NATURAL HISTORY OF HUMAN PAPILLOMAVIRUS INFECTION AMONG YOUNG MEN FROM KISUMU, KENYA

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2010

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ABSTRACT
Danielle M. Backes: Natural history of human papillomavirus infection among young men from Kisumu, Kenya
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Human papillomavirus (HPV) infection is necessary for the development of invasive cervical cancer among women and is a risk factor of other anogenital cancers among women and men. Among men, data on the natural history of HPV infection and on risk factors of HPV-associated penile lesions are limited.

An HPV-ancillary study, nested within a randomized controlled trial (RCT) of male circumcision, was conducted in Kisumu, Kenya from 2002-2007. All participants were human immunodeficiency virus seronegative, uncircumcised and aged 17-24 years at baseline. Penile exfoliated cell specimens were collected at baseline, 12 and 24-month visits from the glans/coronal sulcus and shaft of participating men and tested using GP5+/6+ polymerase chain reaction. An additional visual inspection exam with acetic acid was conducted at the 24-month visit to determine the presence of colposcopy-detected penile lesions.

For incidence and persistence analyses, 949 participants enrolled in the control arm of the main RCT were included. Median follow-up time was 24.2 months (range 11.6-30.4). The incidence of any HPV infection was 24.3/1,000 person-months. A total of 18.3% (95% confidence interval [CI]: 15.0-22.3) of incident HPV infections in the glans persisted from the 12 to 24-month visits and 13.5% (95% CI: 11.1-16.3) of prevalent HPV infections in the glans persisted from baseline until at

least the 12-month visit. HPV clearance was similar for high-risk versus low-risk infections and by age group.

Of 267 men participating in visual inspection exams from both arms of the RCT, 143 were circumcised and 124 uncircumcised. Circumcised men had a lower prevalence of flat penile lesions (0.7%) versus uncircumcised men (26.0%); age-adjusted odds ratio (OR)=0.02; 95% CI: 0.003-0.1. Men with flat lesions had increased odds of HPV DNA positivity and high HPV16/18/31 viral load compared to men without flat lesions.

The natural history of HPV infection among men may be different than that previously described for women, with a relatively high incidence of penile HPV infection found among men. Differences in patterns of high and low-risk HPV clearance between men and women may also exist. This study suggests male circumcision reduces the prevalence of HPV-associated flat penile lesions and may reduce HPV transmission between sexual partners.

ACKNOWLEDGEMENTS

I would first like to thank my advisor Jennifer Smith for her invaluable mentorship over the past five years. She has given me the tools to become a successful and efficient researcher and both her encouragement and strong work ethic have been very inspiring. I would also like to sincerely thank Michael Hudgens for his statistical expertise, dedication to this project and frequent accessibility when I needed advice. I am also very grateful to Bob Millikan, Steve Meshnick and Dirk Dittmer for their guidance and helpful comments throughout my entire dissertation process.

I am thankful to all of my study collaborators including Bob Bailey, Stephen Moses, Peter Snijders and Chris Meijer for all of their work on this project. Additionally, I would like to acknowledge the young men who participated in this study and the clinic staff in Kisumu, without whom this research would not have been possible.

The main randomized controlled trial was funded by the National Institutes of Health (NIH) AI50440 and the Canadian Institutes of Health Research HCT44180. The human papillomavirus study was funded by NIH R01 CA114773-04. I am also grateful for the NIH cancer epidemiology pre-doctoral training grant, which supported my work on this project.

Finally, I would like to thank my parents, family and friends for all of their support over the years and my husband for his unconditional love and encouragement when I needed it most.

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LIST OF ABBREVIATIONS

CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
GEE	Generalized estimating equation
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV-2	Herpes simplex virus type 2
ICC	Invasive cervical cancer
IR	Incidence rate
OR	Odds ratio
PCR	Polymerase chain reaction
RCT	Randomized controlled trial
RLB	Reverse line blot
STI	Sexually transmitted infection
UNIM	Universities of Nairobi, Illinois and Manitoba
VIA	Visual inspection with acetic acid

CHAPTER 1.
STATEMENT OF SPECIFIC AIMS

Human papillomavirus (HPV) infection is a widespread sexually transmitted infection (STI) and the main etiologic cause of invasive cervical cancer (ICC) among women (1). HPV infection is also considered an important risk factor in non-cervical cancers including anal and penile cancers in men (2-4). While numerous studies have investigated HPV infection among women, data on the natural history of HPV infection are limited in men, especially from less developed regions including Africa (5). Data are also needed on risk factors of HPV-associated penile lesions.

An HPV-ancillary study was nested within a randomized controlled trial (RCT) that began in Kisumu, Kenya in February 2002 with the primary aim of determining the effect of male circumcision on human immunodeficiency virus (HIV) incidence (6). All men participating in the main RCT were invited to participate in the HPV study, which involved testing penile exfoliated cell specimens collected from two anatomical sites (the glans/coronal sulcus and shaft) from men at the baseline, 12 and 24-month visits. A sensitive GP5+/6+ polymerase chain reaction (PCR) assay was used to detect a wide range of individual HPV types. An incident or acquired infection was defined as the detection of an HPV type at the 12 or 24-month follow-up visits not detected at baseline. Persistence of incident HPV infection (i.e. incident persistence) was defined as repeat positivity for a type-specific HPV infection at the 12 and 24-month follow-up visits that was not detected at baseline. Persistence of a

prevalent HPV infection (i.e. prevalent persistence) was defined as repeat positivity at two or more consecutive visits for a type-specific HPV infection detected at baseline. An additional clinical examination (a visual inspection of the penis with 3% acetic acid [VIA] and aided magnification), was conducted at the 24-month visit to determine the presence of colposcopy-detected penile lesions (flat lesions, condyloma acuminata, papular lesions, and pearly penile papules) among 267 consenting participants.

With the data collected from this study, the following research aims were addressed:

Specific Aim 1: To investigate the natural history of HPV infection (incidence, incident persistence, prevalent persistence/clearance) in a cohort of uncircumcised Kenyan men aged 17-24.

Hypothesis: The 24-month incidence rate (IR) for the first overall HPV type detected will be within the range of 18-35/1,000 person-months. The overall type-specific persistence over 12 months of both incident and prevalent HPV infections will range between 20-40%.

Rationale: Few studies have been published on the incidence and persistence of HPV infections among men. These data are important given that men are an important factor for increased risk of HPV infection and ICC in their female sexual partners (7), and since HPV infection is associated with penile and anal cancers among men (2;3). Data on the natural history of HPV infection among men

are especially needed from less developed regions, including sub-Saharan Africa where the burden of ICC is among the highest worldwide (8;9).

Specific Aim 2: To determine the association between penile lesions and HPV DNA positivity, HPV viral load and socio-demographic risk factors of ascertained HPV-associated penile lesions.

Hypothesis: Lack of circumcision, higher HPV viral load, lack of condom use and a higher number of sexual partners are risk factors of HPV-associated penile lesions among Kenyan men.

Rationale: Interventions that reduce HPV-associated penile lesions could be important to both men and women, since HPV-associated penile lesions in men may increase the risk of HPV transmission to their sexual partners (10). Risk factor data on HPV-associated penile lesions are needed, especially from less-developed regions with a high burden of HPV-associated cancers. To my knowledge this is the first study to investigate whether circumcision reduces the prevalence of flat penile lesions and the first to assess multiple risk factors of HPV-associated penile lesions among men from sub-Saharan Africa.

CHAPTER 2.
BACKGROUND AND SIGNIFICANCE

HPV and Anogenital Cancers

Cervical cancer is the second most common cancer among women worldwide with an estimated incidence of 493,000 new cases each year and the leading cause of cancer related deaths among women in less-developed countries (8;9). The burden of ICC is particularly high in Eastern Africa, which has the highest annual, age-standardized IR of ICC worldwide (42.7/100,000 women/year) (8;9).

It is now well established that genital HPV infection, a common STI, is a necessary cause for the development of ICC as HPV infection has been identified in almost all biopsy specimens of ICC using sensitive PCR assays (11). Approximately 40 different types of HPV have been associated with anogenital infection, of which 13-15 are classified as oncogenic (i.e. high-risk) types due to their association with ICC (e.g. HPV16 and 18) (12;13). HPV16 and 18 have been found in approximately 60-70% of cervical cancers worldwide and therefore, appear to be the predominant oncogenic types (12). Other HPV types (e.g. HPV6 and 11) are considered non-oncogenic (i.e. low-risk) since they are rarely found in invasive cervical disease (12;14). The prevalence of HPV infection in Kenya has been reported between 17-43% among women 19-55 years of age attending family planning clinics in Nairobi (15;16) and 51%-58% among men aged 17-63 in Kisumu (17;18).

HPV infection is also associated with other female anogenital cancers as HPV DNA has been detected in approximately 40%, 66% and 87% of invasive vulvar, vaginal and anal cancers in women, respectively (3;19). HPV also appears to play a role in both anal and penile cancers in men. HPV DNA has been detected in approximately half (48%) of penile cancers and 77% of anal cancers among men worldwide (2;3).

The Male Role in the Etiology of Cervical Cancer and in HPV Transmission

Men were considered to have an important role in the etiology of cervical cancer even before HPV infection was identified as its central cause (20). As early as 1976, an increased risk of cervical cancer was found among wives of men who were previously married to women with cervical cancer (21). Numerous studies have also shown that a woman's risk of cervical cancer increased with her husband's reported number of lifetime sex partners (22;23) and if he had premarital or extramarital sexual relationships (24). Further, a husband's sexual history was found to be a risk factor of his wife's cervical disease even after adjusting for both her reported number of sex partners and age at first intercourse (25).

Studies investigating HPV prevalence in men among heterosexual couples further supported the importance of the men in the etiology of cervical cancer and their potential to transmit HPV infection to their female partners. Numerous studies have reported a higher prevalence of HPV among husbands of women with cervical cancer compared to those of women without cervical cancer or cervical intraepithelial neoplasia (CIN) (26-28). A wide range of HPV type concordance

among HPV-positive couples have been reported in the literature ranging from 22.7% to 100% (10). HPV type concordance was higher than expected by chance in a study of 50 couples attending an STI clinic (29) and in a study of 238 heterosexual couples where the female partner had CIN (30), providing indirect evidence of HPV transmission among sexual partners. Higher HPV viral loads have also been found among HPV concordant men compared to those that were not HPV concordant with their female partners (30).

Ascertainment of Penile HPV DNA

While many studies have been conducted to characterize HPV infection among women, data on HPV infection in men are more limited. Initial studies of HPV infection in men used aceto-white penile lesions detected by visual inspection as a diagnostic marker for HPV infection (4). However, because of the poor specificity of peniscopy for HPV detection, exfoliated cells or biopsy specimens and molecular techniques such as PCR are strongly recommended in order to accurately assess HPV infection in men (4).

In a systematic review, Dunne et al (5) summarized studies of HPV prevalence in men that used sensitive PCR or hybrid capture assays and were published between 1990-2006. There was a wide range of reported estimates for HPV prevalence among study populations ranging from 1.3% among university students in Japan (31) to 72.9% among male partners of women with CIN in the Netherlands (30). While some of the differences in prevalence estimates can likely be attributed to differences in study populations, it has also been suggested that a

large part of the variability in HPV prevalence estimates is due to incomplete specimen sampling (32).

In a study that included a wide range of anatomical sites for specimen sampling (penile shaft/prepuce, glans/coronal sulcus, scrotum, perianal, anal canal, urethra and semen), HPV prevalence was highest in the penile shaft and glans/coronal sulcus (49.9% and 35.8%, respectively) (32). The authors recommended that the shaft and glans/coronal sulcus should be the minimum sites sampled when estimating HPV prevalence in men. Sampling from the scrotum, perianal or anal canal was also suggested for optimal penile HPV detection. A small decrease in HPV positivity was found by excluding the scrotal, perianal and anal canal samples (2.1%, 2.6% and 3.2%, respectively). There was no decrease in HPV positivity by excluding urethra and semen samples over that found when sampling all other sites.

In another study that evaluated genital sites for HPV DNA detection in men (33), the following sites were recommended for HPV sampling: penile shaft, glans, coronal sulcus, scrotum and urine. Sampling from the scrotum and urine however, did not increase sensitivity for uncircumcised men. Among circumcised men, a small percentage of men had HPV DNA detected in the scrotal or urine sample (2.6% at each site) but not at the shaft or glans. Semen sampling did not increase the sensitivity of sampling for HPV DNA.

