0.13
HPV-31	9/138	6.5	4/190	2.1	**0.04**
HPV-33	2/188	1.1	1/206	0.5	0.46
HPV-35	3/168	1.8	4/196	2.0	0.58
HPV-45	6/150	4.0	1/199	0.5	**0.03**
HPV-52	5/173	2.9	4/199	2.0	0.41
HPV-58	4/182	2.2	2/196	1.0	0.31
Total	**52/210**	**24.8**	**32/217**	**14.7**	**0.009**

n: number of participants that seroconverted at 12 month visit.
t: total number of participants that were HPV antibody negative at baseline visit

5.4 DISCUSSION

In this chapter we present seroprevalence data of South African black women and men. Seroprevalence was assessed by HPV type, gender, age, HIV status, cervical cytology and some sexual behaviour variables. To our knowledge this is the first study to investigate serum antibody responses in men and women to nine different HPV types using the multiplex serology, in South Africa. It is important to note that comparing our data with published data from other laboratories is difficult because of different assays used and different cut-off definitions which many significantly influence seroprevalence and factors associated with seropositivity in different studies. It has been reported that 59% of sera collected from Africans directly bind to beads of the assay used in this study, thus interfering with the assay (Waterboer *et al*., 2006). It was interesting to note that in our study only one study participant was found to have serum that directly binds to the beads in the assay.

We observed that women have higher HPV seropositivity compared to men (53% compared to 31%, P<0.0001), as well as HPV seroconversion over 12-months. For individual HPV types and multiple seropositivity, women were found to have up to a 4-fold higher HPV seroprevalence compared to men. When stratified according to age similar observations were observed. Michael *et al*., (2008) also reported a 5.8-fold and 2.8 fold higher seroprevalence in women respectively compared to men of the same age. The low HPV seroprevalence in men does not necessary mean less HPV infection as we demonstrated higher genital HPV prevalence in HIV-negative men compared to HIV-negative women. In studies in which VLP-based ELISA has been used a lower HPV seroprevalence in men compared to women has been reported (Hopfl *et al*., 2003; Thompson *et al*., 2004). The low seroprevalence in men compared to women can also be explained by the fact that the immune system is less accessible in keratinized epithelium of the penis in men compared to mucosal epithelium of the cervix in women resulting to lower prevalence of seroconversions in heterosexual men compared to women (Stone *et al*., 2002; Thompson *et al*., 2004). HPV antibodies were not associated with HPV clearance in both women and men (chapter 6). The majority of men in our study were circumcised (even though their circumcision was traditionally) an indication that the mucosal epithelium on the penile shaft was removed. A higher rate of transient HPV infection in male genitalia than in female vagina and cervix may also be the reason for the lower prevalence of antibodies in men as persistent exposure to HPV is required for seroconversion (Ho *et al*., 2004).

In our study HPV-16 and -18 seroprevalence was found to be 24% for each type. Vaccarella *et al*., (2010) reported similar observations for HPV-16 and -18 (27% and 24.8% respectively) among Nigerian women. Firnhaber *et al*., (2011) reported a similar HPV-16 seroprevalence (29.3%) and lower HPV-18 seroprevalence (15.9%) in HIV-positive women from Johannesberg South Africa compared to the one observed in our study. HPV-16 and -18 seroprevalence among women from Spain, Argentina, Korea, Thailand and Vietnam were found to be lower than observed in our study (0.8-20.9% for HPV-16 and 0.2-12.2% for HPV-18). Syrjanen *et al*., (2009) presented their data using two different cut-offs, 200 and 400 MFI. When comparing our study with Finnish study, seroprevalence in our study was higher for all HPV types investigated (HPV-11: 34% compared to 13%, HPV-16: 24% compared to 19%, HPV-18: 24% compared to 8% and HPV-45: 22% compared to 2%).

In our study in women we observed a weak association between HPV seroprevalence and HPV DNA and number of sexual partners over the previous year. According to Clifford *et al*., (2007) the presence of genital HPV DNA demonstrated a very strong association with HPV seroprevalence in women (OR: 14.0, 95% CI: 4.7-42.0), even stronger than the association with the number of sexual partners (within the past 12 months, OR: 2.9, 95% CI: 1.8-4.7). Findings from our study and those from Clifford *et al*., (2007) are different probably due to the different demographics of the study participants. In the Clifford *et al*., (2007) study participants had only initiated sexual activity within 12 months before the start of the study. Thus, HPV antibody response they observed was the responses to new infections. Our study participants had been sexually active for more than a year and up to decades. Thus, the antibody responses we observed are probable responses to past and current HPV infection. It is interesting to note that in a study reported by Clifford *et al*., (2007) in which multiplex serology was used to detected antibodies, overall and type-specific HPV seroprevalence in women was not found to differ significantly compared to that of men (15% and 12% respectively). However, in women who were sexually experienced overall HPV seroprevalence was found to be higher compared to that of sexually inexperienced women (25% and 13% respectively). In men sexual experience did not increase HPV seroprevalence. It is interesting to note that when looking at sexually experienced women and men, women displayed significantly higher overall HPV seroprevalence (25% and 13% respectively). In contrast, no difference in seroprevalence was observed (13% compared to 11%) among the sexually inexperienced women and men. Study participants in the Clifford *et al*., (2007) study were between the ages of 15 and 19 years and many of them reported never having had penetrative sexual intercourse or recent (<12 months)

penetrative sexual intercourse. In our study all participants had been sexually active for years or decades.

In our study HPV seroprevalence was associated with the increased number of sexual partners over the previous year in men. It is interesting to note that in the men in Clifford *et al*., (2007) study, higher number of sexual partners (OR: 1.2, 95% CI: 0.6-2.5) and genital HPV DNA (OR: 1.6, 95% CI: 0.4-6.3) had no significant effect on HPV seroprevalence, even though men in their study reported higher sexual activity compared to women. Since the study participants in the Clifford *et al*., (2007) study only recently participated in sexual activities and we know months are required to develop detectable antibodies, the keratinized epithelium of the penis may contribute to the delayed immune response (Thompson *et al*., 2004; Clifford *et al*., 2007). In a Finnish study in which antibodies were detected in the DFKZ laboratory with the same multiplex assay as in our study, type-specific concordance between genital HPV DNA detection and seropositivity was poor. When DNA positivity of oral and genital sites were combined (positive at genital and/or oral site) the type-specific concordance for HPV-16 was improved even though it was still poor (Syrjanen *et al*., 2009).

Unmarried women in our study were found to more likely to be HPV seropositive compared to married women, however, this was not observed in men. Vaccarella *et al*., (2010) also reported that single women were more likely to develop HPV-16 antibodies compared to married women (OR: 1.6, 95% CI: 1.1-2.4). Unmarried women have been previously reported to be more likely to seroconvert compared to married women (Shin *et al*., 2003; Syrjanen *et al*., 2009). These observations can also be explained by increased number of sex partners in single women compared to married women (Shin *et al*., 2003; Syrjanen *et al*., 2009). In our study marital status in men was not associated with seropositivity. However, Lu *et al*., (2010) reported that men, who were divorced, separated or widows were more likely to be HPV-16 or -18 seropositive. In our study increasing numbers of sexual partners over the previous year and a greater number of new sexual partners over the previous year were associated with HPV seroprevalence in men but not in women. It has been reported in several studies that as the number of sexual partners increase in women, the HPV seroprevalence was found to increase significantly as well, while in men it was not (Carter *et al*., 1996; Clifford *et al*., 2007; Sitas *et al*., 2007; Porras *et al*., 2010).

In our study, age at first sexual intercourse and number of sexual partners were not significantly associated with HPV seropositivity. Syrjanen *et al*., (2009) reported an association between HPV seropositivity and age at first sexual intercourse and number of sexual partners, however, the cut-off used in their study was lower than ours (200 MFI compared to 400 MFI) and the study participants in Syrjanen *et al*., (2009) study were a high risk sexual cohort compared with populations analysed in other serological studies where women had about 5 lifetime sex partners. Women who developed a genital ulcer ≥1 month ago and who were smoking were more likely to have detectable antibody. HIV-positive status was not associated with HPV seropositivity in univariate analysis but in multivariate analysis HIV-positive status was found to influence HPV antibody response in both women and men. Other studies have also reported an increased seropositivity among HIV-positive individuals (Viscidi *et al*., 2003; Marais *et al*., 2009).

It was interesting to note that HIV-positive men and women who were using ARVs had a higher HPV-11 and -18 seroprevalence compared to those not using ARVs but not for HPV-16. However, these results should be interpreted with caution as there were too few study participants who were using ARVs to be conclusive on this observation. Hopfl *et al*., (2003) reported that HIV-positive men using highly active antiretroviral therapy (HAART) were more likely to be HPV-16/18/31 seropositive compared to men not using HAART (43.7% compared to 33.3%, OR: 1.6, 95% CI: 0.8-2.9) even though the difference was not statistically significant. These observations may demonstrate the role played by ARVs to restore the immune system (Minkoff *et al*., 2010). The data reporting on the association of HAART on HPV and HPV-associated lesions is not consistent. Some studies have found HAART significantly reducing the burden of HPV infection and HPV-associated lesions (Minkoff *et al.*, 2010). Others studies have found no effect (Moodley *et al*., 2009; Shrestha *et al*., 2010). The period since the participants started using HAART would significantly influence the finding. In this study, the information on period the participants started using ARVs was not available. The role played by ARVs on the prevalence of HPV antibodies still needs further investigation.

Women and men who had acquired a genital ulcer ≥1 month ago were found to be more likely to have antibodies compared to those who had genital ulcer <1 month ago. Mbwana *et al*., (2007) also reported a high HPV seroprevalence among women and men with genital ulcer disease. These observations may indicate high exposure of the virus to the immune system resulting in the elicitation of antibody responses (Mbwana *et al*., 2006). HSV-2 is identified as

the cause of genital ulcer disease (Ahmed *et al*., 2003). HSV-2 data is not yet available in our cohort. This will be generated in future work and the association of HSV2 infection on HPV antibodies in our cohort will be investigated.

Age and cervical abnormal cytology were not found to be associated with HPV seropositivity in our study. Similar observations were reported elsewhere (Syrjanen *et al*., 2009). Firnhaber *et al*., (2011) also did not observe any association between abnormal cervical cytology and HPV seropositivity in South African women from Johannesburg. However, in our study women with abnormal cytology demonstrated seropositivity to multiple types compared to women with normal cytology (64% compared to 47%) and multiple seroprevalence was found to increase with increasing severity of cervical abnormalities. These observations are the reflection of the high prevalence of multiple HPV types detected at the cervix of women with abnormal cytology compared to women with normal cervical cytology (Porras *et al*., 2010).

The multiplex serology method used in this study uses L1 proteins expressed in bacteria as a GST fusion proteins as antigen and these L1 proteins have been reported to display conformational epitopes (Rizk *et al*., 2008). All epitopes displayed on VLPs are also displayed on these L1 antigens (Rizk *et al*., 2008). The HPV antibodies detected by this assay target the L1 major capsid protein of HPV and the L1 major capsid protein is a marker of infection (Zumbach *et al*., 2000). However, antibody cross-reactivity and non-specificity cannot be excluded entirely. Like VLPs, GST fusion L1 proteins present conformational and neutralising epitopes but also display more linear or cross-reacting epitopes than VLPs (Kirnbauer *et al*., 1996; Rizk *et al*., 2008). Michael *et al*., (2008) reported that the assay used in this study detects mainly type-specific antibodies while VLP-based ELISA may detect a higher ratio of specific to non-specific antibodies. These could be the reasons why we observed that some of the factors that were previously found to be positively associated with seropositivity in VLP-based ELISA in other studies, while, in GST-based multiplex serology they are not positively associated with seropositivity. However, a good correlation between GST-L1 fusion protein ELISA and VLP-based ELISA for HPV-16 and -18 antibodies has been reported elsewhere (Sehr *et al*., 2002; Waterboer *et al*., 2005; Rizk *et al*., 2008).

The high antibody response to HPV-11, 16 and 18 in young and old sexual active women and men indicate that they have been exposed to infection. The high HPV-6, -11, α7 and α9 HPV species prevalence and acquisition (chapter 2 and 6) in our cohort indicate that in South Africa

even young women and men are highly exposed to these HPV types indicating that vaccination against HPV-6, -11, -16 and -18 will be of great benefit to women and men's health in our country. The current HPV vaccine are found to be very effective in both women and men (Stanley, 2008). In an ideal situation HPV vaccines should be given before people become sexually active. These are prophylactic vaccines and are not known to have therapeutic activity. HPV sero-epidemiology may inform vaccine policy and although there are misgivings as to what HPV serology actually reflects in terms of HPV exposure, HPV infection and immunity, the more data is available, especially for men where data is very limited the better will be our understanding (Schiffman *et al*., 2009b). Serological data underestimates the exposure to HPV due to low rate of seroconversion after infection; however it is a good epidemiological tool that can be used in young women and men to assess HPV infection and in monitoring the effect of vaccines.

CHAPTER 6: HUMAN PAPILLOMAVIRUS NATURAL HISTORY IN HIV-SEROPOSITIVE AND HIV-SERONEGATIVE WOMEN AND MEN

6.1 INTRODUCTION

The HPV life cycle is linked to the differentiation of the cells which HPV has infected. HPV gains access to basal epithelial cells resulting in productive or abortive infection (Culp *et al*., 2006; Doorbar, 2006). In a productive infection, HPV follows its normal life cycle as described in chapter 2 section 1.4, in which HPV enters the basal cells, genome amplification occurs followed by virus assembly associated with the various layers of differentiating cells and virus release within cells sloughing off the epithelial surface. In an abortive infection HPV gains access to basal cells but some time during replication the viral life cycle stops leaving some cells infected with a low HPV copy number. This condition is referred to as latency. However, the mechanism involved in HPV latency is not yet clear. HPV in a state of latency may get reactivated to resume replication especially in immune suppressed individuals. HPV can also be found in cells in low copy numbers but not sheeding (Stanley, 2006; Doorbar, 2006; Theiler *et al*., 2010; Nicol *et al*., 2010). According to Insinga *et al*., (2010) the risk of HPV reappearance within 3 years of follow-up ranges from 0 - 16% in women.

HPV prevalence and acquisition in both women and men is significantly associated with young age, increased lifetime number of sexual partners, increased number of recent sexual partners, smoking, early age of sexual debut, high-risk sexual partners and the consumption of alcohol (Kjaer *et al*., 2002; Svare *et al*., 2002; Shin *et al*., 2004; Vaccarella *et al*., 2006; Nielson *et al*., 2007; Partridge *et al*., 2007; Goodman *et al*., 2008; Fukuchi *et al*., 2009; Lu *et a*l., 2009). A high prevalence of new HPV infections and persistent infections are reported in immuno-compromised individuals such as in HIV-positive individuals (Piketty *et al*., 2003). Circumcised men have lower HPV prevalence and acquisition rates compared to uncircumcised men (Castellsague *et al*., 2002; Tobian *et al*., 2009; Lu *et al*., 2009).