Other studies are consistent with the findings that a sample from the urethra does not substantially increase the sensitivity of HPV DNA detection (34;35). Urethral sampling is often painful and could likely decrease participation, especially

in follow-up studies. Thus, it has been suggested that urethral sampling is not necessary for inclusion in large studies of HPV detection in men (35).

Natural History of HPV Infection in Men

Seven longitudinal studies (N>20) have been conducted to assess the natural history of HPV infection in men (36-42) and two reports from a RCT of HPV and male circumcision conducted in Rakai, Uganda provide HPV natural history data for men participating in the control arm of the trial (43;44) (Table 2.1). The IR of HPV infections for the first overall type detected has been found to range from 17.9/1,000 person-months among military men from Mexico (37) to 34.9/1,000 person-months among male university students in the United States (39). Type-specific persistence of HPV infection over one year has been found to range between 25-30% among HPV-positive men (36;37). Two previous studies reported a median time to HPV clearance ranging from 3.6-5.9 months (36;42), while one previous study reported a clearance rate of 19.1/1,000 person-months (43).

The same IR of 29.4/1,000 person-months for HPV infection was reported for 290 men from Tucson, Arizona (36) and among 331 women from the same city using a similar study design and method of HPV detection (45). While the 12-month cumulative incidence for acquiring high and low-risk HPV infections were similar among men (0.19 and 0.16, respectively), women were more likely to acquire a high-risk than low-risk infection (0.32 vs. 0.18, respectively). A comparable median time to clearance among men for high and low-risk HPV types was observed (5.8 and 6.0 months, respectively) in comparison with a higher median time to clearance for high-

risk types (9.8 months) compared to low-risk types (4.3 months) among women. In contrast, Lajous et al (37) and Kjaer et al (38) found a higher risk of persistence for high versus low-risk HPV types among men, which is consistent with natural history studies among women (46-48).

Partridge et al reported a 24-month cumulative incidence of 62.4% (95% confidence interval [CI]: 52.6-72.2) among a cohort of 240 male university students (39). This estimate was higher than the corresponding 24-month cumulative incidence found among a cohort of 553 women at the same university (38.8% [95% CI: 33.3-45.0]) (49). The 12-month cumulative incidence among men in this study (35%) (39) was slightly higher than that reported by Giuliano et al (35%) (36).

Four previous studies have investigated multiple risk factors of HPV incidence or persistence in men (37-39;50). Risk factors of incident HPV infection include past smoking (39), recent and lifetime number of sex partners (38;39;50), high socio-economic status (37), anal intercourse with men (37), and condom use (38). Risk factors associated with persistent HPV infection were lack of male circumcision (37;50) and multiple HPV types detected at baseline (37;38).

Additionally, three recent studies investigated the effect of male circumcision on the incidence and clearance of HPV infection (42-44). In a RCT of HPV and male circumcision conducted in Rakai, Uganda, circumcision increased the acquisition rate and decreased the clearance rate of HPV infection among men who were HIV-negative at enrolment (43), although no difference in HPV acquisition or clearance was found by circumcision status among men HIV-positive at enrolment (44).

Hernandez et al (42) also found reduced clearance of HPV infection among uncircumcised men compared to circumcised men in an observational study.

Penile Lesions

Definitions of penile lesions

Flat penile lesions are defined as flat or slightly elevated, well demarcated, aceto-white lesions and are considered a manifestation of productive high-risk HPV infection in men (Table 2.2) (10;51). These lesions are often referred to as subclinical lesions since they are only visible after aceto-white staining. The application of acetic acid can be use to distinguish flat lesions from other conditions that are visible and often white in color without the use of acetic acid, such as psoriasis or yeast infections (10). Condyloma acuminata, commonly known as genital warts, are elevated lesions with an irregular surface, visible without acetic acid application and associated with low-risk HPV infection (4;51). Papular lesions are small elevated papules with a smooth surface usually located near the frenulum, while pearly penile papules are small exophytic lesions located around the corona of the glans penis (51;52). Papular lesions and pearly penile papules are visible without application of 3% acetic acid and have not been previously associated with HPV infection (Table 2.2).

Prevalence of penile lesions

Penile lesions are frequently seen in male sex partners of women with CIN (51;53-55) and a higher prevalence of aceto-white lesions has been found among

male partners of women with CIN compared to women without cervical disease (53;54;56). The prevalence of flat lesions and condyloma acuminata among male partners of women with CIN has been found to range between 29-60% and 5-24%, respectively (10). Among male partners of women without known HPV infection, the prevalence of penile lesions was lower, ranging between 4-36% for flat lesions and 0-6% for condyloma (10). Flat lesions have also been found to be larger in size among male partners of women with CIN compared to a hospital population with no known STIs (56).

HPV and penile lesions

In a study of 175 male sex partners of women with CIN, HPV prevalence was higher in penile exfoliated cells among men with penile lesions (flat, papular, or condyloma acuminata) compared to those without lesions (67 vs. 37%, respectively) (51). HPV infection was associated with flat lesions as HPV DNA was detected in 41 (72%) of 57 flat lesions compared with 16 (25%) flat lesions that were negative for HPV DNA. Of the HPV-positive flat lesions, the majority (93%) contained high-risk HPV DNA. Higher HPV viral loads were also associated with the presence of flat lesions (56). Thus, male sex partners may be a 'reservoir' for high-risk HPV infection and flat penile lesions are likely an important factor in the spread of HPV infection between couples (10).

Further, condom use was associated with the median time to regression of penile lesions (hazard ratio=2.1, 95% CI: 1.2-3.7 for condom use vs. non-use), especially among HPV concordant couples (hazard ratio=2.63, 95% CI: 1.07-6.48)

(57;58). Condom use, therefore, may prevent the spread of HPV between partners, and re-infection with the same HPV type may increase the risk of penile lesion development (10).

More than 90% of genital warts are associated with low-risk HPV types 6 and 11 while high-risk HPV types are rarely detected (4). This is consistent with three studies of penile lesions which reported an HPV6 or 11 prevalence of between 80-100% among HPV-positive genital warts (53;59;60). HPV infection does not appear to be associated with papular lesions as HPV was detected in 5 (62%) of the 8 ascertained papular lesions in a study of 175 men (51). These results are consistent with an earlier study of 270 men which did not find an association between HPV DNA and papular lesions (59), although the number of ascertained papular lesions in both studies was small. Pearly penile papules are also not considered to be related to HPV infection based on a study of 226 male partners of women with CIN where the prevalence of pearly penile papules was not associated with HPV positivity (52). These results were consistent with a previous study that failed to detect HPV DNA in any of the 13 biopsy specimens of pearly penile papules tested by PCR (61).

Table 2.1 Characteristics and findings of natural history studies of human papillomavirus (HPV) in men.

Reference	Study location	Study population	Study size	PCR primer	Sites sampled	Median follow-up, months (range)	HPV type	Incident HPV %*	Cumulative Incidence	Incidence rate/ 1000 person-months	Persistent HPV %†	Median time to clearance (months)
Low-risk populations												
Hernandez 2010 (42)	HI, US	University population	357	PGMY09 /11	glans, coronal sulcus, shaft, scrotum	14.2 (1.2-41.5)	Any	NS	NS	NS	NS	3.6-3.9
Gray 2010 (43)	Uganda	Control arm RCT participants	399	MY09/11 +HMB01	Glans, coronal sulcus	24 (NS)	HR	NS	NS	24.5	NS	19.1‡
							16	NS	NS	4.0	NS	20.1‡
Giuliano 2008 (36)	AZ, US	AZ residents	290	MY09/11	glans, coronal sulcus, shaft, scrotum	15.5 (3.7-24.7)	Any	32.5	29.2 (12 m)	29.4	25.5 (12m)	5.9
							HR	18.2	19	15.5	19.0	5.8
							LR	18.1	16	15.4	29.3	6.0
							16	6.0		4.8		
Partridge 2007 (39)	WA, US	University students	240	MY09/11 +HMB01	glans, shaft, scrotum	12.9 (NS)	Any	41.4	62.4 (24 m)	34.9	49.2	NS
							HR	30.7	47.9	24.7	NS	NS
							LR	28.1	46.6	22.8	NS	NS
							16	13.2	19.5	10.0	NS	NS
Lajous 2005 (37)	Mexico	Military men	336	BGH 20, BPCO4	coronal sulcus, shaft, scrotum, urethral meatus, urethra	12 (NS)	Any	21.4	NS	17.9	29.4	NS
							HR	14.3	NS	11.9	31.0	NS
							LR	12.7	NS	10.6	23.1	NS
							16	2.8	NS	2.3	31.3	NS
Kjaer 2005 (38)	Denmark	Male conscripts	250	Gp5+6+	glans, coronal sulcus	6.6 (mean) (5.4-7.8)	Any	13.8	NS	NS	57.5	NS
							non-16 HR	NS	NS	NS	56	NS
							LR	NS	NS	NS	35	NS
							16	2.6	NS	NS	63	NS

Table 2.1 continued

Reference	Study location	Study population	Study size	PCR primer	Sites sampled	Median follow-up, months (range)	HPV type	Incidence %*	Cumulative Incidence	Incidence rate/ 1000 person-months	Persistent %†	Median time to clearance (months)
Low-risk populations continued												
de Sanjose 2003 (62)	Spain, Columbia	Husbands of study participants	14	PMY 09/11 PCR L1	urethra	108 (84-132)	Any	NS	NS	NS	14.3	NS
High-risk populations												
Serwadda 2010 (44)	Uganda	HIV+ control arm RCT participants	107	MY09/11 +HMB01	Glans, coronal sulcus	NS	Any	57.0	NS	NS	29.0	NS
							16	14.9	NS	NS	36.8	NS
Wikstrom 2000 (40)	Sweden	STD clinic attendees	88	GP5+6+	glans, coronal sulcus	3.5 (0.5-16)	Any	22.4	NS	NS	50	NS
Van Doornum 1994 (41)	the Netherlands	STD clinic attendees	48	TS	coronal sulcus, urethra, anus, rectum	16.4 (NS)	Any	42.4	NS	42**	20††	NS

NS: not specified; RCT: randomized controlled trial; HR: high-risk; LR: low-risk; HIV: human immunodeficiency virus; STD: sexually transmitted disease; TS: type-specific

* Percentage of men who were HPV negative (for specified HPV type) at baseline

† Percentage of HPV-positive (for specified HPV type/group) men at baseline

‡ HPV clearance rate per 1,000 person-months

** Numerator is newly acquired infections (>1 infection/man is possible)

†† Percentage of HPV infections (> 1 infection per man is possible)

Table 2.2 Description and characteristics of different types of penile lesions (51;52)

Type of Lesion	Description	Associated with HPV infection?	Visible without acetic acid application?
Flat lesions	Flat or slightly elevated, well demarcated, aceto-white lesions	Yes	No
Condyloma acuminata (i.e. genital warts)	Elevated lesions with an irregular surface	Yes	Yes
Papular Lesions	Small exophytic papules with a smooth surface usually located near the frenulum	No	Yes
Pearly Penile Papules	Small exophytic papules located around corona of the glans penis	No	Yes

CHAPTER 3.
METHODS

An HPV-ancillary study was nested within a RCT of male circumcision conducted in Kisumu, Kenya (6). Men enrolled in the main RCT were invited to participate in the nested HPV study which involved testing penile exfoliated cell specimens collected at baseline (i.e. randomization visit), and the 12 and 24-month follow-up visits for HPV DNA. RCT participants consented to the HPV study by checking a box on the main RCT consent form.

Men from both arms of the main RCT were also invited to participate in a visual inspection examination with 3% acetic acid at the 24-month visit in order to determine the prevalence of colposcopy-detected penile lesions (e.g. HPV-associated flat lesions, papular lesions). This was an additional exam added on to the main RCT protocol and required a separate informed consent form to be administered at the 24-month visit.

RCT of Male Circumcision for HIV Prevention: Bailey et al, 2007

A RCT with the primary aim of determining the effect of male circumcision on HIV incidence was conducted in Kisumu, Kenya beginning in February 2002 (6). The eligibility criteria for the main trial are listed in Table 3.1.

Recruitment process and screening visit

Study subjects were recruited and enrolled in the study from February 4, 2002 until September 6, 2005. Subjects were recruited from multiple places including STI clinics, workplaces and community organizations. Free transportation was provided to the study clinic. Compensation for time and general health care and treatment were also provided to all recruited subjects. At the screening visit, the study was explained in more detail, HIV testing and counseling was provided, blood hemoglobin levels were tested and a brief examination was conducted to assess circumcision status. Any subject that was HIV negative and had a blood hemoglobin ≥90 g/L was invited to enroll in the study and given a consent form to take with them and read in detail.