In a Zimbabwean study of HIV-negative women, HPV acquisition of any type after 12-months was reported to be 23.3%. HPV-58 was reported to be the most prevalent type followed by HPV-16 (Fukuchi *et al*., 2009). Factors that were associated with HPV acquisition in women include young age, multiple lifetime sexual partners, not using condoms, HSV-2 co-infection, other STIs and having HPV infection at the baseline visit (Fukuchi *et al*., 2009). Goodman *et al*., (2008) reported that the incidence of HR-HPV is not statistically different from that of LR-HPV. It is not yet clear if there is a biological interaction between various HPV types. Possible

infection with a certain type increases the likelihood of acquiring another type in the future or there is a genetic susceptibility to HPV infection (Rousseau *et al*., 2001).

Goodman *et al*., (2008) reported that of all HR-HPV infections observed in women at screening, 69% of HR-HPV and 81% of LR-HPV infections were cleared after 12-months. The α9 HPV species demonstrated lower clearance rate compared to other species types. Women co-infected with multiple HPV types demonstrated lower clearance when compared with women infected with one HPV type. Men with multiple HPV infection also have an elevated risk of HPV persistent infection (Kjaer *et al*., 2002). Women with HPV-16 and/or multiple HPV infection demonstrate lower rates of clearance compared to women infected with other types and with single HPV infection respectively. Women with HPV-16 infection demonstrate a 20% lower clearance than women infected with other HPV genotypes (Louvanto *et al*., 2010). HPV clearance is reported to be lower in uncircumcised men compared to circumcised men (Hernandez *et al*., 2010). According to Hernandez *et al*., (2010) HPV duration is longer in uncircumcised men compared to circumcised men (154 days compared to 91 days, P=0.04).

Older women clear their HPV infection faster than younger women (<25 years). HPV prevalence is found to decrease with increasing age but HPV clearance is reported to increase with increasing age (Castle *et al*., 2005; Louvanto *et al*., 2010). This may indicate that older women may have been exposed to HPV for a longer period of time compared with young women and probably due to immune memory their immune system clears the HPV infection faster than young women who are more likely to be newly infected (Goodman *et al*., 2008). In a study reported by Lu *et al*., (2009) a greater number of lifetime sexual partners (>16) was associated with HPV clearance. People with high number of lifetime sex partners have been highly exposed to genital HPV infection over time resulting in immune responses that clear the infection (Olsen *et al*., 1997; Slavinsky *et al*., 2001). It has been reported that 12-months after HPV infection ~70% of women clear their infection (Ho *et al*., 1998; Dillner, 1999; Giuliano *et al*., 2008a; Oh *et al*., 2008); and after 2 years ~90% of HPV infections are cleared (Moscicki *et al*., 2008).

Castle *et al*., (2009) reported that women that were HR-HPV positive for one year have a 3 year HSIL cumulative incidence of 17% to 22%, while women that were HR-HPV negative and then HR-HPV positive over one year have a 3.4% to 6.8% HSIL incidence. Women that were HR-HPV positive and then HR-HPV negative had an HSIL incidence of 1.2% to 2.5%

and women who remain HR-HPV negative for one year had a 3 years HSIL cumulative incidence of 0.5% to 0.9% (Castle *et al*., 2009). The relative risk of developing cervical neoplasia is reported to be 14.7 among women with transient HPV infection and 42.9 among women with HR-HPV persistent infection for about 1 year (Koshiol *et al*., 2008). Some studies have found that women who tested HR-HPV positive at a first visit and then HPV negative at follow up had a similar risk of developing neoplasia compared to those that were negative at both visits. This may indicate false positive results at the first visit or HPV clearance (Castle *et al*., 2009). In contrast, Kjaer *et al*., (2006) reported a higher risk of developing precancerous lesions in women who tested HR-HPV positive and then negative compared to those who tested HR-HPV negative at both visits. However, the observed risk was still lower than that observed in women who tested HR-HPV positive at both visits. Following HPV in women for one or more years may assist in determining those at higher risk of developing precancerous lesion and thus requiring intervention.

The natural history of different HPV genotypes and factors associated with clearance of different HPV genotypes in men are not well known (Ho *et al*., 1998; Giuliano *et al*., 2008b). Data on the type distribution of HPV and HPV natural history in sub-Saharan Africa is limited and there are no published reports on HPV natural history studies on heterosexually active couples in Africa. The study described here is the first such report in Africa. This is also the first study to describe HPV natural history on HIV-positive; HIV-negative and HIV-discordant couples investigated over a period of 24-months. HPV natural history studies in women are not as limited as those in men. It is important to study HPV in men because of the direct influence men have on genital HPV acquisition and the development of cervical neoplasia and cancer in their female partners.

The aims of the study described below were:

(i) to investigate the rate of HPV acquisition and clearance over a period of 24-months in HIV-positive and HIV-negative women and men;

(ii) to investigate the proportion of HPV acquisition events from the study partner; and

(iii) to investigate factors associated with HPV acquisition and clearance.

6.2 MATERIALS AND METHODS

6.2.1 Study population, specimen collection and HPV genotyping

Cervical and penile specimens were collected as described in chapter 2 section 2.2.1. For the present study, samples from a total of 209 HIV-negative women, 277 HIV-positive women, 333 HIV-negative men and 153 HIV-positive men were available. There were 486 couples in total, 162 couples were both HIV-negative, 115 couples were both HIV-infected, 163 were HIV-discordant where the female partner was HIV-positive and 46 were HIV-discordant where the male partner was HIV-positive. At the 6-month visit samples from 260 couples were available, samples from 197 couples at the 12-month visit, 140 samples from couples at the 18-month visit and from 62 couples at the 24-month visit. At the baseline visit the mean age of women and men participants were 35 years (range: 18-66 years) and 38 years (range: 19-67 years) respectively. HPV genotyping in cervical and penile cells was detected as described in chapter 2 section 2.2.2.

6.2.2 Statistical analysis

Acquisition of an HPV infection was defined as the first positive result for a specific HPV type. HPV clearance was defined as the first time a woman tested negative for HPV following the first positive HPV identification. Time to HPV clearance was measured at 6-month intervals. Rates of HPV acquisition and HPV clearance were calculated for each HPV type. Because follow-up was limited to a maximum of four visits (after baseline), the analysis is limited to the rate at which an HPV type is *first* acquired, or first cleared, over the follow-up period. If an HPV type was present at one visit, absent at the next visit, and then present at the next visit, the negative result was considered to be a false negative and was treated as a positive result for the purpose of calculating rates of HPV clearance. In all other cases, the date of clearance was assumed to be the mid-point between the date of the last positive test and the first negative test, for a given HPV type. Similarly, the date of HPV acquisition for a specified HPV type is assumed to be midway between the last negative visit and the first positive visit. For the purpose of calculating the person months at risk in the acquisition rate denominator, the period at risk is assumed to start when the individual is first observed to be uninfected with the type of interest (or at the second visit at which they are observed to be uninfected if they were previously infected). For the purpose of calculating the person months of infection in the clearance rate denominator, the period of observation begins when the individual is first observed to be infected with the HPV type of interest (and not at the estimated date of acquisition if they were previously uninfected). HPV transmission from the study partner is

defined to be HPV acquisition where the partner is known to be infected with the type of interest at the estimated date of HPV acquisition. This definition of transmission may understate the true proportion of acquisition events that are transmissions from study partners, since it excludes cases in which both partners acquire the same HPV type between two visits, and it excludes cases in which the partner's HPV status was left- or right-censored at the time of the individual's HPV acquisition.

Poisson regression models were used to assess predictors of acquisition rates and clearance rates. Because of the low numbers of acquisition and clearance events for individual HPV types, the regression models were run for all HPV types combined, with variance calculations adjusted to allow for clustering of observations at the individual level. All regression models were controlled for HPV type. All predictors of clearance rates and acquisition rates were defined according to values measured at baseline, with the exception of the partner HPV infection status, which was treated as a time-varying covariate (and calculated separately for each HPV type). All statistical analyses were conducted by Dr Leigh Johnson (Centre for Infectious Disease Epidemiology and Research, University of Cape Town) using STATA 11.0 (StataCorp, College Station, TX, USA). In all analyses P-values ≤ 0.05 were considered significant.

6.3 RESULTS

6.3.1 Cervical and penile HPV genotype and species incidence and infection

The initial analysis of HPV prevalence was done on women and men regardless of their HIV status. At the baseline visit the prevalence of genital HPV infection in women and men was not found to differ significantly between women and men (58% 281/484 compared to 58% 275/471, P=0.92). In women a total of 293 incident cervical HPV infections were observed during follow-up (24-months), 133 incident infections were HR-HPV types and 160 were LR-HPV types. Among men, a total of 459 incident penile HPV infections were observed during follow-up, 211 incident infections were HR-HPV types and 248 were LR-HPV types. There were 42 HPV incident infections in women and 66 in men due to transmission from the study partners. We observed significantly more HR-HPV transmission events in men from their female partner compared to women (18% 38/211 compared to 7% 9/133, P=0.003). In contrast, significantly more LR-HPV transmission events were observed in women from their male partner compared to men (21% 33/160 compared to 11% 28/248, P=0.01; Table 6.1).

The rate of acquiring any type of HPV infection on the cervix was 1.82 (95% CI: 1.53-2.14) per 1000 person months in women. The incidence of HR-HPV types was 1.65 (95% CI: 1.30-2.08) and of the LR-HPV types was 1.98 (95% CI: 1.64-239) per 1000 person-months in women. The rate of acquiring any penile HPV infection was 3.07 (95% CI: 2.65-3.54) per 1000 person-months in men. The incidence of HR-HPV types was 2.80 (95% CI: 2.32-3.36) and of the LR-HPV types was 3.34 (95% CI: 2.81-3.95) per 1000 person-months in men. The most common HR-HPV type acquired in women during follow-up was HPV-35 (2.76 per 1000 person-months) followed by HPV-52 (2.58 per 1000 person-months). While in men, HPV-66 (5.49 per 1000 person-months) was the most acquired type followed by HPV-58 (4.64 per 1,000 person-months) during follow-up. When looking at LR-HPV types, HPV-62 was the most acquired type in both women (5.71 per person-months) and men (10.55 per 1000 person-months) followed by HPV-61 in both women (4.42 per 1000 person-months) and men (7.55 per person-months) during follow-up. Men were found to have a higher rate of acquiring any HPV, HR-HPV or LR-HPV infection during follow-up compared to women (P<0.001 for each; Table 6.1).

When we look at HPV types individually, among HR-HPV types men were found to have a significantly high rate of acquiring HPV-18 compared to women (3.36 per 1000 person-months compared to 1.13 per 1000 person-months, P=0.04); HPV-58 (4.64 per 1000 person-months compared to 2.09 per 1000 person-months, P=0.05); HPV-66 (5.49 per 1000 person-months compared to 2.22 per 1000 person-months, P=0.02) and HPV-68 (4.42 per 1000 person-months compared to 0.89 per 1000 person-months, P=0.004). Among LR-HPV types men were found to have a significantly high rate of acquiring HPV-55 compared to women (3.46 per 1000 person-months compared to 0.65 per 1000 person-months, P=0.01); HPV-62 (10.55 per 1000 person-months compared to 5.71 per 1000 person-months, P=0.004); HPV-70 (4.68 per 1000 person-months compared to 2.04 per 1000 person-months, P=0.004) and HPV-89 (3.16 per 1000 person-months compared to 1.10 per 1000 person-months, P=0.05, Table 6.1).

During follow-up in women, α3 HPV species contributed the most incident cervical infection (97 events of HPV acquisition) followed by α9 HPV species (49 events of HPV acquisition) and α7 HPV species (41 events of HPV acquisition). Among men, α3 HPV species (144 events of HPV acquisition) contributed the most incident infection followed by α7 HPV species (90 events of HPV acquisition) and α6 HPV species (43 events of HPV acquisition). Men were found to have a significantly higher rate of HPV species acquisition compared to women for α3

Table 6.1. The incidence rate of genital HPV infection by genotype and species in women and men

	WOMEN					MEN					
Variable	**rate/1000 PM**	**95% CI**	**Events of acquisition**	**transm. From* study partner**	**PM**	**rate/1000 PM**	**95% CI**	**Events of acquisition**	**transm. from study partner**	**PM**	**P-value**
HR-HPV											
HPV-16	2.04	1.06-3.95	9	0	4407.8	3.16	1.83-5.43	13	4	4113.0	0.32
HPV-18	1.13	0.47-2.73	5	0	4437.7	3.36	1.98-5.66	14	3	4170.3	**0.04**
HPV-26	0.43	0.11-1.74	2	0	4648.4	0.00	-	0	0	4394.2	-
HPV-31	0.44	0.11-1.74	2	1	4580.5	1.40	0.63-3.10	6	2	4294.1	0.15
HPV-33	1.10	0.46-2.65	5	0	4553.2	0.92	0.35-2.44	4	0	4343.3	0.79
HPV-35	2.76	1.56-4.87	12	1	4346.1	3.37	1.98-5.71	14	2	4151.1	0.61
HPV-39	2.21	1.19-4.11	10	2	4524.7	3.08	1.77-5.33	13	2	4215.8	0.43
HPV-45	1.79	0.89-3.57	8	1	4474.6	3.63	2.17-6.07	15	5	4135.5	0.11
HPV-51	2.00	1.03-3.83	9	2	4508.2	3.18	1.85-5.49	13	1	4090.0	0.29
HPV-52	2.58	1.42-4.68	11	0	4264.9	3.17	1.83-5.49	13	3	4106.3	0.62
HPV-53	2.31	1.24-4.28	10	0	4334.3	4.33	2.67-6.98	17	2	3929.7	0.11
HPV-56	1.53	0.73-3.23	7	1	4570.4	0.92	0.34-2.47	4	1	4358.1	0.42
HPV-58	2.09	1.09-4.03	9	0	4315.3	4.64	2.92-7.34	19	5	4097.0	**0.05**
HPV-59	1.11	0.46-2.67	5	0	4503.1	2.62	1.45-4.72	11	2	4194.5	0.11
HPV-66	2.22	1.19-4.15	10	1	4496.8	5.49	3.57-8.35	22	2	4009.1	**0.02**
HPV-68	0.89	0.33-2.37	4	0	4482.3	4.42	2.78-7.05	18	2	4068.1	**0.004**
HPV-73	1.96	1.02-3.79	9	0	4588.5	1.87	0.93-3.75	8	1	4279.3	0.92
HPV-82	1.30	0.58-2.92	6	0	4609.8	1.61	0.77-3.40	7	1	4339.1	0.70
All HR-HPV	1.65	1.30-2.08	133	9	80646.7	2.80	2.32-3.36	211	38	75288.8	**<0.001**
LR-HPV											
HPV-6	1.77	0.88-3.57	8	0	4516.7	1.65	0.78-3.50	7	2	4239.3	0.89
HPV-11	0.43	0.11-1.72	2	0	4670.0	1.85	0.93-3.68	8	1	4332.4	0.07
HPV-40	0.65	0.21-2.02	3	1	4650.5	1.39	0.62-3.07	6	1	4330.9	0.28
HPV-42	1.09	0.45-2.62	5	1	4581.8	1.38	0.61-3.07	6	0	4361.0	0.70
HPV-54	2.29	1.24-4.28	10	1	4367.5	3.15	1.83-5.43	13	3	4128.2	0.45
HPV-55	0.65	0.21-2.04	3	0	4581.0	3.46	1.86-5.54	14	1	4042.6	**0.01**
HPV-61	4.42	2.81-6.98	19	4	4299.3	7.55	5.17-11.05	28	2	3706.4	0.07
HPV-62	5.71	3.83-8.52	24	11	4203.4	10.55	7.56-14.63	37	3	3506.1	**0.02**
HPV-67	0.21	0.03-1.53	1	0	4690.5	0.23	0.03-1.62	1	0	4421.0	0.97
HPV-69	1.75	0.87-3.50	8	1	4571.7	1.61	0.77-3.36	7	1	4337.2	0.88
HPV-70	2.04	1.05-3.95	9	1	4407.0	4.68	2.95-7.41	19	3	4061.8	**0.04**
HPV-71	2.29	1.22-4.28	10	0	4368.0	2.88	1.62-5.12	12	3	4167.0	0.60

	WOMEN					MEN					
Variable	**rate/1000 PM**	**95% CI**	**Events of acquisition**	**transm. From* study partner**	**PM**	**rate/1000 PM**	**95% CI**	**Events of acquisition**	**transm. from study partner**	**PM**	**P-value**
HPV-72	3.66	2.23-6.01	16	5	4374.8	5.19	3.36-8.03	21	4	4049.8	0.30
HPV-81	1.56	0.74-3.26	7	1	4486.1	3.22	1.86-5.60	13	2	4033.1	0.12
HPV-83	2.73	1.56-4.82	12	3	4387.8	3.23	1.86-5.60	13	0	4024.5	0.68
HPV-84	3.14	1.85-5.33	14	4	4458.6	4.72	3.01-7.41	19	1	4022.9	0.25
HPV-89	1.10	0.46-2.65	5	0	4540.9	3.16	1.83-5.43	13	0	4118.7	**0.05**
HPV-IS39	0.87	0.32-2.32	4	0	4613.0	2.54	1.41-4.59	11	1	4331.9	0.07
All LR-HPV	1.98	1.64-2.39	160	33	80768.5	3.34	2.81-3.95	248	28	74214.7	**<0.001**
All HPV	1.82	1.53-2.14	293	42	161415.2	3.07	2.65-3.54	459	66	149503.4	**<0.001**
HPV species											
α1	1.09	0.45-2.62	5	1	4581.8	1.38	0.61-3.07	6	0	4361.0	0.70
α3	3.15	2.54-3.91	97	28	30750.9	5.24	4.32-6.38	144	12	27461.4	**0.001**
α5	1.26	0.85-1.86	29	3	22951.0	1.77	1.26-2.47	38	4	21492.5	0.20
α6	2.01	1.29-3.14	27	2	13401.6	3.50	2.57-472	43	5	12296.8	**0.05**
α7	1.53	1.05-2.21	41	4	26829.4	3.62	2.87-4.59	90	17	24846.1	**<0.001**
α8	0.65	0.21-2.02	3	1	4650.5	1.39	0.62-3.07	6	1	4330.9	0.28
α9	1.57	1.15-2.14	49	2	31158.3	2.37	1.83-307	70	16	29526.0	**0.05**
α10	0.94	0.53-1.69	13	0	13767.7	2.30	1.51-3.26	29	4	12614.3	**0.02**
α11	1.96	1.02-3.79	9	0	4588.5	1.87	0.93-3.75	8	1	4279.3	0.92
α13	2.29	1.24-4.28	10	1	4367.5	3.15	1.83-5.43	13	3	4128.2	0.45
α15	2.29	1.22-4.28	10	0	4368.0	2.88	1.62-5.12	12	3	4167.0	0.60

PM: person-months of follow-up for subject at risk of acquiring HPV infection. * indicates number of acquired events that we possible transmitted from the study partner. **α1** HPV species includes HPV-42. **α3** HPV species includes HPV-61, -62, -72, -81, -83, -84 and -89. **α5** HPV species includes HPV-26, -51, 69, -82 and –IS39. **α6** HPV species includes HPV-53, -56 and -66. **α7** HPV species includes HPV-18, -39, -45, -59, -68 and -70. **α8** HPV species includes HPV-40 . **α9** HPV species includes HPV-16, -31, -33, -35, -52, -58 and -67. **α10** HPV species includes HPV-6, -11 and -55. **α11** HPV species include HPV-73. **α13** HPV species includes HPV-54. **α15** HPV species includes HPV-71.

HPV species (5.24 per 1000 person-months compared to 3.15 per 1000 person-months, P=0.001); α6 HPV species (3.50 per 1000 person-months compared to 2.01 per 1000 person-months, P=0.05); α7 HPV species (3.62 per 1000 person-months compared to 1.53 per 1000 person-months, P<0.001); α9 HPV species (2.37 per 1000 person-months compared to 1.57 per 1000 person-months, P=0.05); and α10 HPV species (2.30 per 1000 person-months compared to 0.94 per 1000 person-months, P=0.02, Table 6.1). The proportion of HPV species acquisition that was due to transmission from the study partner was generally higher in men than in women, with the exception of α3 HPV species which was substantially higher in women.

6.3.2 Factors associated with genital HPV acquisition in women and men

Table 6.2 presents factors associated with acquisition of genital HPV infection during follow-up in the univariate analysis. HIV infection was significantly associated with increased risk of acquiring new HPV types during follow-up in both women (RR: 2.98, 95% CI: 2.1-4.3) and men (RR: 2.0, 95% CI: 1.49-2.69). Having an HIV-positive partner was also significantly associated with increased risk of acquiring a new HPV type during follow-up in both women (OR: 1.67, 95% CI: 1.2-2.3) and men (RR: 1.56, 95% CI: 1.16-2.08). The risk of acquiring genital HPV infection during follow-up was significantly increased in women (RR: 5.71, 95% CI: 3.9-8.4) and men (RR: 9.06, 95% CI: 6.49-12.7) with a sexual partner that was infected with an HPV type similar to the one acquired compared to those with a sexual partner not infected with the same HPV types. Women initiating sexual intercourse at a younger age were found to have an increased risk of acquiring HPV infection compared to those starting sexual intercourse later (P=0.002). In contrast, the risk of acquiring HPV infection during follow-up in men was found to increase with an older age of initiating sexual intercourse. However, the observations were not statistically significant (P=0.06, Table 6.2) but seem to increase with increasing age of sexual debut. Women with an HIV viral load of ≥10 000 copies per mL were found to have a significantly higher risk of acquiring HPV infection during follow-up (RR: 1.46, 95% CI: 1.0-2.2) compared to women with an HIV viral load of <10 000 copies per mL; while in men a significant difference was not observed. Living with the study partner was found to increase the risk of acquiring HPV infection during follow-up in women (RR: 1.45, 95% CI: 1.0-2.1), but not in men. An increased number of lifetime sexual partners was significantly associated with HPV acquisition during follow-up in women (P=0.015), but not in men (Table 6.2).

Table 6.2. Factors associated with genital HPV acquisition in women and men, in the univariate analysis

	WOMEN						MEN					
Variable	**acquisition rate/ 1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**	**acquisition rate/ 1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**
Age group												
<30 years	1.83	76	41426.4	1.0		0.161	3.48	94	26985.6	1.0		0.538
30-39 years	2.18	121	55474.8	1.18	0.77-1.83		2.80	148	52765.2	0.80	0.53-1.19	
40+ years	1.49	96	64513.2	0.81	0.53-1.25		3.11	217	69753.6	0.89	0.63-1.26	
HIV status												
Negative	1.01	95	94480.8	1.0		**<0.001**	2.50	285	114080.4	1.0		**<0.001**
Positive	2.96	198	66934.8	2.98	2.07-4.29		4.91	174	35422.8	2.00	1.49-2.69	
CD4 count (if HIV-positive)												
≥350/mL	2.89	96	33187.2	1.0		0.774	5.37	99	18418.8	1.0		0.449
<350/mL	3.02	102	33747.6	1.05	0.74-1.51		4.46	75	16801.2	0.83	0.51-1.34	
HIV viral load (if HIV-positive)												
<10 000 copies/mL	2.45	71	28950	1.0		**0.05**	4.37	58	13268.4	1.0		0.395
≥10 000 copies/mL	3.57	108	30254.4	1.46	0.99-2.16		5.43	107	19688.4	1.23	0.77-1.96	
Partner HIV status												
Negative	1.57	193	123090	1.0		**0.003**	2.49	215	86326.8	1.0		**0.003**
Positive	2.61	100	38325.6	1.67	1.2-2.33		3.86	244	63176.4	1.56	1.16-2.08	
Partner CD4 count (if partner HIV-positive)												
≥350/mL	2.36	45	19092	1.0		0.419	4.05	126	31094.4	1.0		0.685
<350/mL	2.88	55	19100.4	1.22	0.75-2.0		3.68	118	32082	0.92	0.6-1.40	
Partner HIV viral load (if partner HIV-positive)												
<10 000 copies/ mL	1.80	75	41623.2	1.0		0.844	2.74	48	17546.4	1.0		0.556
≥10 000 copies/mL	1.85	66	35720.4	1.03	0.74-1.45		2.96	74	25006.8	1.11	0.78-1.59	
Partner infected with specific HPV type												
No	1.59	251	157828.8	1.0		**<0.001**	2.67	393	147144	1.0		**<0.001**
Yes	11.71	42	3585.6	5.71	3.88-8.40		27.98	66	2359.2	9.06	6.49-12.65	
Living together with study partner												
No	1.42	84	59197.2	1.0		**0.039**	2.94	159	54006	1.0		0.732
Yes	2.05	209	101784	1.45	1.02-2.07		3.10	294	94867.2	1.06	0.77-1.46	
Duration of relationship with study partner												
<5 years	2.81	68	24206.4	1.0		0.989	3.71	79	21271.2	1.0		0.823
≥5 years	2.78	61	21978	1.00	0.58-1.71		3.47	75	21598.8	0.94	0.54-1.63	

	WOMEN						MEN					
Variable	**acquisition rate/ 1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**	**acquisition rate/ 1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**
Age at first sex												
<16 years	3.12	74	23691.6	1.0		**0.002**	2.30	90	39163.2	1.0		0.062
16-18 years	1.78	178	99800.4	0.57	0.36-0.90		3.43	265	77242.8	1.48	1.06-2.07	
>18 years	1.11	40	36018	0.36	0.2-0.62		3.30	102	30949.2	1.43	0.93-2.20	
Number of lifetime sexual partners												
1-2	1.49	103	69134.4	1.0		**0.015**	3.29	105	31921.2	1.0		0.906
3-5	1.81	125	69226.8	1.22	0.84-1.77		3.06	112	36655.2	0.93	0.62-1.41	
>5	3.03	64	21150	2.05	1.26-3.33		3.02	240	79506	0.92	0.64-1.34	
Number of sex act with study partner in last month												
<5	1.49	173	115839.6	1.0		**<0.001**	3.14	251	79890	1.0		0.762
≥5	2.72	112	41233.2	1.84	1.31-2.58		2.98	196	65685.6	0.95	0.7-1.29	
Ever used a condom with current study partner												
No	1.81	110	60834	1.0		0.955	3.08	175	56859.6	1.0		0.947
Yes	1.78	174	97548	0.99	0.69-1.42		3.08	273	88768.8	1.01	0.75-1.36	
Experienced genital discharge in last 6 months												
No	1.78	251	140631.6	1.0		0.618	3.10	452	145725.6	1.0		0.073
Yes	2.02	42	20784	1.13	0.7-1.82		1.85	7	3777.6	0.60	0.35-1.05	
Experienced genital ulcer in last 6 months												
No	1.75	267	152943.6	1.0		**0.029**	3.07	438	142612.8	1.0		0.901
Yes	3.07	26	8472	1.76	1.06-2.93		3.11	18	5791.2	1.04	0.6-1.8	
Parity (number of live births)												
0	2.59	69	26606.4	1.0		**0.028**						
1	1.24	61	49317.6	0.47	0.29-0.77							
2	1.90	68	35739.6	0.73	0.46-1.16							
3+	1.91	95	49752	0.73	0.46-1.17							
Current use of injectable or oral contraceptives												
No	1.98	208	105039.6	1.0		0.111						
Yes	1.48	78	52665.6	0.74	0.51-1.07							

Acq: acquisition, PM: person-months of follow-up for subject at risk of acquiring HPV infection, RR: relative risk.

Increased numbers of sexual acts with the study partner in the last month prior to a study visit was associated with an increased risk of acquiring HPV infection during follow-up in women (RR: 1.84, 95% CI: 1.3-2.6), but not in men. The presence of a genital ulcer in the last 6 months before the study visit was associated with an increased risk of acquiring HPV infection during follow-up in women (RR: 1.76, 95% CI: 1.1-2.9) but not in men. Increased parity (only number of live births were considered) was associated with a decreased risk of HPV acquisition during follow-up (P=0.028). Age at the baseline visit, CD4 counts among HIV-positive individuals, partner's CD4 count and the HIV viral load if the partner if HIV-positive, duration of relationship with the study partner, ever use of condom with the study partner, experience of genital discharge in last 6 month prior to the study visit and current use of injectable or oral contraceptives were not associated with acquiring infection during follow-up in both women and men (Table 6.2).