Randomization visit

During the randomization, or baseline visit, a trained counselor reviewed the informed consent with the subject in detail and consenting participants had blood drawn, a medical history taken, a medical exam and an interview to obtain socio-demographic and health information. Urine samples were tested for *N. gonorrhea* and *C. trachomatis* infections by PCR-based methods (Roche Diagnostics). Sera were tested for herpes simplex virus type-2 (HSV-2) antibodies using a type-specific enzyme-linked immunosorbent assay (ELISA) (Kalon). Serum specimens were also tested for HIV antibody using two rapid tests (Determine, Abbott Diagnostic Division, Hoofddorp, the Netherlands; Trinity Biotech, Wicklow, Ireland) and confirmed by double ELISA (Adaltis Inc, Montreal, Canada; Trinity Biotech, Wicklow, Ireland) at the University of Nairobi.

A total of 2,784 participants met the eligibility criteria and were randomly assigned by block randomization to the intervention group (i.e. immediate circumcision) and were circumcised (n=1391), or to the control group (i.e. delayed circumcision; n=1393). Circumcision was performed on the same day or within a few days of the baseline visit. Circumcised men were counseled to abstain from sex for 30 days after surgery due to increased risk of HIV and STIs through their open wound. They were also checked for complications at 3 days, 8 days and one month after the circumcision procedure. Both circumcised and uncircumcised participants were provided with HIV testing and counseling one and three months after randomization.

Follow-up visits

Study participants were followed up at approximately 6, 12, 18 and 24 months after randomization. At each follow-up visit, a behavioral risk assessment, HIV testing and counseling, STI testing and treatment, and counseling in HIV and STI prevention were conducted according to the same protocol used at the baseline visit. Men were also allowed to come to the clinic for an unscheduled medical visit at any time during follow-up if needed and all men were offered circumcision at the end of (24-month) follow-up.

Design of the Nested HPV Study

Population

Men enrolling in the main RCT of HIV and male circumcision (6) constituted the source population of the nested HPV study.

There was one additional eligibility criterion for the HPV study:

- Willing and able to give informed consent for the collection of penile exfoliated cell specimens at the baseline, 12 and 24-month visits and for the shipment of these specimens to Amsterdam for HPV testing and storage.

There was one additional eligibility criterion for the VIA sub-study:

- Willing and able to give informed consent for the VIA examination at the 24-month visit.

Collection of penile exfoliated cell specimens

Penile exfoliated cell specimens were collected by a trained physician or clinical officer working as part of the main RCT at the randomization visit prior to circumcision (i.e. baseline) and the 12 and 24-month follow-up visits. Specimens were collected from two different anatomical sites: i. the penile shaft and external foreskin (i.e. shaft specimen) and ii. the glans, coronal sulcus, and the internal tissue of the foreskin (i.e. the glans specimen).

Two sterile 15 ml centrifuge tubes with 2-mL of 0.01 mol/L Tris-HCl 7.4 pH buffer were prepared for each patient and labeled with the participant's ID number, the visit number and type of specimen (glans or shaft). A pre-wetted type 3 Dacron swab was used to collect exfoliated cells for the shaft specimen and then another

separate pre-wetted swab was used to collect exfoliated cells for the glans specimen (18).

For the shaft specimen, exfoliated cells were collected by rubbing the four sides of the external shaft tissue from the proximal to distal penile shaft with sufficient pressure. The shaft specimen also included a sample from the external surface of the foreskin for uncircumcised men.

For the glans specimen, exfoliated cells were collected by swabbing the tip of the urethral opening (completely circling around the urethral orifice 2-3 times). Using the same pre-wetted swab, a sample was taken from the glans (by sampling back and forth from the top to the bottom in a circular motion) and then from the coronal sulcus (by rotating the swab three times around its circumference). For uncircumcised men, the prepuce was gently retracted before sampling and a sample from the inner foreskin tissue was also included.

Processing of collected specimens

On the same day as sample collection, each swab was placed in the previously labeled centrifuge tubes (a different tube for each specimen), processed and centrifuged at high speed for 10 minutes in the clinic laboratory (18). The supernatant was discarded using a Pasteur pipette, and the pellet was resuspended in the same volume of 0.01mol/L of Tris-HCl buffer and vortexed. Diluted cell pellets were frozen and stored at -75°C. All samples were transported via Fedex in a dry shipper (with permission from the Kenyan Ministry of Health) from Kisumu to the Department of Pathology at the VU Medical Center in Amsterdam, the Netherlands for HPV DNA testing.

HPV DNA testing

DNA was isolated from penile exfoliated cell samples using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. In order to assess the quality of the DNA samples, the penile exfoliated cell pellets were evaluated for the presence of human DNA by beta-globin specific PCR using $BGPCO_3$ and $BGPCO_5$ primers (63). An initial 4 minute denaturation step at 94˚C occurred followed by 40 PCR amplification cycles. Each cycle consisted of a 1 minute denaturation step at 94˚C, a 2 minute primer annealing step at 58˚C, and a 1.5 minute chain elongation step at 72˚C. The last elongation step was lengthened by an additional 4 minutes in order to make sure that the DNA was completely amplified.

Each PCR experiment consisted of 86 test samples with the following controls: 2 known HPV-negative samples, 2 PCR reaction mixtures without sample DNA material, and a dilution series of human placental DNA (ranging from 100 pg to 10 ng) serving as a positive control. 10µl of each PCR product was visualised by agarose gel electrophoresis. After agarose gel electrophoresis, positive controls should generate a signal of 209 base pairs and negative controls should not generate a signal. Special precautions were taken to minimize false-positive results in the PCR. Experiments were repeated if positive and negative controls did not give these expected results. Samples showing a specific 209 base pair beta-globin PCR signal were considered beta-globin positive.

All samples underwent PCR analysis to determine HPV positivity and high-risk or low-risk HPV groups prior to individual HPV genotyping. Laboratory testing for HPV positivity was performed using a highly sensitive GP5+/6+ PCR assay followed

by hybridization of PCR products using an enzyme immunoassay (EIA) readout with two HPV oligoprobe cocktail probes that detect the following 44 HPV types: HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), cand85, 86, cand89 (equivalent to CP6108) and JC9710 (63;64).

A total of 40 cycles of PCR amplification with GP5+ and biotinylated GP6+ primers was performed following an initial 4 minute denaturation step at 94˚C. Each cycle consisted of a 20-second denaturation step at 94˚C, a 30-second primer annealing step at 38˚C, and a 80-second elongation step at 71˚C (65). The last elongation step was lengthened by an additional 4 minutes.

Each PCR experiment consisted of 86 test samples with the following controls: 2 known HPV-negative samples, 2 PCR reaction mixtures without sample DNA material, and a dilution series of known HPV 16-positive DNA (ranging from 100 pg to 10 ng) serving as a positive control. This positive control was DNA derived from the cervical cancer cell line SiHa, which contained 1 to 2 copies of HPV 16 per cell. PCR runs or processing steps were repeated (<5%) if positive or negative controls did not give expected results.

PCR products were subsequently detected by EIA hybridization using two HPV oligoprobe cocktail probes to identify high-risk and low-risk HPV DNA (66). Each EIA run contained the 86 test samples, PCR positive and negative controls and 2 EIA positive controls, which were 2 biotinylated PCR products, one derived

from cloned HPV6 DNA plasmids for the low-risk HPV cocktail probe and the other derived from cloned HPV16 DNA plasmids for the high-risk HPV cocktail probe (63;66).

PCR products were captured on streptavidin-coated microwells and subsequently denatured with alkaline treatment and then hybridized to high-risk and low-risk oligonucleotide cocktail probes labelled with digoxigenin. Immunohistochemical staining with anti-digoxigenin polyclonal antibodies was performed and the optical density values at 405 nm were measured to determine if the cocktail probes bound (i.e. if high-risk or low-risk HPV DNA was present). Samples with an optical density at least 3 times the mean value of the 4 negative controls were considered GP5+/6+ PCR positive (66).

HPV genotyping was subsequently performed on GP5+/6+ PCR positive PCR products by reverse line blot (RLB) hybridization (64;65). Oligoprobes specific for each individual HPV type were bound to negatively charged nylon membranes in parallel rows. PCR products were denatured and then pipetted into parallel channels that were perpendicular to the rows of oligoprobes. Hybridization was conducted at 42˚C for 1 hour, followed by incubation of the nylon membrane with antibiotin conjugate, enhanced chemiluminescence detection and exposure to film.

After hybridization of the PCR products, the sensitivity of the GP5+/6+ assay has been previously determined from reconstruction experiments to range from the femtogram level (approximately 70 copies of viral genome per 20,000 cells) for HPV types that were well amplified (e.g.HPV16 and 18) to the picogram level (700,000 copies/20,000 cells) for HPV types that were less well amplified (e.g. HPV39 and 51)

(67;68). The high specificity of RLB has also been demonstrated by the specific hybridization of the type-specific oglioprobes without cross-hybridization in validation experiments (65).

For all analyses, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high-risk types. HPV types detected by EIA but not by RLB genotyping were designated as HPVX, indicating a type, sub-type or variant not detectable with probes used for reverse line blot hybridization. Low-risk types included all other HPV types.

HPV16/18/31 DNA viral load testing

For men participating in the VIA sub-study, HPV DNA viral load testing was performed on penile exfoliated cell samples that contained HPV16, 18 or 31 using a real time PCR assay and a LightCycler instrument (Roche, Mannheim, Germany) (69). DNA extraction and purification were conducted according manufacturer instructions using 100 µl of the remaining cell suspensions. Real time PCR was performed on 100 ng of purified target DNA for each of the three specific HPV types (HPV16, 18 and 31). All samples were run twice and resulting values were averaged. Dilutions of cloned pHPV DNA were used to determine the standard curve for the HPV target. In order to calculate the number of copies per sample, the amount of HPV DNA in one femtogram was multiplied by the dilution factor and divided by the weight of the cloned pHPV viral genome. High HPV DNA viral load was defined as positive when HPV16, 18 or 31 specimen viral loads were >250 copies per scrape in either the glans or shaft specimens (56). All other specimens

containing HPV16, 18 or 31 DNA were considered to have low HPV16/18/31 viral load. This real time PCR assay was tested in validation experiments and found to be more accurate in assessing HPV16 viral load compared to GP5+6+/EIA assays (70).

Visual inspection exams

Consenting study participants were screened by a physician or clinical officer for the presence of penile lesions by visual inspection aided by a colposcope for magnification. The first visual inspection exam was conducted before the application of 3% acetic acid. A second visual inspection exam was performed 2-3 minutes after the application of a 3% acetic acid solution with saturated gauze to the penile shaft, glans, coronal sulcus, frenulum, and the outer and inner foreskin tissue for uncircumcised men. The second exam was conducted to differentiate HPV-associated flat penile lesions, which are usually not visible without the application of 3% acetic acid.

Penile lesions were categorized as described previously (Chapter 2, Table 2.2). The lesion type and location were recorded on a standardized form corresponding to the two anatomical sites: i. the external penile shaft, including the outer foreskin in uncircumcised men and from the base to the circumferential scar for circumcised men and ii. the glans, coronal sulcus, and frenulum, including the inner foreskin (which was retracted during this part of the exam) for uncircumcised men and the part distal to the circumferential scar in circumcised men.

For each visual inspection examination (before and after acetic acid application), two digital photographs were taken of the ventral and the dorsal side of the glans/coronal sulcus with the penile foreskin retracted for uncircumcised men. For quality control purposes, all photographs were also read in Amsterdam without knowledge of HPV data. The percent agreement between Kenya and Amsterdam readings were 83.5%, 80.1%, and 74.2% for flat penile lesions, papular lesions and pearly penile papules, respectively. Final outcome diagnoses were based on the readings by two independent observers in Amsterdam and in case of discrepancies (<10%) a consensus diagnosis was made to determine the final outcome.