Table 6.3 presents factors that were associated with genital HPV acquisition during follow-up in women and men in the multivariate analysis. HIV-positive individuals with a HIV viral load of ≥ 10 000 copies per mL were found to have an increased risk of acquiring any HPV infection during follow-up in women (RR: 3.29, 95% CI: 2.18-4.95) and men (RR: 2.14, 95% CI: 1.46-3.12) compared to those with a HIV viral load of <10 000 copies per mL The risk of acquiring any HPV infection during follow-up was significantly increased in women (RR: 5.25, 95% CI: 3.52-7.81) and men (RR: 8.71, 95% CI: 6.19-12.24) with a study partner infected with an HPV type identical to the one acquired compared to those with a study partner not infected with a similar HPV type. Low age of sexual debut in women was significantly associated with the higher the risk of acquiring any HPV infection during follow-up (P=0.002). The current use of injectable or oral contraceptives was significantly associated with a reduced risk of LR-HPV acquisition during follow-up (OR: 0.54, 95% CI: 1.3-2.6, Table 6.3).

Table 6.3. Factors significantly associated with genital acquisition in both women and men, in multivariate analysis

	Any HPV types			HR types			LR types		
Variable	**RR**	**95% CI**	**P-value**	**RR**	**95% CI**	**P-value**	**RR**	**95% CI**	**P-value**
				WOMEN					
HIV status & viral load									
Negative	1.0		**<0.0001**	1.0		**<0.0001**	1.0		**0.0003**
Positive, VL<10,000 copies	2.31	1.49-3.58		2.58	1.41-4.7		1.95	1.16-3.28	
Positive, VL≥10,000 copies	3.29	2.18-4.95		4.12	2.27-7.46		2.45	1.6-4.02	
Partner infected with same HPV type									
No	1.0		**<0.0001**	1.0		**0.01**	1.0		**<0.0001**
Yes	5.25	3.52-7.81		2.69	1.27-5.7		6.88	4.4-10.77	
Age at first sex									
<16 years	1.0		**0.002**	1.0		**0.01**	1.0		**0.015**
16-18 years	0.6	0.39-0.93		0.55	0.31-0.98		0.58	0.36-0.94	
>18 years	0.4	0.24-0.66		0.31	0.15-0.66		0.43	0.24-0.77	
Current use of injectable or oral contraceptives									
No							1.0		**0.007**
Yes							0.54	0.34-0.84	
				MEN					
HIV status & viral load									
Negative	1.0		**0.0001**	1.0		**0.002**	1.0		**0.001**
Positive, VL<10,000 copies	1.73	1.14-2.62		1.76	1.02-3.03		1.69	0.97-2.95	
Positive, VL≥10,000 copies	2.14	1.46-3.12		2.24	1.4-3.59		2.08	1.38-3.15	
Partner infected with same HPV type									
No	1.0		**<0.0001**	1.0		**<0.0001**	1.0		**<0.0001**
Yes	8.71	6.19-12.24		11.43	7.57-17.26		6.61	4.06-10.75	

Note: multivariate analysis for men and women was done separately

6.3.3 HPV transmission in sexually active couples

HPV transmission was defined as the presence of the same HPV type in the partner at next visit initially detected in the index partner. HPV transmission analysis was restricted to couples in which at least one partner was HPV positive at baseline or HPV discordant. Female to male HPV transmission rate per 1000 person-months was 28.0 while male to female HPV transmission rate per 1000 person-months was 11.7. In male to female transmission events, LR-HPV transmission rate per 1000 person-months was more common (15.8) compared HR-HPV transmission rate per 1000 person-months (6.0). In contrast in female to male transmission events, HR-HPV transmission rate/1000 person-months were similar (29.2) to LR-HPV transmission rate per 1000 person-months (26.4).

HIV-positive women were found to be at high risk of HPV infection transmitted from their male partners compared to HIV-negative women (RR: 2.31 95% CI: 1.08-4.92, P=0.03). In contrast, HIV-positive men were not found to be at high risk of HPV infection transmitted from their female partners compared to HIV-negative men (RR: 1.23, 95% CI: 0.58-2.63, P=0.585). HIV-positive men with <350mL CD4 counts had high risk of HPV infection transmitted from female partners compared to HIV-positive men ≥350/mL CD4 counts (RR: 3.17, 95% CI: 1.05-9.55, P=0.04). However, HIV-positive women with <350mL CD4 counts were not found to have significantly higher risk of HPV infection transmitted from their male partners compared to HIV-positive women with 350/mL CD4 counts (RR: 1.57, 95% CI: 0.65-3.77, P=0.313). In women the risk of HPV transmission from the male partners was significantly associated with young age of sexual debut (P=0.03, Table 6.4). In men the risk of HPV infection transmitted from the female partner was associated with increasing age but it was not statistically significant (P=0.09). Women that have experienced genital ulcers in last 6-months before the study visit were found to be at higher risk of HPV infection from their male partner compared to those who did not (RR: 2.91, 95% CI: 1.05-8.07, P=0.04). Men with female partners that were currently using injectable or oral contraceptives were found to be less likely to have HPV infection transmitted from their female partner compared to men with female partners that were not using injectable or oral contraceptives (RR: 0.39, 95% CI: 0.18-0.86, P=0.019, Table 6.4).

In women, the risk of HPV infection transmitted from their male partners was found to increase with increasing age. While in men the risk of HPV infection transmitted from their female partners was found to decrease with increasing age; however these observations were

Table 6.4. Factors associated with HPV transmission to female and men over a period of 24 months, in the univariate analysis

	MALE TO FEMALE[#]						FEMALE TO MALE[+]					
Variable	**Transmission rate/1000 PM**	**Events**	**PM ***	**RR**	**95% CI**	**P-value**	**Transmission rate/1000 PM**	**Events**	**PM***	**RR**	**95% CI**	**P- value**
Age group												
<30 years	6.6	6	911	1.0		0.11	36.1	18	499	1.0		0.7
30-39 years	11.2	15	1338	1.86	0.59-5.88		26.7	21	786	0.74	0.26-2.08	
40+ years	15.7	21	1337	3.01	1.02-8.87		25.1	27	1074	0.69	0.29-1.63	
HIV status												
Negative	7.3	12	1645	1.0		**0.03**	25.7	44	1710	1.0		0.585
Positive	15.5	30	1942	2.31	1.08-4.92		33.9	22	649	1.23	0.58-2.63	
CD4 count (if HIV-positive)												
≥350/mL	14.0	16	1139	1.0		0.313	21.5	10	466	1.0		**0.04**
<350/mL	17.4	14	803	1.57	0.65-3.77		66.2	12	181	3.17	1.05-9.55	
HIV viral load (if HIV-positive)												
<10 000 copies/mL	21.7	15	691	1.0		0.194	26.5	6	227	1.0		0.316
≥10 000 copies/mL	14.4	14	972	0.52	0.20-1.39		40.1	17	424	2.03	0.51-8.03	
Partner HIV status												
Negative	13.0	27	2077	1.0		0.612	33.3	20	600	1.0		0.268
Positive	9.9	15	1510	0.82	0.37-1.79		26.1	46	1759	0.69	0.36-1.33	
Partner CD4 count (if partner HIV-positive)												
≥350/mL	11.0	6	545	1.0		0.363	28.4	24	845	1.0		0.618
<350/mL	9.4	9	962	1.78	0.52-6.11		24.1	22	913	0.80	0.32-1.96	
Partner HIV viral load (if partner HIV-positive)												
<10 000 copies/mL	14.7	6	408	1.0		0.716	25.4	18	709	1.0		0.671
≥10 000 copies/mL	10.8	10	925	1.28	0.34-4.90		26.6	23	865	1.22	0.49-3.02	
Living together with study partner												
No	6.9	10	1451	1.0		0.129	23.5	25	1062	1.0		0.472
Yes	15.0	32	2135	2.02	0.81-5.01		31.6	41	1297	1.32	0.62-2.80	
Duration of relationship with study partner												
<5 years	14.9	11	739	1.0		0.834	17.5	12	688	1.0		0.179
≥5 years	16.8	8	476	0.89	0.32-2.53		38.3	14	366	2.35	0.68-8.21	

	MALE TO FEMALE[#]						FEMALE TO MALE[+]					
Variable	**Transmission rate/1000 PM**	**Events**	**PM ***	**RR**	**95% CI**	**P-value**	**Transmission rate/1000 PM**	**Events**	**PM***	**RR**	**95% CI**	**P- value**
Age at first sex												
<16	21.4	13	607	1.0		**0.03**	21.1	18	852	1.0		0.092
16-18	11.2	24	2152	0.44	0.18-1.07		18.6	20	1074	1.32	0.63-2.77	
>18	5.0	4	802	0.21	0.06-0.69		65.1	26	400	2.7	1.10-6.60	
Number of lifetime sexual partners												
1-2	7.8	11	1406	1.0		0.184	42.7	15	352	1.0		0.238
3-5	14.0	23	1646	2.16	0.94-4.92		22.8	13	571	0.51	0.21-1.25	
>5	13.8	7	509	1.77	0.62-5.03		25.7	36	1403	0.6	0.29-1.23	
Number of sex acts with study partner in last month												
<5	10.0	25	2492	1.0		0.358	24.2	34	1406	1.0		0.479
≥5	13.4	14	1042	1.42	0.67-2.99		31.2	28	899	1.31	0.62-2.74	
Ever used a condom with current study partner												
No	11.6	15	1290	1.0		0.95	35.8	26	726	1.0		0.343
Yes	11.1	25	2251	0.98	0.44-2.15		24.0	38	1584	0.72	0.36-1.42	
Experienced genital discharge in last 6 months												
No	10.4	33	3186	1.0		0.075	28.2	63	2238	1.0		0.458
Yes	22.5	9	400	2.28	0.92-5.62		24.8	3	121	0.64	0.19-2.1	
Experienced genital ulcer in last 6 months												
No	10.2	34	3318	1.0		**0.04**	28.5	64	2245	1.0		0.995
Yes	29.9	8	268	2.91	1.05-8.07		23.1	1	43	1.01	0.13-8.09	
Current use of injectable or oral contraceptives												
No	12.4	31	2504	1.0		0.089						
Yes	7.7	8	1037	0.44	0.17-1.13							
Partner's current use of injectable or oral contraceptives												
No							34.0	52	1530	1.0		**0.019**
Yes							16.3	13	799	0.39	0.18-0.86	
Partner's cytology												
Normal							24.2	29	1200	1.0		0.64
Abnormal							33.0	35	1062	1.17	0.60-2.28	

PM*: person-month of exposure to infected partner [#] In women, factors associated with HPV transmission from male partner. [+] In men, factors associated with HPV transmission from female partner.

not statistically significant (Table 6.4). The individual's HIV viral load, partner's HIV viral load partners' HIV status and CD4 count level, living together with the study partner, duration of relationship with the study partner, number of lifetime sexual partners, number of sexual acts with study partner in last month, condom usage with study partner and experienced genital discharge in last month before the study visit was not significantly associated with HPV transmission to women and men. In women the use injectable or oral contraceptives was not significantly associated with HPV transmission from their male partners. Men with female partners with abnormal cervical cytology were not found to have a significantly higher risk of HPV infection transmitted from the female partner compared to men with female partners with normal cervical cytology (Table 6.4).

In the multivariate analysis, increasing age in women was significantly associated with HPV infection transmitted from their male partner (P=0.003). In women, the risk of HPV transmission from the male partner was significantly associated with age at sexual debut (P=0.012). HIV-positive women with ≥350 CD4/mL counts were found to have 1.76 relative risk of HPV transmission from male partners (95% CI: 0.7-4.42) and HIV-positive women with <350/mL CD4 counts were found to have 3.60 risk of HPV infection transmitted from their male partners (95% CI: 1.42-9.09) compared to HIV-negative women (Table 6.5).

Table 6.5. Factors associated with HPV transmission to women, in the univariate analysis

	MALE TO FEMALE*		
Variable	**RR**	**95% CI**	**P-value**
Age group			
<30 years	1.0		**0.003**
30-39 years	1.74	0.5-6.04	
40+ years	4.44	1.58-12.47	
HIV status/CD4 count			
HIV-negative	1.0		**0.021**
HIV-positive, CD4 ≥ 350	1.76	0.7-4.42	
HIV-positive, CD4 < 350	3.6	1.42-9.09	
Age at first sex			
<16	1.0		**0.012**
16-18	0.41	0.17-0.96	
>18	0.18	0.06-0.58	

* In women, factors associated with HPV transmission from male partner. RR: relative risk

6.3.4 Clearance of genital HPV infection in women and men

In women a total of 319 clearance events were observed, 166 of them were LR-HPV types and 153 were HR-HPV types. In men a total of 528 clearance events were observed, 285 of them were LR-HPV types and 243 were HR-HPV types. The rate of clearing any HPV infection was 95.12 (95% CI: 83.3-108.1) per 1000 person-months in men and 66.95 (95% CI: 57.0-78.5) per 1000 person-months in women. Men demonstrated a significantly higher rate of clearing any HPV infection compared to women (P=0.001). The rate of clearing HR-HPV infection was significantly higher in men 103.22 (95% CI: 88.5-120.6) per 1000 person-months compared to 62.28 (95% CI: 51.1-76.2) per 1000 person-months in women (P<0.001). The rate of clearing LR-HPV was 89.15 (95% CI: 76.2-10.38) per 1000 person-months in men and 71.92 (95% CI: 58.1-89.4) per 1000 person-months in women but the difference in the rate of clearing LR-HPV between women and men was not statistically significant (P=0.111, Table 6.6).

In women, the three HR-HPV types most cleared during follow-up were HPV-26 (258.63 per 1000 person months) followed by HPV-18 (106.55 per 1000 person-months) and HPV-33 (100.21 per 1000 person-months). In women HPV-16 was found to be the least cleared when compared with other HR-HPV and LR-HPV types during follow up (32.29 per 1000 person-months). In men, three HR-HPV types most cleared included HPV-82 (290.83 per 1000 person-months), HPV-33 (148.73 per 1000 person-months) and HPV-18 (133.20 per 1000 person months). In men HPV-59 was found to be the least cleared when compared with other HR-HPV and LR-HPV types during follow up (50.79 per 1000 person-months). When we look at HR-HPV types individually, men were found to have a significantly higher clearance rate compared to women for HPV-16 (101.23 per 1000 person-months compared to 32.30 per 1000 person-months, P=0.02); HPV-35 (133.21 per 1000 person-months compared to 48.95 per 1000 person-months, P=0.014); HPV-45 (110.02 per 1000 person-months compared to 38.52 per 1000 person-months, P=0.032); HPV-52 (115.26 per 1000 person-months compared to 52.58 per 1000 person-months, P=0.034) and HPV-82 (290.83 per 1000 person-months compared to 99.02 per 1000 person-months, P=0.001, Table 6.6).

In women, the three LR-HPV types most cleared were HPV-54 (105.31 per 1000 person-months); followed by HPV-42 (94.39 per 1000 person-months) and HPV-84 (92.85 per 1000 person-months). Among all LR-HPV types, HPV-11 was found to be the least cleared type in women (46.29 per 1000 person-months). In men HPV-42 was found to be the most cleared LR-

Table 6.6. The clearance rate of genital HPV infection by genotype and species in women and men

	WOMEN				MEN				
Variable	**Clearance rate/ 1000 PM**	**95% CI**	**Events of clearance**	**PM**	**Clearance rate/ 1000 PM**	**95% CI**	**Events of clearance**	**PM**	**P-value**
HR-HPV									
HPV-16	32.39	13.9-75.4	7	216.1	101.23	62.3-164.5	17	167.9	**0.02**
HPV-18	106.55	71.0-159.6	16	150.2	133.30	91.2-195.0	14	105.0	0.425
HPV-26	258.63	174.7-384.8	4	15.5	66.90	11.1-404.6	2	29.9	0.104
HPV-31	66.33	28.3-154.9	5	75.4	108.99	65.6-180.0	9	82.6	0.313
HPV-33	100.21	38.6-260-6	7	69.9	148.73	88.5-250.3	5	33.6	0.462
HPV-35	48.95	28.3-85.0	11	224.7	133.21	73.9-240.5	16	120.1	**0.014**
HPV-39	71.53	36.0-141.6	7	97.9	132.07	87.6-198.9	15	113.6	0.125
HPV-45	38.52	15.7-94.0	6	155.8	110.02	74.7-161.2	18	163.6	**0.032**
HPV-51	80.68	39.4-164.5	9	111.6	70.94	40.6-124.3	14	197.3	0.777
HPV-52	52.58	29.8-93.0	15	285.3	115.26	72.4-183.6	21	182.2	**0.034**
HPV-53	81.58	47.6-140.2	17	208.4	100.82	68.2-148.8	27	267.8	0.528
HPV-56	32.45	9.2-113.6	3	92.5	72.38	16.7-312.0	3	41.4	0.394
HPV-58	54.23	31.0-94.9	14	258.1	76.44	41.4-140.2	14	183.2	0.411
HPV-59	73.27	39.8-134.7	10	136.5	50.79	22.7-113.6	8	157.5	0.471
HPV-66	65.12	30.0-141.6	8	122.9	113.91	80.9-161.2	26	228.3	0.187
HPV-68	44.86	19.5-102.8	7	156.0	99.35	62.4-159.6	18	181.2	0.096
HPV-73	75.22	23.6-240.5	3	39.9	112.15	61.7-202.9	8	71.3	0.527
HPV-82	99.02	52.1-189.2	4	40.4	290.83	231.1-366.1	8	27.5	**0.001**
All HR-HPV	62.28	51.1-76.2	153	2456.8	103.22	88.5-120.6	243	2354.1	**<0.001**
LR-HPV									
HPV-6	68.19	30.7-151.8	7	102.7	84.02	44.8-156.5	10	119.0	0.681
HPV-11	46.29	4.9-442.7	1	21.6	97.21	28.6-331.2	5	51.4	0.483
HPV-40	64.01	12.6-324.7	2	31.2	177.11	87.6-358.8	9	50.8	0.229
HPV-42	94.39	43.5-205.0	6	63.6	188.47	71.0-499.1	5	26.5	0.254
HPV-54	105.31	67.5-164.5	18	170.9	105.56	66.2-167.8	18	170.5	0.994
HPV-55	51.38	15.7-167.8	4	77.8	110.90	73.2-167.8	22	198.4	0.207
HPV-61	66.35	39.0-112.5	16	241.1	71.40	50.0-101.8	32	448.2	0.821
HPV-62	60.60	37.8-97.8	18	297.0	72.88	54.2-98.8	42	576.3	0.516

	WOMEN				MEN				
Variable	**Clearance rate/ 1000 PM**	**95% CI**	**Events of clearance**	**PM**	**Clearance rate/ 1000 PM**	**95% CI**	**Events of clearance**	**PM**	**P-value**
HPV-67			1	5.2			1	3.0	
HPV-69	84.23	32.9-215.5	6	71.2	128.97	72.4-228.8	7	54.3	0.435
HPV-70	91.17	51.6-161.2	17	186.5	117.63	81.7-169.5	22	187.0	0.455
HPV-71	74.31	41.0-134.7	15	201.9	91.17	55.3-150.3	14	153.6	0.602
HPV-72	55.51	30.0-101.8	12	216.2	85.53	50.0-147.4	18	210.5	0.293
HPV-81	47.18	24.6-90.3	7	148.4	97.60	65.6-145.9	22	225.4	0.057
HPV-83	64.72	36.0-117.1	13	200.9	68.80	39.8-118.3	17	247.1	0.88
HPV-84	92.85	46.7-183.6	12	129.2	94.54	64.3-138.8	21	222.1	0.964
HPV-89	81.79	44.4-150.3	8	97.8	71.46	40.6-125.6	15	209.9	0.746
HPV-IS39	66.72	19.5-228.8	3	45.0	116.62	60.5-224.3	5	42.9	0.416
All LR-HPV	71.92	58.1-89.4	166	2308.2	89.15	76.2-103.8	285	3196.9	0.111
All HPV	66.95	57.0-78.5	319	4765.0	95.12	83.3-108.1	528	5551.0	**0.001**
HPV species									
α1	94.39	43.5-205-0	6	63.6	188.47	71.0-499.1	5	26.5	0.254
α3	64.63	50.0-83.3	86	1330.6	78.06	64.9-94.0	167	2139.4	0.241
α5	91.67	60.5-138.8	26	283.6	102.30	72.4-144.0	36	351.9	0.691
α6	66.08	43.9-99.8	28	423.7	104.18	79.3-137.4	56	537.5	0.068
α7	71.37	53.1-95.9	63	882.8	104.63	85.0-128.1	95	907.9	**0.038**
α8	64.01	12.6-324.7	2	31.2	177.11	87.6-358.8	9	50.8	0.229
α9	52.88	40.2-69.6	60	1134.7	107.43	85.0-136.0	83	772.6	**<0.001**
α10	59.38	30.4-115.9	12	202.1	100.32	71.0-141.6	37	368.8	0.165
α11	75.22	23.6-240.5	3	39.9	112.15	61.7-202.9	8	71.3	0.527
α13	105.31	67.5-164.5	18	170.9	105.56	66.2-167.8	18	170.5	0.528
α15	74.31	41.0-134.7	15	201.9	91.17	55.3-150.3	14	153.6	0.602

PM: person-months of follow-up for subject at risk of acquiring HPV infection. **α1** HPV species includes HPV-42. **α3** HPV species includes HPV-61, -62, -72, -81, -83, -84 and -89. **α5** HPV species includes HPV-26, -51, 69, -82 and –IS39. **α6** HPV species includes HPV-53, -56 and -66. **α7** HPV species includes HPV-18, -39, -45, -59, -68 and -70. **α8** HPV species includes HPV-40 . **α9** HPV species includes HPV-16, -31, -33, -35, -52, -58 and -67. **α10** HPV species includes HPV-6, -11 and -55. **α11** HPV species include HPV-73. **α13** HPV species includes HPV-54. **α15** HPV species includes HPV-71.

HPV type (188.47 per 1000 person-months) followed by HPV-40 (177.11 per 1000 person-months) and HPV-69 (128.97 per 1000 person-months). HPV-61 was found to be the least cleared LR-HPV type in men during follow-up (71.40 per 1000 person-months). When we look at the LR-HPV individually the clearance rate of LR-HPV types was not found to differ significantly between women and men (Table 6.6).

When HPV clearance was investigated according to each HPV species types, in women the three most cleared HPV species types during follow-up were α13 HPV species (105.31 per 1000 person-month); α1 HPV species (94.39 per 1000 person-month) and α5 HPV species (91.67 per 1000 person-month). In men HPV types within the α1 HPV species (188.47 per 1000 person-month) were most cleared followed by α8 HPV species (177.11 per 1000 person-month) and α11 HPV species (112.15 per 1000 person-month). In women α9 HPV species were the least cleared HPV species (52.88 per 1000 person-month) and in men α3 HPV species (76.06 per 1000 person-month) were the least. Men were found to have significantly higher HPV clearance rates compared to women for α7 HPV species types (104.63 per 1000 person-month compared to 71.37 per 1000 person-month) and for α9 HPV species types (107.43 per 1000 person-month compared to 52.88 per 1000 person-month, Table 6.6).

6.3.5 Factors affecting clearance of genital HPV infection in women and men

HIV-positive women were found to have a significantly lower clearance of HPV infection compared to HIV-negative women (RR: 0.46, 95% CI: 0.34-0.62, P<0.001). Women with abnormal cervical cytology were also found to have a significantly lower HPV clearance when compared with women with normal cervical cytology (RR: 0.68, 95% CI: 0.48-0.96, P=0.029). HIV-positive men were also found to have a significantly lower clearing of HPV infection compared to HIV-negative men (RR: 0.71, 95% CI: 0.55-0.93, P=0.013). All other variables demonstrated no statistically significant effect on HPV clearance (Table 6.7). In multivariate analysis, the only factor that remained significantly associated with the rate of HPV clearance in both men and in women was HIV status. The effect of HIV infection on HPV clearance was similar for women infected with HR-HPV types (RR: 0.44, 95% CI: 0.29-0.65) and women infected with LR-HPV types (RR: 0.48, 95% CI: 0.33-0.71). However, in men the effect of HIV infection on HPV clearance was significant only for high risk types (RR: 0.59, 95% CI: 0.43-0.81) but not for LR-HPV types (RR: 0.84, 95% CI: 0.61-1.16). The presence of HPV antibodies was not significantly associated with the rate of HPV clearance

Table 6.7. Factors associated with genital HPV clearance in women and men, in the univariate analysis

	WOMEN						MEN					
	Clearance rate/1000 PM	**Events**	**PM**	**RR**	**95% CI**	**P-value**	**Clearance rate/1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**
Age group												
<30	65.4	104	1590.0	1.0		0.133	95.0	119	1252.8	1.0		0.782
30-39	59.7	114	1910.4	0.9	0.59-1.39		90.8	193	2125.2	0.92	0.64-1.33	
40+	79.9	101	1263.6	1.31	0.88-1.97		99.4	216	2173.2	1.02	0.74-1.42	
HIV status												
Negative	110.1	114	1035.6	1.0		**<0.001**	106.8	331	3098.4	1.0		**0.013**
Positive	55.0	205	3729.6	0.46	0.34-0.62		80.3	197	2452.8	0.71	0.55-0.93	
CD4 count (if HIV-positive)												
≥350/mL	63.8	91	1425.6	1.0		0.155	81.0	82	1012.8	1.0		0.987
<350/mL	49.5	114	2302.8	0.77	0.53-1.1		79.7	114	1430.4	1.00	0.64-1.56	
HIV viral load (if HIV-positive)												
<10 000 copies	59.9	80	1335.6	1.0		0.211	94.3	61	646.8	1.0		**0.063**
≥10 000 copies	48.4	96	1984.8	0.77	0.51-1.16		71.9	116	1612.8	0.67	0.44-1.02	
Partner HIV status												
Negative	73.5	222	3021.6	1.0		0.085	100.7	215	2134.8	1.0		0.373
Positive	55.6	97	1743.6	0.74	0.52-1.04		91.6	313	3416.4	0.89	0.7-1.14	
Partner CD4 count (if partner HIV-positive)												
≥350/mL	51.4	52	1011.6	1.0		0.73	82.9	134	1616.4	1.0		0.236
<350/mL	60.7	44	724.8	1.11	0.62-2.0		99.4	179	1800	1.25	0.86-1.80	
Partner HIV viral load (if partner HIV-positive)												
<10 000 copies	55.4	27	487.2	1.0		0.784	103.8	116	1117.2	1.0		0.165
≥10 000 copies	57.0	69	1209.6	0.92	0.5-1.68		80.6	154	1910.4	0.74	0.49-1.13	
Antibody to HPV strain												
No	57.8	67	1159.2	1.0		0.933	113.2	110	972	1.0		0.114
Yes	60.9	16	262.8	0.98	0.58-1.65		70.9	12	169.2	0.58	0.29-1.14	
Living together with study partner												
No	64.9	117	1803.6	1.0		0.924	98.9	194	1962	1.0		0.758
Yes	68.2	202	2960.4	1.02	0.72-1.43		92.7	329	3547.2	0.95	0.71-1.28	
Duration of relationship with study partner												
<5 years	71.9	85	1182	1.0		0.366	111.5	121	1084.8	1.0		0.287
≥5 years	64.9	63	970.8	0.77	0.44-1.36		137.9	115	834	1.24	0.84-1.83	

	WOMEN						MEN					
	Clearance rate/1000 PM	**Events**	**PM**	**RR**	**95% CI**	**P-value**	**Clearance rate/1000 PM**	**Events**	**PM**	**RR**	**95% CI**	**P-value**
Age at first sex												
<16	77.4	59	762	1.0		0.595	104.2	161	1545.6	1.0		0.77
16-18	66.5	206	3097.2	0.84	0.52-1.35		91.4	255	2791.2	0.89	0.64-1.23	
>18	59.6	52	872.4	0.76	0.45-1.28		95.3	109	1143.6	0.90	0.62-1.32	
Number of lifetime sexual partners												
1-2	69.2	118	1704	1.0		0.961	79.3	98	1236	1.0		0.335
3-5	65.2	129	1977.6	0.98	0.66-1.44		104.5	155	1483.2	1.27	0.9-1.78	
>5	66.7	70	1050	1.05	0.63-1.75		98.5	272	2762.4	1.23	0.89-1.68	
Number of sex acts with study partner in last month												
<5	68.4	194	2838	1.0		0.622	94.9	306	3225.6	1.0		0.923
≥5	62.4	115	1842	0.91	0.63-1.31		94.5	206	2180.4	1.01	0.77-1.34	
Ever used a condom with current study partner												
No	76.3	107	1402.8	1.0		0.196	89.5	171	1910.4	1.0		0.816
Yes	64.0	203	3170.4	0.78	0.54-1.14		96.0	338	3522	1.03	0.78-1.38	
Experienced genital discharge in last 6 months												
No	65.7	278	4232.4	1.0		0.557	94.9	502	5288.4	1.0		0.959
Yes	77.0	41	532.8	1.15	0.72-1.85		98.9	26	262.8	0.98	0.5-1.94	
Experienced genital ulcer in last 6 months												
No	67.1	297	4426.8	1.0		0.786	96.2	500	5196	1.0		0.336
Yes	65.2	22	337.2	0.93	0.54-1.60		72.1	23	319.2	0.71	0.35-1.43	
Parity (number of live births)												
0	82.4	84	1018.8	1.0		0.157						
1	60.8	92	1513.2	0.76	0.48-1.20							
2	52.5	54	1028.4	0.57	0.34-0.96							
3+	73.8	89	1206	0.92	0.58-1.45							
Current use of injectable or oral contraceptives												
No	66.7	225	3370.8	1.0		0.946						
Yes	65.5	88	1342.8	1.01	0.72-1.43							
Cervical cytology												
Normal	78.6	193	2454	1.0		**0.029**						
Abnormal	51.9	113	2175.6	0.68	0.48-0.96							

PM: person-months of follow-up for subject at clearing HPV infection, RR: relative risk

after controlling for HIV status, either in women (RR: 1.05, 95% CI: 0.59-1.86) or in men (RR: 0.58, 95% CI: 0.29-1.17).