Statistical Methods

Natural history of HPV infection

Of the 1,102 men enrolled in the control arm of the RCT with HPV DNA results at baseline, 954 had an HPV result from the 12-month visit, of which 949 remained uncircumcised until the 12-month visit and were included in subsequent statistical analyses. Men who were circumcised between the 12 and 24-month visits (n=39) contributed data until the visit date prior to circumcision (i.e. their 12-month visit). HPV prevalence at baseline and IRs for the first HPV type detected over the 24-month follow-up period were estimated for individual HPV types and for groups of HPV types (any HPV, high-risk HPV and low-risk HPV). A prevalent HPV infection was defined as a HPV infection detected at baseline. An incident or acquired infection was defined as the detection of a type-specific HPV infection at either the 12 or 24-month visit that was not detected at baseline.

For incidence analyses, individual subjects were the unit of analysis. The time at risk for an incident HPV infection was estimated from the date of the baseline visit until the time of the first HPV-positive result at the glans or shaft or until a participant was censored. Participants were censored on the date of their last visit prior to circumcision if they were circumcised before their 24-month visit or on the date of their last study visit if they remained HPV negative. IRs for each HPV type or HPV group were estimated only among participants who were negative for the given individual HPV type or group of types at baseline. Men with HPV infections of multiple HPV types were considered to have a high-risk HPV infection if one or more high-risk types were detected and a low-risk infection if only low-risk types were detected. Men with untyped HPV infections (i.e. HPVX) were excluded from analyses involving high-risk and low-risk HPV categorizations unless they had a high-risk HPV type detected. Incidence analyses were repeated stratified by anatomical site for each group of HPV types and HPV16, among participants who were negative for the particular HPV type or group of types at the given anatomical site.

The 95% CIs for all IRs were estimated by modeling the number of incident HPV infections as a Poisson variable (36;71). Hazard ratios comparing IRs for i. high-risk versus low-risk HPV infections and ii. HPV infections in the glans versus the shaft were estimated using time in days and the Win-Lin-Weissfeld method to adjust for correlation within subjects (72).

Given that each participant could have more than one HPV infection at a given time, the units of analysis for all persistence analyses were individual HPV

infections. Persistence of an incident HPV infection (i.e. incident persistence) was defined as repeat positivity for a type-specific HPV infection at the 12 and 24-month visits that was not detected at baseline. Only incident infections at the 12-month visit were included in the incident persistence analysis since it was unknown whether incident infections detected at the 24-month visit persisted after the end of follow-up. Persistence of a prevalent HPV infection (i.e. prevalent persistence) was defined as repeat positivity on two or more consecutive visits for a type-specific HPV infection detected at baseline. For each individual HPV type and group of types, i. the proportion of incident 12-month HPV infections that persisted until the 24-month visit and ii. the proportion of prevalent baseline HPV infections that persisted from baseline until at least the 12-month visit were calculated, stratified by anatomical site. HPV infections from 169 (17.8%) of the 949 participating men were excluded from only the incident persistence analysis if the participant did not have a 24-month HPV result (n=130) or was circumcised before the 24-month visit (n=39). HPVX infections were excluded from high-risk and low-risk HPV categorizations in all persistence analyses due to their unknown oncogenic status. For each group of HPV types, 95% CIs for incident and prevalent persistence were estimated using generalized estimating equations (GEEs) to account for correlation within subjects (72). Results did not differ substantially when pooling glans and shaft infections due to the low number of persistent HPV infections in the shaft (data not shown).

The proportion of remaining prevalent HPV infections at the end of 24-month follow-up (i.e. proportion of HPV infections detected at all 3 visits) was estimated using the Kaplan-Meier method for the glans and shaft separately. The time at risk

for the detection of a cleared HPV infection was estimated from the date of the baseline visit until the time of the first HPV-negative result or until an infection was censored. Infections were censored on the visit date prior to circumcision for men who were circumcised before their 24-month visit or on the date of the last HPV-positive result. Differences in time (in days) to detection of HPV clearance in the glans by HPV type (high-risk vs. low-risk) and by age group (≤19, 20-21, and ≥22 years) were assessed using the Win-Lin-Weissfeld method to adjusted for correlation within subjects (72).

Beta-globin positivity in the glans and shaft specimens was 56.9% and 35.9% at baseline, 69.7% and 45.9% at the 12-month visit, and 78.4% and 50.5% at the 24-month visit. Reported analyses utilized HPV DNA data from all penile exfoliated cell specimens regardless of beta-globin positivity. Results did not differ substantially when analyses were restricted to beta-globin positive samples from both the glans and the shaft, except that persistence of incident HPV infections in the glans was slightly higher compared to analyses that included all samples.

Risk factors of penile lesions

Pearson's χ^2 and Fisher's exact tests were used to assess differences in i. baseline risk factors between men participating in the VIA exam and all other men enrolled in the parent RCT; ii. 24-month risk factors between circumcised and uncircumcised men; and iii. flat penile lesion prevalence stratified by circumcision status. Prevalence odds ratios (ORs) and corresponding 95% CIs for potential risk factors of flat penile lesions were estimated via age-adjusted, univariate logistic

regression models. A multivariate logistic regression model was used to estimate the OR for flat penile lesions and HPV DNA positivity adjusted for age and male circumcision status since these variables were believed a priori to be potential confounders. Analyses were repeated for papular lesions and pearly penile papule outcomes.

HPV infections with multiple HPV types were considered high-risk if one or more high-risk HPV types were detected. Low-risk types included all other known HPV types. Men with untyped HPV infections (i.e. HPVX) were excluded from analyses involving high-risk and low-risk HPV categorizations unless they also had a known high-risk HPV type detected.

Of 151 circumcised men who consented to VIA exams, 8 (5.3%) were originally assigned to the control arm of the trial but were circumcised before their 24-month visit and therefore excluded from analyses. Results were similar with the inclusion of these 8 men. Beta-globin positivity was 87.2% in the glans specimens and 73.6% in the shaft specimens. Reported analyses utilized HPV DNA data from all penile exfoliated cell specimens regardless of beta-globin positivity. Results did not differ substantially when analyses were restricted to beta-globin positive glans and shaft samples.

Table 3.1 Inclusion and exclusion criteria for a randomized controlled trial of male circumcision conducted in Kisumu, Kenya (6).

Inclusion Criteria	Exclusion Criteria
Uncircumcised	Foreskin covering less than half of the glans
HIV negative	Hemophilia or other bleeding disorder
Sexually active within the past 12 months	High prothrombin time index
Aged 18-24 years	Hypospadias
Resident of Kisumu, Kenya	Other medical condition contraindicating surgery
Not planning to move in the next 2 years	Absolute indication for circumcision
Blood hemoglobin ≥90 g/L	
Willing and able to give informed consent	

CHAPTER 4.
INCIDENCE AND PERSISTENCE OF PENILE HUMAN PAPILLOMAVIRUS INFECTION AMONG UNCIRCUMCISED MEN FROM KENYA

Abstract

Background: Data on the natural history of HPV infection in men are limited, especially from less developed regions including Africa.

Methods: Penile exfoliated cell specimens were collected at the baseline, 12 and 24-month visits from the glans/coronal sulcus and shaft of men enrolled in the control arm of a RCT of male circumcision in Kenya between 2002-2007. All participants were HIV seronegative and aged 17-24 years at baseline. Specimens were tested with GP5+/6+ PCR to detect 44 HPV types.

Results: Among 949 uncircumcised participants, median follow-up time was 24.2 months (range 11.6-30.4). The IR of any HPV infection was 24.3/1,000 person-months. The incidence of HPV infection was higher for high versus low-risk HPV (hazard ratio=1.7; 95% CI: 1.4-2.1) and in glans versus shaft (hazard ratio=2.1; 95% CI: 1.8-2.5). The proportion of incident glans HPV infections that persisted from the 12 to 24-month visits was 18.3% (95% CI: 15.0-22.3). The percentage of prevalent HPV infections in the glans that persisted from baseline until at least the 12-month visit was 13.5% (95% CI: 11.1-16.3). HPV clearance was similar for high-risk versus low-risk infections and by age group.

Conclusions: The natural history of HPV infection among men may be different than that previously described for women, with a relatively high incidence of penile HPV infection found among men. Differences in patterns of high and low-risk HPV clearance between men and women may also exist.

Introduction

Carcinogenic HPV infections are necessary for the development of ICC, and are also considered etiologic agents of other non-cervical cancers among both men and women (1-4). While many previous studies have described HPV infection among women, data on the prevalence and natural history of HPV infection among men are relatively limited (5). A greater understanding of HPV infection among men is needed given that men are an important factor for increased risk of HPV infection and ICC in their female sexual partners (7), and since HPV infection is associated with penile and anal cancers among men (2;3).

Few prospective, follow-up studies have been published on HPV infection among men from North America, Europe and Africa (36-44). The IR of penile HPV infections for the first overall HPV type detected has ranged from 17.9 to 34.9/1,000 person-months (36;37;39). Type-specific persistence of HPV infection over one year has been found to range between 25-30% among HPV-positive men (36;37). Data are especially needed on the acquisition and persistence of HPV infection among men in less-developed geographical regions including sub-Saharan Africa, where the incidence of ICC is among the highest worldwide (8;9).

The aim of this study was to characterize the type-specific incidence and persistence of HPV infection over a 24-month period among 949 uncircumcised men participating in the control arm of a RCT of male circumcision in Kisumu, Kenya.

Methods

Study population and enrolment

Uncircumcised men were screened between February 4, 2002 and September 6, 2005 in Kisumu, Kenya to participate in a RCT of male circumcision (6). The primary aim of the RCT was to determine the effectiveness of male circumcision in reducing HIV incidence. In brief, inclusion criteria included being uncircumcised, aged 18-24 years, HIV seronegative, sexually active, and having blood hemoglobin ≥90 g/L. Study participants were recruited from STI clinics, workplaces, and community organizations. The study protocol was approved by the Institutional Review Boards of the Universities of Illinois at Chicago, Manitoba, Nairobi, and North Carolina; RTI International; and the VU University Medical Center.

Specimen collection

After undergoing informed consent, participants were administered a standardized questionnaire on sociodemographic characteristics and sexual behavior by a trained male interviewer. Penile exfoliated cells were collected for HPV DNA detection at the baseline, 12 and 24-month medical visits. At each visit, specimens were taken from two anatomical sites: i. shaft and external foreskin tissue

(shaft specimen) and ii. the glans, coronal sulcus and inner foreskin tissue (glans specimen) using prewetted Type 3 Dacron swabs and placed in separate conical tubes (18).

Penile cell samples were placed in individual 15-mL centrifuge tubes containing 2-mL 0.01 mol/L Tris-HCl, 7.4 pH, buffer, and processed on the day of collection at the UNIM (Universities of Nairobi, Illinois, and Manitoba) clinic laboratory by centrifugation at high speed (maximum, 3000g) for 10 minutes. Excess Tris-HCl buffer was discarded using a Pasteur pipette, and the remaining cell pellet was resuspended in the same volume of 0.01 mol/L Tris-HCl buffer, and vortexed. Diluted cell pellets were then frozen at -75°C. All samples were sent using a dry shipper to the Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands, for HPV DNA testing.

HPV DNA and STI testing

DNA was isolated from penile exfoliated cell samples using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. Presence of human DNA was evaluated by beta-globin specific PCR, followed by agarose gel electrophoresis. HPV positivity was assessed by GP5+/6+ PCR followed by hybridisation of PCR products using an EIA readout with two HPV oligoprobe cocktail probes that, together, detect 44 HPV types: HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82

(IS39 and MM4subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), cand85, 86, cand89 (equivalent to CP6108) and JC9710. Subsequent HPV genotyping was performed by RLB hybridisation of PCR products, as described previously in Chapter 3 (64;65).

HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high-risk types. HPV types detected by EIA but not by RLB genotyping were designated as HPVX, indicating a type, sub-type or variant not detectable with probes used for RLB hybridization. Low-risk types included all other HPV types.

Urine samples were tested for *N. gonorrhea* and *C. trachomatis* infections by PCR-based methods (Roche Diagnostics) at baseline. Serum specimens were tested for HSV-2 antibody (Kalon) and for HIV antibody using two rapid tests (Determine, Abbott Diagnostic Division, Hoofddorp, Netherlands; and Unigold, Trinity Biotech, Wicklow, Ireland) and confirmed by double ELISA (Adaltis Inc, Montreal, Canada; Trinity Biotech, Wicklow, Ireland) at the University of Nairobi.

Statistical methods

Of the 1,102 men enrolled in the control arm of the RCT with HPV DNA results at baseline, 954 had an HPV result from the 12-month visit, of which 949 remained uncircumcised until the 12-month visit and were included in subsequent statistical analyses. Men who were circumcised between the 12 and 24-month visits (n=39) contributed data until the visit date prior to circumcision (i.e. their 12-month visit). HPV prevalence at baseline and IRs for the first HPV type detected over the 24-month follow-up period were estimated for individual HPV types and for groups of HPV types (any HPV, high-risk HPV and low-risk HPV). A prevalent HPV infection

was defined as a HPV infection detected at baseline. An incident or acquired infection was defined as the detection of a type-specific HPV infection at either the 12 or 24-month visit that was not detected at baseline.

For incidence analyses, the individual subject was the unit of analysis. The time at risk for an incident HPV infection was estimated from the date of the baseline visit until the time of the first HPV-positive result at the glans or shaft or until a participant was censored. Participants were censored on the date of their last visit prior to circumcision if they were circumcised before their 24-month visit or on the date of their last study visit if they remained HPV negative. IRs for each HPV type or HPV group were estimated only among participants who were negative for the given individual HPV type or group of types at baseline. Men with HPV infections of multiple HPV types were considered to have a high-risk HPV infection if one or more high-risk types were detected and a low-risk HPV infection if only low-risk types were detected. Men with untyped HPV infections (i.e. HPVX) were excluded from analyses involving high-risk and low-risk HPV categorizations unless they had a high-risk HPV type detected. Incidence analyses were repeated stratified by anatomical site for each group of HPV types and HPV16, among participants who were negative for the particular HPV type or group of types at the given anatomical site.

The 95% CIs for all IRs were estimated by modeling the number of incident HPV infections as a Poisson variable (36;71). Hazard ratios comparing IRs for i. high-risk versus low-risk HPV infections and ii. HPV infections in the glans versus

the shaft were estimated using time in days and the Win-Lin-Weissfeld method to adjust for correlation within subjects (72).

Given that each participant could have more than one HPV infection at a given time, the units of analysis for all persistence analyses were individual HPV infections. Persistence of incident HPV infection (i.e. incident persistence) was defined as repeat positivity for a type-specific HPV infection at the 12 and 24-month visits that was not detected at baseline. Only incident infections at the 12-month visit were included in the incident persistence analysis since it was unknown whether incident infections detected at the 24-month visit persisted after the end of follow-up. Persistence of a prevalent HPV infection (i.e. prevalent persistence) was defined as repeat positivity on two or more consecutive visits for a type-specific HPV infection detected at baseline. For each individual HPV type and group of types, i. the proportion of incident 12-month HPV infections that persisted until the 24-month visit and ii. the proportion of prevalent baseline HPV infections that persisted from baseline until at least the 12-month visit were calculated, stratified by anatomical site. HPV infections from 169 (17.8%) of the 949 participating men were excluded from only the incident persistence analysis if the participant did not have a 24-month HPV result (n=130) or was circumcised before the 24-month visit (n=39). HPVX infections were excluded from high-risk and low-risk HPV categorizations in all persistence analyses due to their unknown oncogenic status. For each group of HPV types, 95% CIs for incident and prevalent persistence were estimated using GEEs to account for correlation within subjects (72). Results did not differ substantially when

pooling glans and shaft infections due to the low number of persistent HPV infections in the shaft (data not shown).

The proportion of remaining prevalent HPV infections at the end of 24-month follow-up (i.e. proportion of HPV infections detected at all 3 visits) was estimated using the Kaplan-Meier method for the glans and shaft separately. The time at risk for the detection of a cleared HPV infection was estimated from the date of the baseline visit until the time of the first HPV-negative result or until an infection was censored. Infections were censored on the visit date prior to circumcision for men who were circumcised before their 24-month visit or on the date of the last HPV-positive result. Differences in time (in days) to detection of HPV clearance in the glans by HPV type (high-risk vs. low-risk) and by age group (≤19, 20-21, and ≥22 years) were assessed using the Win-Lin-Weissfeld method to adjusted for correlation within subjects (72).

Beta-globin positivity in the glans and shaft specimens was 56.9% and 35.9% at baseline, 69.7% and 45.9% at the 12-month visit, and 78.4% and 50.5% at the 24-month visit. Reported analyses utilized HPV DNA data from all penile exfoliated cell specimens regardless of beta-globin positivity. Results did not differ substantially when analyses were restricted to beta-globin positive samples.

Results

The median age of the 949 included men was 20 years (range 17-24) at baseline (Table 4.1). The median numbers of reported lifetime female partners and female partners in the year prior to enrolling in the RCT were 4 (range 1-86) and 2

(range 0-28), respectively. Only 1 (0.1%) participant reported a male sexual partner. Most men did not live with their sexual partner (93.9%), had at least a secondary education (63.0%) and no source of income (63.9%). Approximately one-third (34.8%) of men reported never using a condom in the past 6 months. The prevalence of HSV-2 seropositivity was 27.1%, with laboratory-diagnosed *C. trachomatis* (4.4%) and *N. gonorrhea* (1.3%) infections being less common.

Baseline prevalence of HPV infection was 49.7%, with multiple HPV infections found among 28.6% of participants (Table 4.2). High-risk and low-risk HPV types were detected in 35.8% and 10.1% of participants, respectively. The six most common individual HPV types were HPV16 (9.2%), 56 (6.8%), 67 (5.6%), 52 (5.6), 66 (5.1%) and 45 (5.0%) within single or multiple infections. All other known HPV types detected had a prevalence of less than 5%. HPVX infections were detected in 5.4% of participants.

The median time follow-up time was 24.2 months (range 11.6-30.4). The median time between study visits was 12.2 months (range 10.4-17.2) from the baseline to 12-month visits and 12.0 months (range 7.0-17.1) from the 12 to 24-month visits.

Incidence analysis

The IR of any HPV infection was 24.3/1,000 person-months for the first HPV type detected at either the glans or the shaft (Table 4.2). Incident infections of multiple HPV types were common (14.1/1,000 person-months). The IR of high-risk HPV was higher compared to low-risk HPV (hazard ratio=1.7; 95% CI: 1.4-2.1). The

four individual HPV types with the highest incidence were HPV16 (IR=5.5/1,000 person-months), JC9710 (IR=3.7/1,000 person-months), HPV56 (IR=3.6/1,000 person-months) and HPV35 (3.5/1,000 person-months). All other HPV types had an incidence less than 3.5/1,000 person-months.

The incidence of any HPV infection was higher in the glans than in the shaft (hazard ratio=2.1; 95% CI: 1.8-2.5) (Table 4.3). The incidence of high-risk and low-risk infections in the glans were 16.2 and 9.6/1,000 person-months respectively, with lower corresponding rates for high-risk and low-risk HPV infections in the shaft (6.2 and 4.6/1,000 person-months, respectively). Incident infections of multiple HPV types were also more common in the glans compared to the shaft (hazard ratio=3.2; 95% CI: 2.5-4.0).

Persistence of incident HPV infections

A total of 550 incident HPV infections were detected in the glans at the 12-month visit, of which 100 (18.2%) persisted until the 24-month visit (Table 4.4). The proportions of high-risk and low-risk incident HPV infections that persisted from the 12 to 24-month visits were 16.7% (n=43) and 21.0% (n=57), respectively. Among the high-risk types, HPV51 was most persistent with 5 (27.8%) of 18 incident infections persisting until the 24 month visit, followed by HPV56 (23.1%), HPV16 (20.0%), HPV59 (20.0%) and HPV66 (20.0%).

A total of 192 incident HPV infections were detected in the shaft at the 12-month visit, of which 9 (4.7%) persisted until the 24-month visit. Three (3.4%) of 88 incident high-risk infections and 6 (6.5%) of 92 incident low-risk infections persisted.

Persistence/clearance of prevalent HPV infections

A total of 944 HPV infections were detected in the glans at baseline (Table 4.4), with 13.5% persisting until at least the 12-month visit. The proportions of high-risk and low-risk prevalent HPV infections that persisted until the 12-month visit were 14.4% and 13.4%, respectively. Among the high-risk prevalent HPV types, the highest proportions of persistence were found for HPV56 (22.4%) and HPV51 (21.2%). A total of 26 (3.2%) of 805 prevalent HPV infections with a 24-month result persisted from the baseline to 24-month visits. There did not appear to be a difference in the time until detection of clearance by HPV type during the 24-month follow-up (Figure 4.1) (hazard ratio=0.91; 95% CI: 0.81-1.04 for high vs. low-risk infections). The cumulative probability of HPV persistence was also similar across age groups (Figure 4.2). Compared to younger men (aged ≤19), the cumulative probability of persistence of HPV infections in the glans of men aged 20-21 and ≥22 clearance were similar (hazard ratios=0.9; 95% CI: 0.7-1.2 and 0.9; 95% CI: 0.7-1.2, respectively).

Of the 284 HPV infections detected in the shaft at baseline, only 4 (1.4%) infections persisted until the 12-month visit and 2 (0.7%) persisted until the 24-month visit.

Discussion

Incident HPV infections were common among men from Kenya with an IR of almost 25/1,000 person-months. The incidence of high-risk HPV infections in the glans was especially high compared to low-risk infections and HPV infections in the

shaft. The proportion of persistent incident HPV infections in the glans (18.2%) was slightly higher compared to the persistence of prevalent HPV infections in the glans (13.5%). The persistence of both incident and prevalent infections in the shaft over 12 months was low (<5%). Detection of HPV clearance in the glans throughout follow-up was similar for high-risk and low-risk prevalent infections and did not differ by age.

This is the largest follow-up study of HPV infection, to our knowledge, among uncircumcised men to date and the first to characterize the natural history of low-risk HPV infections among men from sub-Saharan Africa. A sensitive GP5+/6+ PCR assay was used to detect a wide range of HPV types in a central laboratory allowing for comparisons with natural history studies among women. Separate HPV testing for glans and shaft specimens also allowed for stratified analyses by anatomical site.

The IR of 24.3/1,000 person-months found in our study is consistent with previously reported IR estimates among men ranging from 17.9/1,000 person-months among military men from Mexico to 34.9/1,000 person-months among male university students in the United States (36;37;39). Our IR estimate for high-risk HPV infection in the glans (16.2/1,000 person-months), was lower than the corresponding IR in the glans among HIV-negative, married men aged 15-49 participating in the control arm of an RCT of male circumcision in Uganda (24.5/1,000 person-months) (43). Compared to our study, analyses in the Ugandan study utilized a shorter interval between study visits, which may help explain the disparity in the observed IRs. The IR of high-risk HPV infection among HIV-negative women from Uganda (7.2/1,000 person-months) (73) and Brazil (6.1/1,000 person-

months) (48) was lower than the rates observed among men in our study and in the Uganda RCT, indicating that men may be more likely to acquire HPV infections compared to women.

HPV16, the most prevalent HPV type (9.2%), had the highest incidence in this population with a rate of 5.5/1,000 person-months. A high incidence of HPV16 has also been reported in other studies among both men (36;38;39) and women (73). The high rate of acquisition of HPV16 has a clear implication for cancer risk among men and their sexual partners as HPV16 is the most common HPV type found in penile cancer among men (2), cervical, vulvar and vaginal cancers among women (1;19) and anal and oropharyngeal cancers among both sexes (3;4).

HPV IRs among men in our study were higher in the glans compared to the shaft (hazard ratio=2.1; 95% CI: 1.8-2.5). These results are in contrast to the findings of a study of 240 men from the United States (39) wherein the cumulative probability of incident HPV infections did not differ by anatomical site. Over 75% of the latter study population was circumcised and so there may be a larger disparity in HPV acquisition by penile site among uncircumcised men.

Given that a participant could have more than one HPV infection at a given time, we analyzed persistence of both incident and prevalent HPV infections using the individual HPV infection as the unit of observation. The proportions of men who had at least one type-specific persistent HPV infection in the glans were 29.3% and 22.3% for incident and prevalent HPV infections, respectively. These proportions are expectedly higher than the percentages of incident and prevalent HPV infections in the glans that persisted (18.2 and 13.5%, respectively) given that men with multiple

HPV infections had more than one opportunity to be defined as having a persistent HPV infection.

HPV clearance was similar for both high-risk and low-risk HPV infections and among age groups. These findings are similar to those of a previous study in the United States of 290 men aged 18-49 that found no difference in the median time to clearance for high-risk and low-risk HPV infections and no clear association between HPV clearance and age group (36). In contrast, natural history studies among women have generally found a lower clearance of high-risk HPV infections compared to low-risk types (47;48).