6.4 DISCUSSION

In this chapter we presented the HPV acquisition and clearance rate data for women and men as well as factors associated with HPV acquisition and clearance over a period of 24-months. We also demonstrated the effect of a women's abnormal cervical cytology and the effect of HIV co-infection in one or both partners on sexual HPV transmission between partners. To our knowledge this is the first study to investigate HPV transmission in heterosexually active couples that are both HIV-positive and HIV-discordant.

Men demonstrated a higher rate of total HPV acquisition compared to women (459 compared to 293 acquisition events). In chapter 2 we demonstrated that HIV-negative men have significantly higher HPV prevalence compared to HIV-negative women; however, HIV-positive men demonstrated higher HPV prevalence compared to HIV-positive women but not statistically significant. In our study, HPV prevalence at baseline was not different between women and men (58.1% and 58.4% respectively). Giuliano *et al*., (2008a) also observed similar baseline HPV prevalence between women and men (53.8% and 52.8% respectively). However, total HPV acquisition during follow-up was higher in our study in men compared to women (3.07 per 1000 person-months compared 1.82 per 1000 person-months, P<0.001). In contrast, Giuliano *et al*., (2008a) did not observed similar rates of total HPV acquisition between women and men (29.4 per 1000 person-months in both women and men). In our study among men, the acquisition of HPV-16 was 3.16 per 1000 person-months and for HPV-6 it was 1.65 per 1000 person-months; however Giuliano *et al*., (2008a) reported a higher HPV-16 and -6 acquisition during follow-up (4.8 per 1000 person-months and 2.8 per 1000 person-months respectively).In our study among men, the acquisition of HPV-18 and 11 was (3.36 per 1000 person-months and 1.85 per 1000 person-months respectively). In a Giuliano *et al*., (2008a) report a far lower HPV-18 and -11 acquisitions compared to our study (0.8 per 1000 person-months and 0.5 per 1000 person-months respectively). The rate of LR-HPV acquisition in men was higher than the rate of HR-HPV acquisition (3.34 per 1000 person-months compared to 2.8 per 1000 person-months) in our study; while Giuliano *et al*., (2008a) reported a comparable rate of acquiring LR and HR-HPV types. However other studies reported that HR-HPV types are acquired faster compared to LR-HPV types (Franco *et al*., 1999; Giuliano

et al., 2002; Winer *et al*., 2008). The difference between studies may be caused by the different sampling methods used, different HPV genotyping assays and different demographics of the cohort. The high rate of LR-HPV acquisition in men in our study also explains the observed high LR-HPV prevalence at baseline. The different epithelium on samples site in women and men could be the result of different HPV acquisition rate observed in this study.

It was interesting to note that the events of HR-HPV acquisition from the study partner were significantly higher in men compared to women (18% compared to 7%, P=0.003) while for LR-HPV acquisition were significantly higher in women compared to men (21% compared to 11%, P=0.01). HR-HPV types were more commonly transmitted from female to male transmission events while LR-HPV types were more commonly transmitted from male to female transmission events. The findings that LR-HPV types were more prevalent at baseline among men compared to women and also the events of LR-HPV acquisition during follow-up were higher in men compared to women may also explain the high rate of LR-HPV in male to female transmission and the fact that in women only the cervix was sampled while in men the penile shaft, glans and foreskin in uncircumcised men were samples.

The female to male HPV transmission rate was found to be higher compared to the male to female HPV transmission (28.0 compared to 11.7 HPV transmission rate per 1000 person-months). Hernandez *et al*., (2008) observed high female to male HPV transmission and male to female HPV transmission compared to our study (174.0 and 49 HPV transmission rate per 1000 person-months). It is interesting to note that female to male HPV transmission rate is higher than male to female HPV transmission rate while for most STIs male to female transmission rate is usually higher than female to male transmission rate (Weinstock *et al*., 2004; Arora *et al*., 2011). The different sampling interval in our study compared to Hernandez *et al*., (2008) is probable the reason we observed different HPV transmission rates (6-month interval in our study compare to 2-month interval). The high rate of HPV transmission from female to male explains the high rate of HPV acquisition observed in men compared to women. HPV transmission from male partners to female partners was associated with their HIV-positive status and low CD4 count and but HPV transmission from female partners to male partners was only associated with low CD4 counts. The high risk of HPV transmission to women and men with low CD4 count is due to suppressed immune system condition (Strickler

et al., 2005). HPV transmission was not associated with increased number of sexual act with study partner a month prior the visit suggesting that the observed transmissions were not just HPV deposited by the partner from previous sexual act but true infection.

Women who started participating in sexual debut at young age were found to have high risk of HPV infection transmitted by their male partner. Women who engaged in sexual activity at young age probably have high number of lifetime sex partners compared to those who started at older age, therefore the high risk of HPV transmission we observed in women who started participating in sexual debut at young age is the reflection of high number of lifetime sex partners. It has been reported that the cervix of young women is still immature with an inadequate production of protective cervical mucus and increased cervical ectopy increasing the susceptibility to HPV infection; thus they are at increased risk of HPV transmission (Collins *et al*., 2005). However, its not clear if old women who started participating in sexual debut at <16 years of age would still be at high risk of HPV transmission compared to those who started participating in sexual debut at <18 years of age.

Women who experienced genital ulceration 6-months before the study visit had 2.9 risk of acquiring HPV infection transmitted from their male partners compared to women who did not experience genital ulceration 6-months before the study visit. The association of genital ulcerations with HPV transmission can be explain by the fact that the wounds caused by the ulcer provide an easy access to the genital mucosa compared to women without genital ulcers (Shafti-Karamat *et al*., 2003). Ulceration can be a complete loss of the epithelial surface but sometimes it can be a lesion, then that lesion will give HPV an access to basal cells (Shafti-Karamat *et al*., 2003). Women who experienced genital ulcers in the last 6-months before their study visit had an increased risk of acquiring new HPV infection during follow-up. The development of a genital ulcer increased the risk of HPV acquisition because HPV is able to access the genital mucosa more easily through the wounds caused by ulcers (Shafti-Karamat *et al*., 2003; Culp *et al*., 2003). However, the development of a genital ulcer over the last 6-months before the study visit did not influence HPV acquisition in men.

It was interesting to note that men with female partners using injectable or oral contraceptives were less likely to have HPV transmission compared to those with female partners not using injectable or oral contraceptives (P=0.019). However the biological explanation of this still needs further investigation. Abnormal cervical cytology was not significantly associated with female to male HPV transmission in our study. Since women with abnormal cervical cytology have high HPV viral load it was expected that female to male transmission will be high in men with female partner with abnormal cervical cytology compared to those with female partner with normal cervical cytology. The small number of HPV transmission events and women with abnormal cervical cytology observed in our study could be the reason for the lack of statistical significance.

It is important to note that in this study samples from women were only collected at the cervix while a single swab from men sampled penile shaft, glans and foreskin (in uncircumcised men). It has been reported that more LR-types are likely to be detected in specimens from the vagina than in samples from the cervix (Jones *et al.*, 2007). Therefore the higher LR-HPV prevalence in men compared to women and male to female transmission events which were more likely to be LR-HPV types could be due to the fact that women were only sampled at the cervix not the vagina. The high rate of LR-HPV acquisition in women due to transmission from the study partner can be explained by high prevalence of LR-HPV types observed in men and the fact that in men we sampled more than one genital site (penile shaft, glans as well as foreskin in uncircumcised men). HPV species α3, α7 and α9 were the most prevalent species in both women and men. All transmitted HPV types were higher in men but not for α3 HPV species which was higher in women. The α3 HPV species group contains seven LR-HPV types and since we find that LR-HPVs were more commonly transmitted from male to female women would be expected to harbour more α3 HPV types.

We observed that women and men with HIV-positive partners were found to have a significantly increased risk of HPV acquisition. However, the CD4 count and HIV viral load of their partner were not found to influence HPV acquisition in either women or men. The risk of acquiring HPV infection was significantly increased in women and men with partners infected with the same HPV type that was acquired. This possibly indicates that those types acquired

were transmitted from their sexual partner. It was interesting to note that women living with their male partner had an increased risk of acquiring new HPV types not found in their partners. These observations may indicate that even though the couples were staying together they may have other partners, as it has been reported that having a high risk partner who has other partners, can increase the rate of HPV acquisition (Fukuchi *et al*., 2009). Among men or women in monogamous relationships the rate of HPV acquisition was not increased compared with polygamous relationship (Bosch, 2008). Having a new sexual partner apart from the study partner was associated with an increased risk of acquiring new HPV infection (Bosch *et al*., 2008). Oh *et al*., (2008) conducted a study on women that were virgins, and women who had a first sexual encounter at baseline visit and found a 2.9 higher risk of acquiring new HPV infection in those who had new or changed sexual partners compared to those who remained virgins. Bosch *et al*., (2008) suggested that the large age gaps between partners in Africa may indicate that staying together with or married to the partner does not necessarily mean "safe sex" and that men who might have multiple partners are more likely to be the source of STIs transmitted to their partners (Bosch *et al*., 1996; Castellsague *et al*., 2002).

The duration of a couple's relationship was not associated with HPV acquisition for both women and men. The more sexual acts a woman had with their study partner in last month increased the risk of acquiring new HPV infection. In contrast, the number of sex acts did not influence the rate of HPV acquisition in men. In our study condom use was not found to play a role in decreasing the rate of HPV acquisition in both women and men. These findings may suggest that the study participants were not using condoms for every sexual act. Using condoms has been found to provide protection against HPV acquisition in women (Kjaer *et al*., 2005; Partridge *et al*., 2007). Consistent condom use is reported to provide some level of protection against HPV infection as well as genital lesions (Bleeker *et al*., 2005b; Nielson *et al*., 2010).

In our study women initiating sexual intercourse after 16 years had a lower risk of acquiring HPV compared to those initiating sex before the age of 16 years. Fukuchi *et al*., (2009) also reported that initiation of sex at age >16 years was associated with HPV acquisition during 12-months. However, in men we observed that HPV acquisition seem to increase with the age

years they initiated sexual intercourse however these finding were not statistically significant. These observations are in contrast to what has been reported by Shin *et al*., (2004) in which they observed an increased risk of acquiring HPV with younger age of initiating penetrative sexual intercourse. It has been reported that women have their first sexual intercourse at younger age compared to men (Bosch *et al*., 2008). In women we observed that having more lifetime sexual partners is associated with increased risk of HPV acquisition, similar findings are reported elsewhere (Ho *et al*., 1998; Winer *et al*., 2003; Munoz *et al*., 2004; Goodman *et al*., 2008; Lu *et al*., 2009). Women with male partners with a large number of other sexual partners have a significantly increased risk of cervical disease compared to women in monogamous relationships (Bosch *et al*., 1996; Castellsague *et al*., 2002). In men, the high number of lifetime sexual partners was not associated with HPV acquisition. Our findings are in contrast with other studies in which large numbers of lifetime sex partners are associated with high risk of HPV acquisition in men (Hippelainen *et al*., 1993; Franceschi *et al*., 2002; Giuliano *et al.,* 2008a).

In our study the use of injectable or oral contraceptives was not associated with acquisition of new HPV during follow-up. Vaccarella *et al*., (2006) reported that the use of oral contraceptives even in long term is not associated with increased risk of HPV acquisition and persistence but the association of oral contraceptives, smoking and alcohol drinking with HPV acquisition may indicate high risk behaviour (Goodman *et al*., 2008). The high rate of HPV acquisition in women who use injectable or oral contraceptives could be an indication of high risk behaviour. It was interesting to note that in the multivariate analysis the use of injectable or oral contraceptives was associated with reduced risk of only LR-HPV types (RR: 0.54, 95% CI: 0.34-0.84, P=0.007) in our study. The explanation of this will require further investigation. It is important to note that in our study the majority of women were using injectable contraceptives compared to oral contraceptives and in our analysis we combined both the injectable and oral contraceptives as a single variable. In our study, having multiple parity was associated with a reduced risk of acquiring HPV infection, however in parity was only restricted to women with live births which could impact on HPV risk. In other studies high parity has been reported to be associated with cervical cancer (Castellsague & Munoz, 2003). An increasing number of pregnancies are associated with an increased risk of cervical cancer (Hildesheim *et al*., 2001; Moreno *et al*., 2002; Munoz *et al*., 2002).

The increased risk of detection of HPV acquisition in HIV-positive women and men could be the result of suppressed immune system or reactivation of latent HPV infections, not new HPV acquisition. It is not possible to define whether a new HPV detection is a new infection or reactivation of latent HPV infection (Palefsky *et al*., 1999; Strickler *et al*., 2005; Bleeker *et al*., 2005a). Strickler *et al*., (2005) reported that 22% of HIV-positive women with a CD4 count <200/mL having no sexual activity had new HPV infection during follow-up. This may indicate reactivation of latent HPV infection (Theiler *et al*., 2010). In our study the risk of acquiring new HPV infection and the ability to clear HPV infection were not found to differ significantly between women or men with CD4 count ≥350/mL and <350/mL. However some studies have indicated the risk of acquiring HPV infection among HIV-positive individuals is affected by CD4 count (Strickler *et al*., 2005). HPV infections that persist for longer periods are not easy to clear and may lead to the development of precancerous lesions and cancer (Ho *et al*., 1998; Wu *et al*., 2006). HIV-positive women with a HIV viral load ≥10 000/mL were found to have a higher risk of HPV acquisition. However, high HIV viral load was not associated with HPV acquisition in our study in men.