The 12-month interval between each visit was a limitation of this study. Two previous studies reported a median duration of clearance among men of 3-6 months (36;42). Participants in our study could have therefore acquired a new HPV infection and cleared it before the subsequent follow-up visit. Incident HPV infections could have also occurred before they had the opportunity to be detected at the 12 or 24-month follow-up visits. Thus, IRs for the first HPV type detected likely underestimate the actual IR of HPV infection. In our analysis, the incidence of detected HPV infections ranged from 0.05/1,000 person-months for HPV44 to 5.5/1,000 person-months for HPV16. Reanalyzing our data assuming a new HPV infection was acquired at the midpoint of the interval as in one recent study (43), the IR of HPV infections increased slightly ranging from 0.1 to 7.7/1,000 person-months for HPV44 and HPV16, respectively. Further, we were also unable to determine if a participant had a persistent HPV infection, or cleared and re-acquired another HPV infection of the same type within the 12-month interval, which may have led to an overestimation

of HPV persistence over a 12-month period. A reliable median duration of clearance could also not be estimated due to the length of the interval between study visits.

The generalizability of our findings may be limited, given that included participants were a select population of uncircumcised men, who met eligibility criteria for an RCT that entailed circumcision (e.g. age 18-24 years, HIV seronegative at enrolment). Uncircumcised men may be more likely to acquire (43) and less likely to clear HPV infections than circumcised men (42;43), thus our findings should be compared to those of circumcised populations with caution. It has been suggested that age may be a proxy for sexual behavior and immune response among women (74-76). The relatively small age range of included participants in this study, however limited our assessment of HPV clearance by age among men.

In conclusion, we found a high incidence of high-risk HPV in the glans but no difference in HPV clearance between high-risk and low-risk HPV infections or by age. The IR of HPV infection among men appears higher than in women. Differences in patterns of high-risk and low-risk HPV clearance between men and women may also exist. Future studies using similar study designs are needed to confirm potential differences in the natural history of HPV between men and women by investigating comparable populations of both men and women.

Table 4.1 Baseline characteristics of 949 uncircumcised men from Kisumu, Kenya

Variable	
	Median (range)
Age (years)*	20 (17-24)
Lifetime number of female sexual partners	4 (1-86)
Number of female partners in last 12 months	2 (0-28)
	n (%)
Marital Status	
Not living with partner	888 (93.9)
Living with partner	58 (6.1)
Missing	3
Education	
Secondary or tertiary	598 (63.0)
Primary or none	351 (37.0)
Employment Status	
No income	606 (63.9)
Income	343 (36.2)
Condom Use in last 6 months	
>50%	223 (26.6)
≤50%	323 (38.5)
Never	292 (34.8)
Missing	111
HSV-2 seropositive	
Yes	248 (27.1)
No	668 (72.9)
Missing	33
Presence of *C. trachomatis*	
Yes	41 (4.4)
No	898 (95.6)
Missing	10
Presence of *N. gonorrhea*	
Yes	12 (1.3)
No	927 (98.7)
Missing	10

HSV: Herpes simplex virus
*The study inclusion criteria required participants be aged 18-24 years; there was one protocol violation resulting in one 17 year-old included in this study

Table 4.2 Prevalence and incidence of human papillomavirus infection among 949 uncircumcised men in Kisumu, Kenya

HPV type	Baseline Prevalence, %	Incident infections, n	Subjects at risk, n	Person-months	IR*	(95% CI)
Any HPV	49.7	218	477	8,970.7	24.3	(21.2, 27.8)
Multiple†	28.6	196	678	13,945.2	14.1	(12.2, 16.1)
HR HPV†	35.8	219	609	12,314.6	17.8	(15.5, 20.3)
16	9.2	104	862	18,815.8	5.5	(4.5, 6.7)
18	3.3	47	918	20,314.1	2.3	(1.7, 3.1)
31	3.6	35	915	20,359.9	1.7	(1.2, 2.4)
33	1.8	23	932	20,799.7	1.1	(0.7, 1.7)
35	3.3	71	918	20,165.5	3.5	(2.7, 4.4)
39	1.8	28	932	20,754.7	1.3	(0.8, 1.9)
45	5.0	32	902	20,038.8	1.6	(1.1, 2.3)
51	4.2	45	909	20,173.3	2.2	(1.6, 3.0)
52	5.4	42	898	19,975.0	2.1	(1.5, 2.8)
56	6.8	70	884	19,480.8	3.6	(2.8, 4.5)
58	4.2	41	909	20,223.5	2.0	(1.5, 2.8)
59	4.3	33	908	20,206.1	1.6	(1.1, 2.3)
66	5.1	51	901	19,886.4	2.6	(1.9, 3.4)
68	1.2	10	938	21,047.4	0.5	(0.2, 0.9)
LR HPV†	10.1	178	802	17,163.3	10.4	(8.9, 12.0)
6	3.3	52	918	20,310.3	2.6	(1.9, 3.4)
11	1.9	28	931	20,751.0	1.3	(0.9, 2.0)
26	1.4	15	936	20,943.9	0.7	(0.4, 1.2)
30	1.6	18	934	20,901.4	0.9	(0.5, 1.4)
32	1.4	14	936	20,983.4	0.7	(0.4, 1.1)
34	0.1	1	948	21,299.3	0.0	(0.0, 0.3)
40	3.6	45	915	20,337.1	2.2	(1.6, 3.0)
42	4.8	62	903	19,895.7	3.1	(2.4, 4.0)
43	3.9	42	912	20,256.6	2.1	(1.5, 2.8)
44	0.0	1	949	21,324.1	0.0	(0.0, 0.3)
53	1.1	0	939	21,092.5		
54	1.1	4	939	21,055.8	0.2	(0.1, 0.5)
55	1.4	14	936	20,941.9	0.7	(0.4, 1.1)
67	5.6	58	896	19,789.8	2.9	(2.2, 3.9)
69	0.7	21	942	21,005.0	1.0	(0.6, 1.5)
70	2.3	36	927	20,598.0	1.7	(1.2, 2.4)

Table 4.2 continued

HPV type	Baseline Prevalence, %	Incident infections, n	Subjects at risk, n	Person-months	IR*	(95% CI)
72	0.8	9	941	21,103.4	0.4	(0.2, 0.8)
73	1.4	27	936	20,881.1	1.3	(0.9, 1.9)
81	3.2	29	919	20,441.8	1.4	(1.0, 2.0)
82	0.6	15	943	21,151.7	0.5	(0.4, 1.1)
83	1.8	28	932	20,688.1	1.4	(0.9, 2.0)
84	0.3	6	946	21,226.6	0.3	(0.1, 0.6)
85	0.2	12	947	21,179.3	0.6	(0.3, 1.0)
86	0.0	12	949	21,276.7	0.6	(0.3, 1.0)
89	2.0	20	930	20,691.3	1.0	(0.6, 1.5)
JC9710	4.1	74	910	19,983.4	3.7	(2.9, 4.6)
X	5.4	55	898	19,773.8	2.8	(2.1, 3.6)

HPV: Human papillomavirus; HR: high risk; LR: low risk; IR: incidence rate; CI: confidence interval
Note: HPV57, 61, 64, 71 were not detected at baseline nor during follow-up
*Incidence rate per 1,000 person-months for the first type detected in the glans or shaft
[†]Multiple HPV included >1 HPV type. Infections with multiple HPV types were considered high-risk if one or more HR HPV types were detected. All other multiple infections were considered LR types unless they included HPVX.

Table 4.3 Incidence of human papillomavirus infection stratified by anatomical site among 949 uncircumcised men from Kisumu, Kenya

HPV type‡	Incident HPV infections in glans, n	IR (95% CI)*	Incident HPV infections in shaft, n	IR (95% CI)*	Hazard Ratio (95% CI)†
Any HPV	217	21.9 (19.1, 25.0)	181	11.1 (9.5, 12.8)	2.1 (1.8, 2.5)
Multiple	182	12.4 (10.6, 14.3)	77	3.9 (3.1, 4.9)	3.2 (2.5, 4.0)
HR HPV	211	16.2 (14.0, 18.5)	113	6.2 (5.1, 7.5)	2.7 (2.2, 3.3)
LR HPV	167	9.6 (8.2, 11.1)	90	4.6 (3.7, 5.6)	2.1 (1.7, 2.7)
HPV16	83	4.3 (3.5, 5.3)	45	2.2 (1.6, 2.9)	2.0 (1.4, 2.7)

HPV: Human papillomavirus; HR: high risk; LR: low risk; IR: incidence rate; CI: confidence interval

*IR=incidence per 1,000 person-months

†Hazard ratio comparing glans versus shaft

‡Multiple HPV included >1 HPV type. Infections with multiple HPV types were considered high-risk if one or more HR HPV types were detected. All other multiple infections were considered LR unless they included HPVX.

Table 4.4 Persistence of 550 incident human papillomavirus infections detected at the 12-month visit and 944 prevalent HPV infections detected at baseline in the glans of men from Kisumu, Kenya

HPV type	Incident Infections	Incident Persistent HPV Infections*		Prevalent Infections	Prevalent Persistent HPV Infections†	
	N	n	% (95% CI)	N	n	% (95% CI)
Any HPV	550	100	18.2 (15.0, 22.3)	944	127	13.5 (11.1, 16.3)
HR HPV	257	43	16.7 (12.6, 22.2)	487	70	14.4 (11.2 18.4)
16	35	7	20.0 (8.4, 36.9)	72	12	16.7 (8.9, 27.3)
18	19	2	10.5 (0.8, 22.8)	26	4	15.4 (4.4, 34.9)
31	13	2	15.4 (1.9, 45.4)	27	5	18.5 (6.3, 38.1)
33	8	1	12.5 (0.3, 52.7)	15	3	20.0 (4.3, 48.1)
35	32	6	18.8 (7.2, 36.4)	29	1	3.4 (0.1, 17.8)
39	14	2	14.3 (1.7, 42.8)	15	3	20.0 (4.3, 48.1)
45	16	1	6.3 (0.2, 30.2	39	3	7.7 (1.6, 20.9)
51	18	5	27.8 (9.7, 53.5)	33	7	21.2 (9.0, 38.9)
52	15	1	6.7 (0.2,3.2)	46	4	8.7 (2.4, 20.8)
56	26	6	23.1 (9.0, 43.6)	58	13	22.4 (12.5, 35.3)
58	15	1	6.7 (0.01,31.9)	39	7	17.9 (7.5, 33.5)
59	15	3	20.0 (4.3, 48.1)	36	5	13.9 (4.7, 29.5)
66	30	6	20.0 (7.7, 38.6)	42	3	7.1 (1.5,19.5)
LR HPV	272	57	21.0 (16.5, 26.6)	424	57	13.4 (10.4, 17.3)
6	19	7	36.8 (16.3, 61.6)	28	3	10.7 (2.3, 28.2)
11	11	1	9.1 (0.2, 41.3)	14	2	14.3 (1.7, 42.8)
26	8	1	12.5 (0.3, 52.7)	13	0	0.0 (0.0, 24.7)
32	2	0	0.0 (0.0, 84.2)	13	2	15.4 (1.9, 45.4)
40	17	4	23.5 (6.8, 49.9)	31	8	25.8 (11.9, 44.6)
42	27	8	29.6 (13.8, 50.2)	42	5	11.9 (4.0, 25.6)
43	16	2	12.5(1.6, 38.3)	35	5	14.3 (4.8, 30.3)
55	6	0	0.0 (0.0, 45.9)	13	1	7.7 (2.0, 36.0)
67	23	8	34.8 (16.4, 57.3)	47	10	21.3 (10.7, 35.7)
69	11	3	27.3 (6.0, 61.0)	7	2	28.6 (3.7, 71.0)
70	15	1	6.7 (0.2, 31.9)	20	6	30.0 (11.9, 54.3)
72	2	1	50.0 (1.3, 98.7)	8	0	0.0 (0.0, 36.9)
73	13	2	15.4 (1.9, 45.4)	13	0	0.0 (0.0, 24.7)
81	15	2	13.3 (1.7, 40.5)	25	3	12.0 (2.5, 31.2)
83	15	1	6.7(0.2, 31.0)	15	1	6.7 (0.2, 31.9)
85	7	4	57.1 (18.4, 90.1)	2	1	50.0 (0.1, 98.7)
86	4	2	50.0 (6.8, 93.2)	0	0	
89	12	4	33.3 (9.9, 65.1)	18	2	11.1 (1.4, 34.7)

Table 4.4 continued

HPV type	Incident Infections	Incident Persistent HPV Infections*		Prevalent Infections	Prevalent Persistent HPV Infections†	
	N	n	% (95% CI)	N	n	% (95% CI)
JC9710	32	6	18.8 (7.2, 36.4)	39	6	15.4 (58.6, 30.5)
X‡	21	2		33	1	

HPV: Human papillomavirus; HR: high risk; LR: low risk;
Note: No incident infections at the 12-month visit, nor prevalent infections at baseline were detected at the glans for low-risk HPV34, 44, 57, 61, 64 or 71. HPV86 was not detected at baseline. No incident or prevalent persistent HPV infections were detected for HPV68, 30, 53, 54, 82, or 84.
*Defined as repeat positivity for a type-specific HPV infection at the 12 and 24-month follow-up visits that was not present at baseline.
†Defined as repeat positivity on two or more consecutive visits for a type-specific HPV infection present at baseline.
‡HPVX infections were excluded from HR and LR categorizations due to their unknown oncogenic status. 2 incident persistent and 1 prevalent persistent HPVX infections were excluded from the any HPV category since type-specificity of persistent HPVX infections could not be determined.