The decreased ability to clear HPV infection in HIV-positive women and men may be the result of decreased immune competency and the high prevalence of multiple HPV infection. It has been reported that women with multiple HPV infections have a reduced HPV clearance compared to women with a single HPV infection (Goodman *et al*., 2001; Rousseau *et al*., 2003). Men demonstrated greater total HPV clearance compared to women (528 compared to 319 HPV clearance events). In men the HPV clearance was found to be greater for HR-HPV types compared to LR-HPV types (103.22 per 1000 person-months compared to 89.15 per 1000 person-months) in our study. This is in contrast to what has been reported by previous studies in which LR-HPV are reported to have a higher rate of clearance compared to HR-HPV types, and HR-HPV persistence is reported to be higher than that of LR-HPV types (Moscicki *et al*., 1998; Franco *et al*., 1999; Giuliano *et al*., 2008a). However, Oh *et al*., (2008) did not find a significant difference in the clearance of LR and HR-HPV. In women, HPV clearance was higher for LR-HPV compared to HR-HPV (72.92 per 1000 person-months compared to 62.28 per 1000 person-months). Assessing LR-HPV types in women, HPV-11 was the least cleared type. This may be explained by the high prevalence of HPV-11 DNA and antibodies at baseline (chapter 2 and 5 respectively). It is reported that persistent HPV infection and high viral load is required to activate the immune system (Stanley *et al*., 2006). Among women in our study, HPV-16 was found to have a lower rate of clearance compared to other HPV types.

Louvanto *et al*., (2010) reported that women with HPV-16 or with multiple HPV infection are less likely to clear HPV infection during follow-up compared to women with single HPV infection or not infected with HPV-16.

Amongst women in our study, α9 HPV species were the least cleared HPV types. Trottier *et al*., (2008) reported that α7 and α9 HPV species have a lower clearance rate compared to other HPV species. HPV-16 is the most dominant HPV type and is responsible for ~50% cervical cancer cases and belongs to the α9 HPV species, while HPV-18 is the second most dominant type and is responsible for ~20% cervical cancer cases. HPV-18 belongs to α7 HPV species. The eight most common HPV types reported in cervical cancers belong to α9 HPV species (HPV-16, -31, -33, -35, -52 and -58) and α7 HPV species (HPV-18 and -45). The lower clearance rate of α7 and α9 HPV species could be the reason why they are the most common HPV types associated with cervical cancer. The HPV persistence is necessary for the development of precancerous lesions and cancer. In men, α3 HPV types were found to be the least cleared HPV species followed by α7 and α9 HPV species. The reason for the low clearance rate of α3 HPV species in men could be that α3 HPV species contains LR-HPV types and in men we observed a higher prevalence of LR-HPV types (as single or multiple infections) and a higher rate of LR-HPV acquisition. The rate of HPV clearance in our study was higher than the rate of HPV acquisition in both women and men and similar observations were observed elsewhere (Goodman *et al*., 2008). Even though HPV acquisition in men is higher compared to women, men are reported to clear their HPV infection faster than women (Giuliano *et al*., 2008a; Lu *et al*., 2009). The incidence of HPV associated cancer is lower in men compared to that observed in women (Parkin & Bray, 2006). The high rate of HPV clearance in men could be the reason why there is a low incidence of HPV-associated cancer in men compared to that observed in women.

HIV co-infection was found to significantly influence HPV clearance in both women and men and these observations were observed in both univariate and multivariate analyses. The decreased ability to clear HPV infection in HIV-positive women and men could be the result of a suppressed immune system caused by HIV infection, a resultant high prevalence of multiple HPV infection and a high HPV viral load (Ho *et al*., 1998; Goodman *et al*., 2001; Rousseau *et al*., 2003; Stricker *et al*., 2005; Wu *et al*., 2006). When we controlled for HIV status, HPV antibodies were not associated with HPV clearance in our study. HPV acquisition and clearance was not significantly associated with age in our study for both women and men.

Giuliano *et al*., (2008a) also reported no significant difference in the acquisition of HPV in men according to age. Dunne *et al*., (2006) reported a constant HPV prevalence in men of different ages. In our study we also observed that in men HPV prevalence did not significantly increase with increasing age. So the lack of association between age and HPV acquisition and clearance in men during follow-up was a reflection of what was observed at baseline. In women the lack of association between age and HPV acquisition or clearance during follow-up in our study was in contrast to what has been reported by several studies (Munoz *et al*., 2004). However, Castle *et al*., (2005) did not observe a significant increase of HPV acquisition with age in women. However in the Syrjanen *et al*., (2005) study, HPV clearance was reported to be greater from 25 years age and older compared to HPV acquisition, HPV acquisition was higher in younger women substantiating why clearance can sometimes be associated with increasing age.

In our study the number of lifetime sexual partners and recent partners was not associated with clearing HPV infection. Lu *et al*., (2009) found that men with many lifetime sex partners had an increased (5-fold higher) likelihood of clearing HPV infection. However, these individuals are highly exposed to genital HPV resulting in the elicitation of antibody responses against HPV which could assist in HPV clearance. In our study antibody responses were not found associated with HPV clearance. In support of our results, HPV persistent infection is associated with seropositivity but not with HPV transient infection (Wideroff *et al*., 1995; Lu *et al*., 2009). Persistent HPV infection may elicit a good immune response while transient HPV infection may not. According to Trottier *et al*., (2010) HPV re-infection does not seem to be controlled by natural immunity.

It has been reported that traditional and medical circumcision differs. A high percent of traditionally circumcised men are incompletely circumcised with some foreskin still covering part of the glans; resulting in reduced protection by circumcision against HPV and other STI (Morris, 2007; Bailey *et al*., 2008). In our study 96.6% of men were circumcised and the majority of them had had a traditional circumcision, which could mean the presence of some residual foreskin. Clearance of HPV infection is reported to be 3-6 times more likely in circumcised men compared to uncircumcised men (Lu *et al*., 2009). According to Auvert *et al*., (2009), HR-HPV prevalence was significantly higher in uncircumcised men (22.3%) compared to circumcised men (14.8%, P=0.002) and these observations were not affected by age, sexual behaviour, marital status and HIV status. Tobian *et al*., (2009) also reported a higher

prevalence of HR-HPV in uncircumcised men compared to circumcised men (27.9% compared to 18%, P=0.009). Circumcision may be of great benefit in men making the penis less susceptible to HPV infections due to increased keratinized epithelium, reduced viral shedding and viral persistence compared to uncircumcised men (Lu *et al*., 2009). Monogamous women with uncircumcised male partners with ≥6 other sexual partners were found to have a 5.6 times greater risk of developing cervical cancer compared to monogamous women with circumcised male partners with ≥6 other sexual partner (Castellsague *et al*., 2002). Clinically confirmed circumcision tends to provide protection differently from traditional circumcision. The majority of the men in our study were traditionally circumcised and their circumcision was not confirmed by clinicians.

It is importantat to note that in this study median duration of HPV infection was not investigated because study participants were folowed up for 24-months at 6-month interval, therefore the data was available for only four visits after baseline visits. If the study participants were followed at 3-month interval the median duration of infection would be better. The definition for acquisition and clearence used in this study was very stringent which could result in missing some of HPV acqusisition and clearence, this should be taked as one of the limitations of the study. It is important to note that there were some study participants with 2 visits and in first visit there were HPV positive and then HPV negative in second visit. So in these study participants it was possible to confirm that it was a true HPV clearence or just a false negative.

In conclusion, findings from this study indicate that HIV infection increases the risk of HPV acquisition in women and men. HIV-positive women and men with high HIV viral load have an increased risk of HPV acquisition. HIV co-infection in women and men also decreased the ability to clear genital HPV infection in both women and men. Data from this study also indicates that the high rate of HPV transmission between sexual active couples more especially in partnerships were one or both partners are HIV-positive. Understanding the natural history of HPV in both women and men is important to arm ourselves with the knowledge of who to target for the best methods of cervical cancer prevention and other HPV-associated cancers. This report will assist in understanding HPV natural history in both women and men. Determining factors influencing genital HPV acquisition, clearance and transmission are necessary in understanding HPV natural history. Data from this study will also assist in informing HPV prevention strategies.

CHAPTER 7: SUMMARY OF FINDINGS AND CONCLUSIONS

This thesis constitutes the largest study of the genital transmission of HPV amongst heterosexual couples that has been described worldwide. To add to the importance of the study, participants were from a local community not an STI clinic. We were able to investigate the impact of the co-infection by HIV in one or both members of a partnership on the presence of HPV and the ability of participants to clear the virus. This also constitutes the first report on type-specific HPV concordance, transmission and factors associated with type-specific HPV concordance and transmission in heterosexually active couples and the impact of HIV co-infection. This is also the first report on the seroprevalence of antibodies to nine different HPV types in black South African HIV-positive and HIV-negative women and men.

Data from this project clearly indicates that HIV co-infection increases HPV prevalence, viral load and the risk of acquisition in women and men. The risk of HPV infection in men was influenced by their own HIV status and that of the female partner. However, in women HPV infections was influence by their own HIV status not that of their male partner. The three most prevalent HPV types found at baseline infecting HIV-positive men were HPV-62, -61 and -70; HIV-negative men HPV-62, -61 and -66; HIV-positive women HPV-62, -52 and -58; and HIV-negative women HPV-11, -6 and -52. It is important to note that HPV-16 was not the most dominant type in our cohort, however, it was the least cleared type in women. LR-HPV types were most dominant in our cohort. The α3 HPV species were most prevalent in women but in women with abnormal cervical cytology the HPV-16 and HPV-18, α9 and α7 HPV species were the second dominant HPV species. Worldwide α9 and α7 HPV species are the most prevalent HPV types in cervical cancer cases.

HPV-16 and -18 were not the most dominant types in our study even though they are known to be the most dominant HPV types in cervical cancer. Many cross-sectional or longitudinal studies have shown that the distribution of HPV types in a normal population does not reflect the distribution of HPV types in women with cervical cancer. Franceschi *et al*., (2005) reported that as the lesion severity (LSIL-HSIL-SCC) increases, HPV-16 also increases while other HPV types seem to decrease. In our study the majority of women had normal cervical cytology and only 2.2% had HSIL so we would not expect to find as HPV-16 the most prominent type. A total of 7/12 (58%) of women with HSIL were found to have HPV-16/18 infection. The

remaining five women were infected with HPV-31/33/45/52/58. Even though were had few women with HSIL in our study, but these findings confirm that HPV-16/18 and HPV types that are related to HPV-16 and -18 are more dominant in precancerous lesions. Therefore introducing HPV vaccines in our country will reduce the high percentage of precancerous HPV-associated lesions in South Africa.

HIV-positive individuals were found to have a higher HPV prevalence compared to HIV-negative individuals across all age groups. Importantly we noted that HIV co-infection resulted in women displaying a second peak of HPV infection 10 years earlier than uninfected women, at 36-45 years. The increase of HPV infection in HIV-negative women at >45 years of age is thought to be due to immune senescence. Clearly the reduced immune competence of HIV-positive individuals reduces their ability to clear the virus. HIV-positive women are reported to progress to cervical cancer 10 years earlier compared to HIV-negative women (Lomalisa *et al.*, 2000). These findings suggest that providing HIV-positive women with regular cervical screening will help to reduce the prevalence of cervical cancer among them.

We also demonstrated that more men than women acquired HPV but also cleared their infection. The clearance of HPV was diminished in HIV positive individuals. Women and men acquired similar LR-HPV types while they acquired different HR-HPV types. In women α3 and α9 HPV species were most acquired while in men α3 and α7 HPV species were most acquired HPV. HPV-11 was found to be the least cleared of all LR-HPV types and HPV-16 was the least cleared of all types followed by HPV-11. HIV-positive women and men with high HIV viral load have an increased risk of HPV acquisition.

Type-specific HPV concordance amongst partners was related to HIV co-infection and high HPV viral load; suggesting that more virus increases the chance of transmission. In men, type-specific HPV concordance was associated with their own HIV-positive status and that of their female partner. While in women type-specific HPV concordance was only associated with their own HIV-positive status and not that of their male partner. The risk of acquiring HPV increased when a partner was infected with the same type and in men with partners with abnormal cervical cytology. This data suggest that men with a female partner with abnormal cervical cytology are at great risk of HPV infection and possible HPV associated genital warts and cancer. It was interesting to note that HIV-discordant couples' type-specific HPV concordance

decreased with time during follow-up suggesting that they changed their sexual behaviour, by started using condoms, after counselling

HPV transmission from female to male was more common than male to female HPV transmission. Women were more likely to transmit HR-HPV to their male partners while men were more likely to transmit LR-HPV types to their female partners. We cannot conclude that women transmit more of HR-HPV and men transmit more of LR-HPV because in this study in women only the cervix was sampled. Including a vaginal sample from women might have increased the amount of LR-HPV found transmitted to men. HIV-co-infection and low CD4 counts were found associated with HPV transmission to both women and men from their sexual partners. HPV transmission was not associated with increased number of sexual act couples had a month before the visit indicating that the observed HPV transmission was the true HPV transmission not just HPV DNA deposited by the partner from previous sexual act.

More women were found to mount a serum antibody response compared to men to all nine HPV types investigated. HPV seropositivity was not significantly associated with age but HIV co-infection was associated with HPV seropositivity. Using ARVs among HIV-positive women and men was found to be associated with HPV-11 seropositivity. However, the number of study participants that were using ARVs was too small to make conclusive conclusions. The association of ARVs with HPV seropositivity still requires further investigation and the reason why the use of ARVs was not associated with seropositivity of the other HPV types is not clear. In both women and men HPV HPV-11 antibodies were most common. HPV antibodies were not associated with HPV clearance.

Data from this study indicate that South African women and men are highly exposed to numerous HPV types including HPV-6, -11, -16 or -18 which Gardasil® HPV vaccine effectively protect against, indicating that vaccines in our country will be of great benefit in reducing HPV associated cancers in women and men. South Africa has high prevalence of HIV, cervical cancer and genital warts mostly in HIV-positive individuals. Therefore introduction of vaccine in our country to both HIV-positive and HIV-negative individuals will reduce the cost used in treating the high rate of cervical cancer and genital warts. Data from this study will add considerably to the very limited data available on HPV infection and the impact of HIV co-infection especially in men and sexual active couples. Data from this study

will also help to inform the government on HPV vaccine policy as well as cervical screening policies.

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APPENDIX 1

Prof Anna-Lise Williamson HPV laboratory participated in the 2010 WHO HPV LabNet Proficiency study. In which samples were provided by WHO and I (Z.Z.A Mbulawa) performed the DNA extraction and HPV genotyping. A test was regarded as proficient in typing if it can detect 50 International Units (IU) / 5 µl of HPV 16 and HPV 18 DNA, and 500 genome equivalents (GE) / 5 µl of the other HPV types included in the panel both in samples with single and multiple plasmids. In addition, the specificity of the reported types should be >97 % (i.e. at most 1 false positive result).

Our laboratory was proficient for detection of HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66 and 68 using Roche Linear Array HPV genotyping assay.

Table 1. WHO HPV LabNet Proficiency results comparison of WHO results and our results

Panel ID	HPV type(s) in the panel	Content (IU or GE per 5µl)	Our results Roche Linear Array HPV genotyping, 50µl input volume
1	59	50	59
2	31	500	31
3	6, 16, 18, 51	500	6, 16, 18, 51
4	45	500	45
5	16	50	16
6	35, 59, 66, 68ME	500	35, 59, 66, 68;*
7	56	500	56
8	18	50	18
9	35	500	35
10	68ME	500	68
11	11, 16, 31, 33, 58	50	11, 16, 31, 33, 58;*
12	51	50	51
13	6	500	6
14	58	500	58;*
15	66	50	66
16	39, 45, 52, 56, 68[a]	500	39, 45, 52, 56
17	33	500	33; *
18	39	50	39
19	52	500	52
20	68	50	negative
21	11	500	11
22	11, 16, 31, 33, 58	500	11, 16, 31, 33, 58;*
23	16	5	16
24	56	50	56
25	33	50	33; *
26	6, 16, 18, 51	50	6, 16, 18, 51
27	Negative	0	negative
28	35	50	35; *
29	18	5	18
30	58	50	58; *
31	68ME	50	68
32	39, 45, 52, 68	50	39, 45, 52
33	6	50	6
34	45	50	45
35	68	500	negative
36	66	500	66
37	11	50	11
38	59	500	59
39	52	50	52, 70
40	35, 59, 66, 68ME	50	35, 59, 66, 68; *
41	31	50	31
42	39	500	39
43	51	500	51
A	16	25	negative
B	none	0	no isolate
C	16	2500	16

*cross reactive probe HPV52 cannot be excluded

HPV 68a cannot be detected using PGMY 09/11 based primer systems or primers targeted to other regions than L1.

BASELINE FEMALE QUESTIONNAIRE
FOR HPV COUPLES STUDY
Version 2 (from 02 Oct 2007)

Place sticker here

Use this questionnaire for
1. New recruits entering the HPV study from 02/10/07
2. All ex-PIP study participants recruited into HPV study
3. All new partners of existing participant in the HPV study

Form Number □□□-□ STUDY NUMBER_____□□□-□

Interviewer Initials □

□ **Date of Interview** **DDMMYY_____□□/□□/□□**

HIV Test Result from PIP _____1. Positive 2. Negative □

IF POSITIVE: Go to next section on Demographic characteristics

IF NEGATIVE:
HIV pretest counseling_____1. Yes 2. No □
HIV Test Result _____ _____1. Positive 2. Negative □
HIV Post test counseling_____1. Yes 2. No □

DEMOGRAPHIC CHARACTERISTICS
1. Date of Birth _____DDMMYY □□/□□/□□
2.Age (Years)_____□□

I would like to ask you a few more details about your education and employment.

3.What is the highest level of education that you have completed?_____□□

0. Did not attend school at all	7 Std 5 Grade 7
1. Sub A Grade 1	8 Std 6 Grade 8
2. Sub B Grade 2	9 Std 7 Grade 9
3. Std 1 Grade 3	10 Std 8 Grade 10
4. Std 2 Grade 4	11 Std 9 Grade 11
5 Std 3 Grade 5	12 Std 10 Grade 12
6 Std 4 Grade 6	

4. Did you have any training of a year or more after school 1. Yes 2. No_____□

IF YES:

4a. How many years at

1. **University_____ years □**
2. **Technical College/Technikon _____years □**
3. **Other, Specify _____years □**

5. Do you do work that you are paid for 1. Yes 2. No_____ _____□

IF YES:

5a.What work do you do? Specify:_____□

6. IF NO: Are you:

1. **Unemployed-looking for work**
2. **Unemployed-not looking for work**
3. **Home-maker (by choice)**
4. **Full-time student**
5. **Disabled (physically or mentally) or a pensioner (government or private civil pension/not working due to old age)**

7. What is the approximate total household income per month?

(This money could be coming from grants and donations from various sources)

R□□□□□

PARTNERSHIP CHARACTERISTICS

Now I am going to ask you some questions about your relationships.

1. Are you married to the partner that you came to the clinic with? 1. Yes 2. No_____□

2. For how long have you been having a sexual relationship with the current partner?

MONTHS □□ YEARS□□

3. Including the one at the clinic, how many regular sexual partners do you have?□□

4. In addition to you, does your partner have any other regular partners 1. Yes 2. No□

5. Do you live together with your current partner? 1. Yes 2. No_____□

SEXUAL PRACTICES

1. At what age did you have your first sexual encounter? (Age in years)_____□□

2. How many sexual partners have you had in your life? _____□□

3. How many sexual partners have you had in the past 1-year?_____□□

4. How many NEW sexual partners have you had in the past 1-year?_____□□

5. How many times have you had sex with your current partner in the past 1-month?

□□

6. Are you or your partner currently using any kind of contraception?

Yes=1 No =2 Don't know =3_____□

7. Which of the following methods of contraception are you (or your partner) <u>using currently</u>?

(Read all, 1. YES 2. NO)

Oral contraceptive pill	□
3-month injectable ('depo')	□
2-month injectable ('nuristerate')	□
Injectable but don't know which one	□
Female sterilization	□
Male condom	□
Female condom	□
Other methods	□ **Specify:**

8. Have you ever used condoms with your current partner? 1. Yes 2. No_____□

SMOKING HISTORY

1. Do you now or have you ever smoked cigarettes?

1. Never
2. Ex-smoker □

3. Present smoker

If never go to next section on STI infections

IF EX-SMOKER: (STOPPED MORE THAN ONE YEAR PREVIOUSLY)
2. How many cigarettes did you smoke a day?_____□□
3 For how long did you smoke?_____months □□ years □□

IF PRESENT SMOKER: (SMOKING SOMETIME DURING THE PAST YEAR)
4. How many cigarettes do you smoke a day?_____□□
5. For how long have you been smoking?_____months □□ years □□

SEXUALLY TRANSMITTED INFECTIONS (STI)

I would like to talk about diseases or conditions that may affect the genital area. These include: a discharge from the vagina or penis - sometimes this discharge causes itching or may be foul smelling or may cause you some worry; a sore, warts or blisters on your private parts

Have you had any of the following symptoms:

1. Vaginal discharge that has caused you some worry. 1. YES 2. NO_____□

IF YES:
1a. When was the last time it occurred: _____□
in the last week =1
more than 1 week but less than a month ago =2
more than 1 month but less than 6 months =3

more than 6 months ago =4

2. Ulcers/blisters/warts on the genitals 1. YES 2. NO_____□

IF YES:

2a. When was the last time it occurred: _____□

in the last week =1
more than 1 week but less than a month ago =2
more than1 month but less than 6 months =3
more than 6 months ago =4

To be completed if participant is HIV +

3. Are you taking anti-retrovirals (ARVs) 1. YES 2. NO___ _____□

IF YES:

3a. What are the names of the ARVS that you are taking

_____□

PREGNANCY HISTORY

I am now going to ask you about pregnancies.

1. Have you ever been pregnant? 1. YES 2. NO_____ _____□

If no → go to next section on Pap smears

1a. IF YES: How many live children do you have?_____ □□

2. Have any of your children died 1. YES 2. NO_____□

IF YES:

2a. How many_____□

3. Have you had any stillborn infants? 1. YES 2. NO_____□

IF YES:

3a. How many_____□

4. Were any of these babies born by Caesarian section? 1. YES 2. NO_____□

IF YES:

4a. How many_____□

5. Have you had any miscarriages? 1. YES 2. NO_____ □
(abortions/ectopic pregnancies)

IF YES:

5a. How many_____□

PAP/CERVICAL SMEARS

I am now going to talk about PAP smears

Do you know what a Pap smear is? Could you describe this for me?

If the person fully understands don't give an explanation again.

Otherwise say, "Let me go over it again" and then give explanation*:*

It is a test to detect abnormal cells in the mouth of the womb that could lead to cancer.

When performing this test the doctor or nurse places an instrument called a speculum (spoon) in the woman's vagina so that he/she can see the mouth of the womb and the test is done.

then interviewer to ask:

Did you ever have this test done? 1. YES 2. NO 9.UNKNOWN _____□

If No: go to Locator information sheet

IF YES:

2. How many times have you ever had a PAP?_____□□

3. Age at first?_____ _____ _Years□□

4. Age at last?_____ Years □□

5. Did you ever get the result of any of your PAP smears/tests? 1. YES 2. NO_____□

IF YES:

5a. What were you told:_____ □

6. **Have you ever been told that there was something wrong with the mouth of your womb? 1. YES 2. NO_____□**

IF YES:

6a. What were you told:_____□

Complete Locator information sheet (back page)

EXAMINATION AND SPECIMEN CHECK LIST

Form Number____□□□-□
DYAD STUDY NUMBER__□□□-□

1. Pelvic Examination

Symptoms	Present/	Absent	Comment	Code
Warts				
Discharge				
Other observations				

2. Specimens for TESTS - tick when completed

WOMEN	
Cytobrush for PAP smear	□
Cytobrush for HPV	□
Buccal swab	□
Orasure test	□
Blood: 1 Red top test tube	□
For HIV +: 2 Purple top test tubes	□

INFORMATION FOR FOLLOW-UP
(KEEP SEPARATE FOR REASONS OF CONFIDENTIALITY)

Form Number _____□□□-□
DYAD STUDY NUMBER □□□-□

Participants Name: _____

Participants Address: _____
_____
_____
_____
□□□□

Patient's Tel no *(□□□)-□□□-□□□□*

Cell phone *(□□□)-□□□-□□□□*

Information on neighbour, family member or close friend (For follow up purposes)

Name: _____

Address: _____
_____
_____
_____
□□□□

Tel no *(□□□)-□□□-□□□□*

Cell phone **(□□□)-□□□-□□□□**

Date of Follow up visit_____DD/MM/YY □□/□□/□□

BASELINE MALE QUESTIONNAIRE
FOR HPV COUPLES STUDY
Version 2 (from 02 Oct 2007)

Use this questionnaire for
1. New recruits entering the HPV study from 02/10/07
2. All ex-PIP study participants recruited into HPV study
3. All new partners of existing participant in the HPV study

Place sticker here

Form Number □□□-□

STUDY NUMBER_____□□□-□

Interviewer Initials □

□ **Date of Interview** **DDMMYY_____**□□/□□/□□

HIV Test Result from PIP _____1. Positive 2. Negative □

IF POSITIVE: Go to next section on Demographic characteristics

IF NEGATIVE:
HIV pretest counseling_____1. Yes 2. No □
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DEMOGRAPHIC CHARACTERISTICS
1. Date of Birth _____DDMMYY □□/□□/□□
2.Age (Years)_____□□

I would like to ask you a few more details about your education and employment.

3.What is the highest level of education that you have completed?_____□□

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1. Sub A Grade 1	8 Std 6 Grade 8
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5 Std 3 Grade 5	12 Std 10 Grade 12
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4. Did you have any training of a year or more after school 1. Yes 2. No_____□

IF YES:
4a. How many years at
1. University_____ years □
2. Technical College/Technikon _____years □
3. Other, Specify _____years □
5. Do you do work that you are paid for 1. Yes 2. No_____ _____□

IF YES:
5a.What work do you do? Specify:_____□

6. IF NO: Are you:

1. Unemployed-looking for work
2. Unemployed-not looking for work
3. Home-maker (by choice)
4. Full-time student
5. Disabled (physically or mentally) or a pensioner (government or private civil pension/not working due to old age)

7. What is the approximate total household income per month?

(This money could be coming from grants and donations from various sources)

R□□□□□

PARTNERSHIP CHARACTERISTICS

Now I am going to ask you some questions about your relationships.

1. Are you married to the partner that you came to the clinic with? 1. Yes 2. No_____□

2. For how long have you been having a sexual relationship with the current partner?
MONTHS □□ YEARS□□

3. Including the one at the clinic, how many regular sexual partners do you have?□□
4. In addition to you, does your partner have any other regular partners 1. Yes 2. No□
5. Do you live together with your current partner? 1. Yes 2. No_____□

SEXUAL PRACTICES

1. At what age did you have your first sexual encounter? (Age in years)_____□□
2. How many sexual partners have you had in your life? _____□□
3. How many sexual partners have you had in the past 1-year?_____□□
4. How many NEW sexual partners have you had in the past 1-year?_____□□
5. How many times have you had sex with your current partner in the past 1-month?
□□
6. Are you or your partner currently using any kind of contraception?
Yes=1 No =2 Don't know =3_____□

7. Which of the following methods of contraception are you (or your partner) <u>using currently</u>?
(Read all, 1. YES 2. NO)

Oral contraceptive pill	□
3-month injectable ('depo')	□
2-month injectable ('nuristerate')	□
Injectable but don't know which one	□
Female sterilization	□
Male condom	□
Female condom	□
Other methods	□ **Specify:**

8. Have you ever used condoms with your current partner? 1. Yes 2. No_____□
9. Are you circumsized? 1. Yes 2. No_____□

SMOKING HISTORY
1. Do you now or have you ever smoked cigarettes?
1. Never
2. Ex-smoker □
3. Present smoker

If never go to next section on STI infections

IF EX-SMOKER: (STOPPED MORE THAN ONE YEAR PREVIOUSLY)
2. How many cigarettes did you smoke a day?_____□□
3 For how long did you smoke?_____months □□ years □□

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SEXUALLY TRANSMITTED INFECTIONS (STI)
I would like to talk about diseases or conditions that may affect the genital area. These include: a discharge from the vagina or penis - sometimes this discharge causes itching or may be foul smelling or may cause you some worry; a sore, warts or blisters on your private parts

Have you had any of the following symptoms:

1. Penile discharge that has caused you some worry. 1. YES 2. NO____□
IF YES:
1a. When was the last time it occurred: _____□
in the last week =1
more than 1 week but less than a month ago =2
more than 1 month but less than 6 months =3
more than 6 months ago =4

2. Ulcers/blisters/warts on the genitals 1. YES 2. NO_____□

IF YES:
2a. When was the last time it occurred: _____□
in the last week =1
more than 1 week but less than a month ago =2
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To be completed if participant is HIV +
3. Are you taking anti-retrovirals (ARVs) 1. YES 2. NO___ _____□

IF YES:
3a. What are the names of the ARVS that you are taking
_____□

Complete Locator information sheet (back page

EXAMINATION AND SPECIMEN CHECK LIST

Form Number____□□□-□
DYAD STUDY NUMBER__□□□-□

1. Pelvic Examination

Symptoms	Present/	Absent	Comment	Code
Warts				
Discharge				
Other observations				

2. Specimens for TESTS - tick when completed

MEN	
Penile swab for HPV	□
Buccal swab	□
Orasure test	□
Blood: 1 Red top test tube	□
For HIV +: 2 Purple top test tubes	□