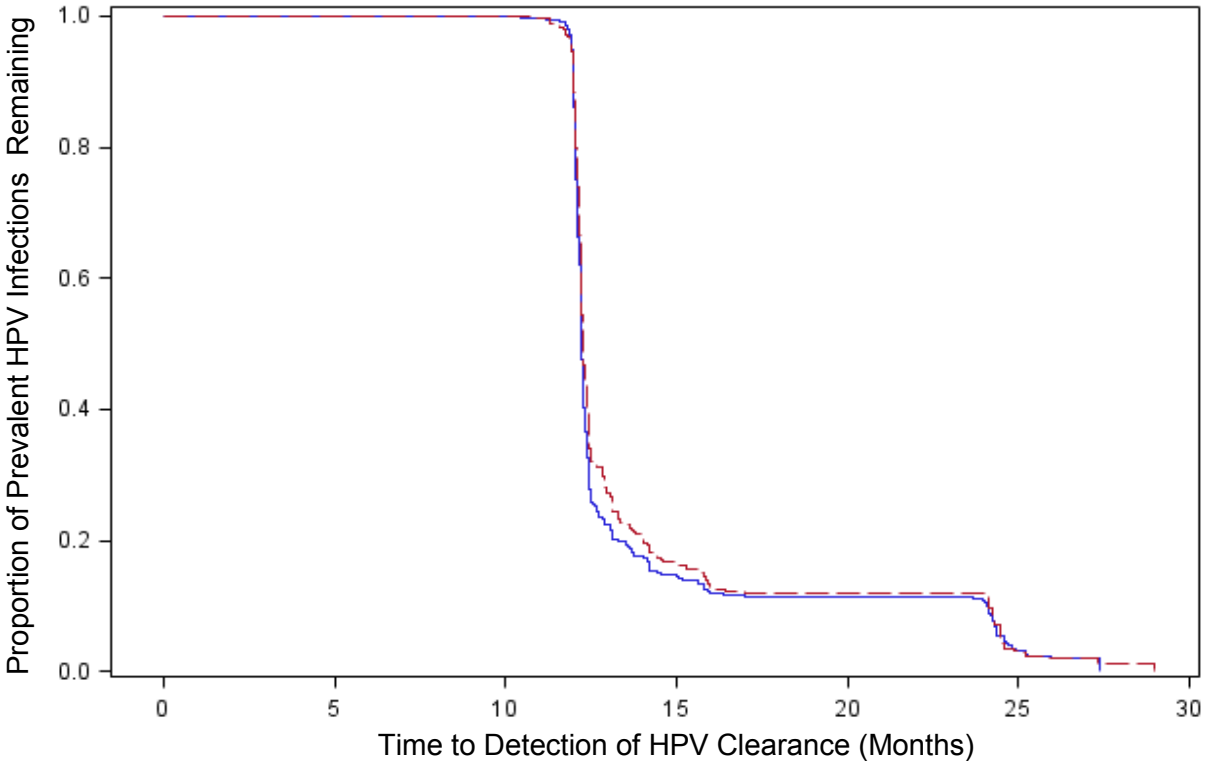

Figure 4.1 Kaplan-Meier curves for the proportion of prevalent high-risk human papillomavirus (HPV) infections (dotted line) versus low-risk HPV infections (solid line) remaining positive through follow-up. High-risk types included HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Low-risk types included HPV6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, cand85, 86, cand89 and JC9710.

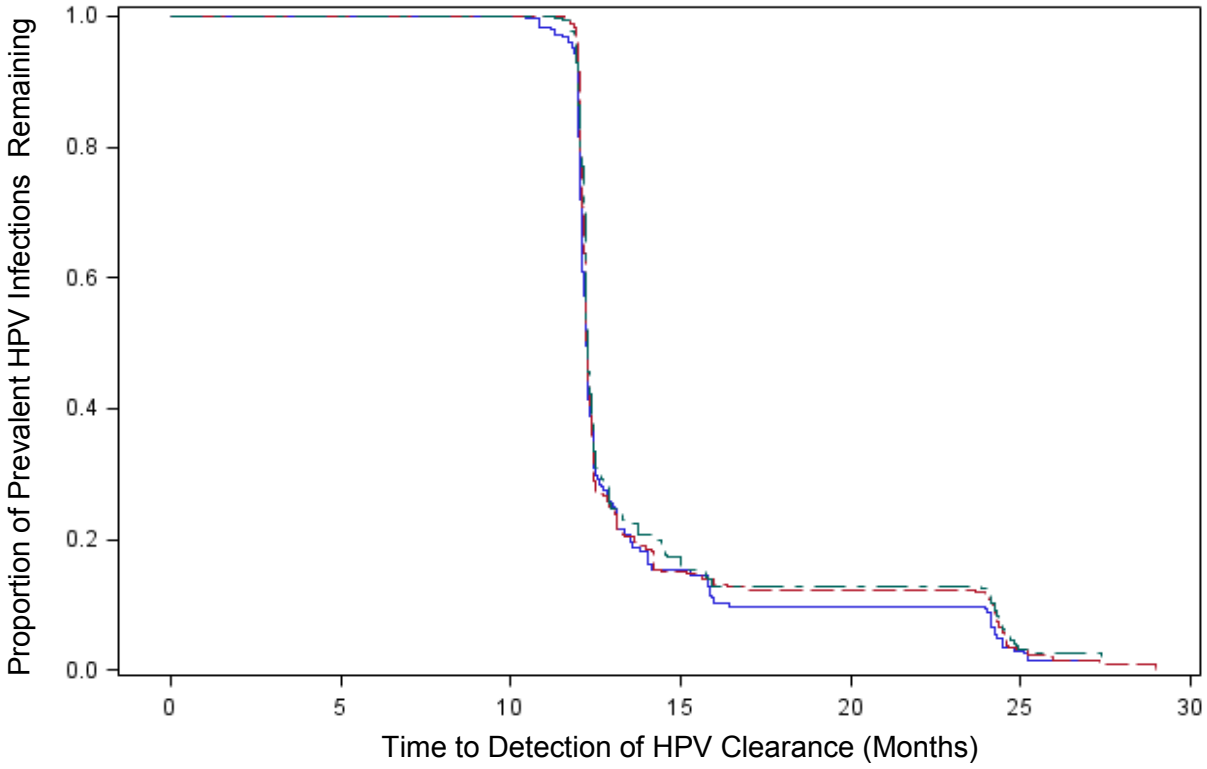

Figure 4.2 Kaplan-Meier curves for the proportion of prevalent human papillomavirus (HPV) infections remaining by age group (aged ≤19 years = solid line; 20-21 years = dashed line (— — —); ≥22 years = dashed line (— - —).

CHAPTER 5.
MALE CIRCUMCISION IS ASSOCIATED WITH A LOWER PREVALENCE OF HUMAN PAPILLOMAVIRUS-ASSOCIATED PENILE LESIONS AMONG KENYAN MEN

Abstract

Background: HPV-associated penile lesions in men may increase the risk of HPV transmission to their female partners. Risk factor data on HPV-associated penile lesions are needed from regions with a high burden of cervical cancer.

Methods: Visual inspection of the penis was conducted using a colposcope at the 24-month visit for a RCT of male circumcision in Kenya, from May 2006 to October 2007. All photos were read independently by two observers for quality control.

Results: Of 267 men, 143 were circumcised and 124 uncircumcised. The median age was 22 years. Circumcised men had a lower prevalence of flat penile lesions (0.7%) versus uncircumcised (26.0%); age-adjusted OR=0.02; 95% CI: 0.003-0.1. Men with flat lesions had increased odds of HPV DNA positivity (OR=5.5; 95% CI: 2.2-13.3) and high HPV16/18/31 viral load (OR=7.6; 95% CI: 1.8-32.8) compared to men without flat lesions. Among men with flat penile lesions, HPV56 (29.0%) and 16 (25.8%) were the most common types.

Conclusions: Flat penile lesions are much more frequent in uncircumcised men, and associated with increased odds of HPV positivity and high viral load. This

study suggests circumcision reduces the prevalence of HPV-associated flat lesions and may ultimately reduce male to female HPV transmission.

Introduction

HPV infection is the central cause of cervical cancer in women and plays an important role in other anogenital cancers, including penile and anal cancers in men (1-4). Interventions that reduce HPV-associated penile lesions could be important to both men and women, since HPV-associated penile lesions in men may increase the risk of HPV transmission to their sexual partners (10). Risk factor data on HPV-associated penile lesions are needed, especially from less-developed countries with a high burden of cervical cancer.

Flat penile lesions are commonly found among male partners of women with CIN, but often go unnoticed without the application of acetic acid (51;53;56). Positive associations have been found between flat penile lesions, lack of condom use, HPV positivity and high HPV viral loads (51;57;58). More data however are needed to determine the type-specific distribution of HPV infection and whether other risk factors, including male circumcision, are associated with penile lesions. While circumcision has been previously associated with a lower point-prevalence of HPV infection (77-79) and a decreased incidence of high-risk HPV (43), it is unknown whether circumcision reduces the prevalence of flat penile lesions. Other types of common penile lesions, including papular lesions and pearly penile papules, have not been associated with HPV infection (51;52). It is unclear whether risk factors

other than HPV infection differ between flat penile lesions, papular lesions and pearly penile papules.

The primary aim of this study was to determine the association between male circumcision status and HPV-associated flat penile lesions among high-risk men from Kisumu, Kenya. We also sought to investigate risk factors of ascertained penile lesions, including HPV viral load and type-specific HPV infection.

Methods

Study population and enrolment

Uncircumcised men were screened between February 4, 2002 and September 6, 2005 in Kisumu, Kenya to participate in a RCT of male circumcision (6). The primary aim of the RCT was to determine the effectiveness of male circumcision in reducing HIV incidence. In brief, inclusion criteria included being uncircumcised, aged 18-24 years, HIV seronegative, sexually active, and having blood hemoglobin ≥90 g/L. Study participants were recruited from STI clinics, workplaces, and community organizations. For men assigned to the intervention group in the main RCT, circumcision was performed on the same day after HPV specimen collection or within a few days of the enrolment visit.

Beginning May 5, 2006, RCT participants were invited to participate in a visual inspection examination of the penis with 3% acetic acid at the 24-month (final) follow-up visit. Of the 1,398 men enrolled in the RCT who had a 24-month visit date after May 5, 2006, 267 (19%) consented to participate in the VIA exam and were included in this sub-study. The study protocol was approved by the Institutional

Review Boards of the Universities of Illinois at Chicago, Manitoba, Nairobi, and North Carolina; RTI International; and the VU University Medical Center.

Questionnaire, clinical examination, and specimen collection

After undergoing informed consent, participants were administered a standardized questionnaire on sociodemographic characteristics and sexual behavior by a trained male interviewer at baseline and the 24-month visit. Participants also underwent a clinical examination by a trained physician or clinical officer at each visit (6). Penile exfoliated cells were collected for HPV DNA detection at baseline prior to circumcision and at the 24-month visit from two anatomical sites: i. shaft and external foreskin tissue (shaft specimen) and ii. glans, coronal sulcus and inner foreskin tissue (glans specimen) using prewetted Type 3 Dacron swabs in separate conical tubes (18).

Both penile cell samples were placed in individual 15-mL centrifuge tubes containing 2-mL of 0.01 mol/L Tris-HCl, 7.4 pH buffer, and processed on the day of collection at the UNIM clinic laboratory in Kisumu by centrifugation at high speed (maximum, 3000g) for 10 minutes. Excess Tris-HCl buffer was discarded using a Pasteur pipette, and the remaining cell pellet was resuspended in the same volume of 0.01 mol/L Tris-HCl buffer, and vortexed. Diluted cell pellets were then frozen at -75°C. All samples were sent using a dry shipper to the Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands, for HPV DNA testing.

Visual inspection exams

Consenting study participants were screened at the 24-month visit by a physician or clinical officer for the presence of penile lesions by visual inspection aided by a colposcope for magnification. The first visual inspection exam was conducted before the application of 3% acetic acid. A second visual inspection exam was performed 2-3 minutes after the application of a 3% acetic acid solution with saturated gauze to the penile shaft, glans, coronal sulcus, frenulum, and the outer and inner foreskin tissue for uncircumcised men. The second exam was conducted to differentiate HPV-associated flat penile lesions, which are usually not visible without the application of 3% acetic acid. The lesion type and location were recorded on a standardized form corresponding to the two anatomical sites: i. the external penile shaft, including the outer foreskin in uncircumcised men, and from the base to the circumferential scar for circumcised men; and ii. the glans, coronal sulcus and frenulum, including the inner foreskin (retracted during this part of the exam) for uncircumcised men, and the part distal to the circumferential scar in circumcised men. Two digital photographs were taken of the ventral and dorsal side of the glans/coronal sulcus with the penile foreskin retracted for uncircumcised men both before and after acetic acid application. All photographs were also read in Amsterdam without knowing HPV data and in case of discrepancies (<10%) a consensus diagnosis was made to determine the final outcome.

Penile lesions were categorized as follows: i. flat lesions (flat or slightly elevated, well demarcated, aceto-white lesions in which a capillary pattern can be seen); ii. condyloma acuminata (exophytic lesions with an irregular surface

commonly known as genital warts); iii. papular lesions (small exophytic papules usually located nearby the frenulum of the penis with a smooth surface on which a hyperkeratinized layer could be present) (51); and iv. pearly penile papules (small exophytic papules located around the corona of the glans penis and presenting in 1 to 4 rows) (52).

HPV DNA, HPV viral load and STI testing

DNA was isolated from penile exfoliated cell samples using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. Presence of human DNA was evaluated by beta-globin specific PCR, followed by agarose gel electrophoresis. HPV positivity was assessed by GP5+/6+ PCR followed by hybridization of PCR products using an EIA readout with two HPV oligoprobe cocktail probes that, together, detect 44 HPV types HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), cand85, 86, cand89 (equivalent to CP6108) and JC9710. Subsequent HPV genotyping was performed by RLB hybridization of PCR products, as described previously in Chapter 3 (64;65). HPV16, 18 and 31 viral loads were subsequently determined using a real time PCR assay and a LightCycler instrument (Roche) as previously described in Chapter 3 (69). High HPV DNA viral load was defined as positive when HPV16, 18 or 31 specimen viral loads were >250 copies per scrape in either the

glans or shaft specimens (56). All other specimens containing HPV 16, 18 or 31 DNA were considered to have low HPV16/18/31 viral load.

HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high-risk types. HPV types detected by EIA but not by RLB genotyping were designated as HPVX, indicating a type, sub-type or variant not detectable with probes used for RLB hybridization. HPV infections with multiple HPV types were considered high-risk if one or more high-risk HPV types were detected. Low-risk types included all other known HPV types. Men with untyped HPV infections (i.e. HPVX) were excluded from analyses involving high-risk and low-risk HPV categorizations unless they also had a known high-risk HPV type detected.

Urine samples were tested for *N. gonorrhea* and *C. trachomatis* infections by PCR-based methods (Roche Diagnostics). Serum specimens were tested for HSV-2 antibody (Kalon) and for HIV antibody using two rapid tests (Determine, Abbott Diagnostic Division, Hoofddorp, Netherlands; and Unigold, Trinity Biotech, Wicklow, Ireland) and confirmed by double ELISA (Adaltis Inc, Montreal, Canada; Trinity Biotech, Wicklow, Ireland) at the University of Nairobi.

Statistical methods

Pearson's χ^2 and Fisher's exact tests were used to assess differences in: i. baseline risk factors between men participating in the VIA exam and all other men enrolled in the parent RCT; ii. 24-month risk factors between circumcised and uncircumcised men; and iii. flat penile lesion prevalence stratified by circumcision status. Prevalence ORs and corresponding 95% CIs for potential risk factors of flat

penile lesions were estimated via age-adjusted, univariate logistic regression models. A multivariate logistic regression model was used to estimate the OR for flat penile lesions and HPV DNA positivity adjusted for age and male circumcision status since these variables were believed a priori to be potential confounders. Analyses were repeated for papular lesions and pearly penile papule outcomes.

Of 151 circumcised men who consented to VIA exams, 8 (5.3%) were originally assigned to the control arm of the trial but were circumcised before their 24-month visit and therefore excluded from analyses. Results were similar with the inclusion of these 8 men. Beta-globin positivity was 87.2% in the glans specimens and 73.6% in the shaft specimens. Reported analyses utilized HPV DNA data from all penile exfoliated cell specimens regardless of beta-globin positivity. Results did not differ substantially when analyses were restricted to beta-globin positive samples.

Results

At baseline, men who participated in the VIA exam (N=267) were more likely to have ≥2 sex partners during the past 12 months ($p<0.001$), live with their female sexual partner ($p=0.01$) and be randomized to the intervention arm ($p=0.06$), as compared to all other RCT participants (N=2,517) (Table 5.1). All other baseline risk factors assessed were similar between men who did and did not participate in the VIA study.

At the 24-month visit, of the 267 men consenting to undergo the penile lesion examination, 143 (53.6%) were circumcised and 124 (46.4%) were uncircumcised.

The median age was 22 years (range 20-26). One participant was HIV seropositive (0.37%), while 27 were seropositive for HSV-2 (14.6%) at the 24-month visit. Less than 2% of men had laboratory diagnosed *N. gonorrhea* or *C. trachomatis* infections, or reported having an STI within the last 6 months. The median number of female sexual partners in the previous 12 months was 2 women (range 0-14). No men reported having a male sexual partner in their lifetime. The vast majority of men (99%) reported bathing daily. Risk factors assessed at the 24-month visit did not differ between circumcised and uncircumcised men except circumcised men were more likely to be HPV negative (p=0.01), and have two or more female sexual partners in the previous 12 months (p=0.04) (Table 5.2).

Prevalence of penile lesions

A total of 33 (12.4%) men had flat penile lesions detected after application of 3% acetic acid. The foreskin (or foreskin remnant among circumcised men) was the most common site where flat lesions were detected (10.2%), followed by the frenulum (3.4%) and then the glans (2.6%) (Table 5.3). The prevalence of flat lesions was higher among uncircumcised men (26.0%) than circumcised men (0.7%), respectively (p<0.001), with only one circumcised participant having a flat lesion, which was on the foreskin remnant. Compared to circumcised men, men who were uncircumcised were more likely to have flat lesions detected at the foreskin (p<0.001), frenulum (p<0.001) and glans (p=0.004). No flat lesions were detected on the penile shaft of any participant. Two uncircumcised participants were diagnosed with condyloma acuminata. Papular lesions (n=129; 48.3%) and pearly penile

papules were common (n=183; 68.5%). Uncircumcised men had a lower prevalence of papular lesions (34.7% vs. 60.1% in circumcised; p<0.001) and pearly penile papules (61.3% vs. 74.8%; p=0.02).

Risk factors of flat penile lesions

After age adjustment, circumcised men were less likely to have flat penile lesions versus uncircumcised men (0.7% vs. 26.0%; OR=0.02; 95% CI: 0.003-0.1) (Table 5.4). Flat penile lesions were more common among men with who were HPV DNA positive at the glans or shaft (21.6% vs. 4.8% in HPV-negative men; OR=5.5; 95% CI: 2.2-13.3). A strong association was also found between flat penile lesions and high-risk HPV DNA (OR=8.0; 95% CI: 3.2-19.9 vs. HPV-negative) and HPV DNA detected at the glans only (OR=6.3; 95% CI: 2.7-15.0) (Table 5.4). In a multivariate model, high-risk HPV DNA remained a strong risk factor of flat penile lesions even after controlling for age and male circumcision status (OR=8.3; 95% CI: 3.0-22.8 vs. HPV-negative).

Compared to men who were HPV DNA negative, participants with high HPV16, 18 or 31 viral loads were much more likely to have flat penile lesions (OR=7.6; 95% CI: 1.8-32.8). Even men with low viral loads were more likely to have flat penile lesions compared to HPV-negative men (OR=4.3, 95% CI: 1.1-16.6). Compared to men who were HPV DNA negative, a particularly strong association was found between high HPV16/18/31 viral load in the glans and the presence of flat penile lesions (OR=12.3; 95% CI: 2.6-58.8) with a weaker association found in the shaft (OR=5.1; 95% CI: 0.8-31.4). A higher reported number of female sexual

partners in the last 12 months was associated with reduced odds of flat penile lesions (OR=0.5; 95% CI: 0.2-1.0 for 0-1 vs. ≥2 partners) (Table 5.4), although this association was no longer significant after adjusting for age and circumcision status (OR=0.5, 95% CI: 0.2-1.2). Other risk factors assessed including education, income, condom use in the last 6 months and years of sexual activity were not significantly associated with prevalence of flat penile lesions (Table 5.4).

Risk factors of papular lesions and pearly penile papules

Circumcised men were more likely to have papular lesions (OR=2.9; 95% CI: 1.7-4.7 vs. uncircumcised) in the age-adjusted model (Table 5.4). High-risk HPV was not significantly associated with papular lesions (OR=0.6, 95% CI: 0.3-1.1 vs. HPV-negative) after controlling for age and circumcision status. Male circumcision and younger age (22-23 vs. ≥24 years) were associated with pearly penile papules in age-adjusted models (ORs=1.9; 95% CI: 1.1-3.2 and 2.2; 95% CI: 1.2-4.2) (Table 5.4).

HPV type distribution among men with and without flat penile lesions

Among men with flat penile lesions, 30 individual HPV types were detected, with high-risk HPV 56 (29.0%) and 16 (25.8%) the most common HPV types within single or multiple HPV infections. The next three most common HPV types were high-risk HPV52 (19.4%), 35 (12.9%) and 66 (12.9%). All other HPV types detected (n=25) had an HPV prevalence of less than 10%: 18, 31, 33, 45, 51, 58, 59, 68, 6, 26, 30, 34, 40, 42, 43, 55, 61, 67, 72, 73, 81, 82 83, 89 and JC9710.

A total of 41 HPV types were detected among men without flat penile lesions, with HPV16 the most common type (9.3%) within single or multiple infections. The next four most common known HPV types were HPV45 (4.0%), HPV51 (3.6%), HPV58 (3.6%) and HPV66 (3.6%). HPVX infections were found in 4.4% of men without penile lesions. All other HPV types detected (n=35) were found in ≤3.1% of participants, and included HPV18, 31, 33, 35, 39, 52, 56, 59, 68, 6, 11, 26, 32, 40, 42, 43, 44, 53, 54, 55, 61, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, 85, 86, 89 and JC9710.

The prevalences of the 5 most common individual HPV types among men with flat penile lesions were higher than the corresponding HPV prevalences among men without flat penile lesions (Figure 5.1). Multiple HPV types (defined as having ≥ 2 different individual HPV types) were less common among men without flat penile lesions compared to men with flat penile lesions detected (19.6% vs. 67.7%, respectively; $p<0.001$).

Discussion

Male circumcision was strongly associated with reduced odds of flat penile lesions. Men with flat penile lesions had a higher prevalence of HPV infection, than men without flat lesions. High-risk HPV infection and high HPV16/18/31 viral load in the glans were particularly strong risk factors of flat penile lesions.

The prevalence of flat penile lesions among uncircumcised men in our study population (26%) was higher than that found among uncircumcised participants in a previous study (17%) of men in the Netherlands who self-reported no known STI

infections (56). The prevalence of flat lesions among circumcised men in both studies was <1%. The higher flat lesion prevalence among uncircumcised men in our study could also potentially be due to the younger age of our study population.

Our results are also consistent with previous studies that found an association between flat penile lesions and HPV using PCR or *in situ* hybridization methods (51;54). HPV prevalence among men with flat penile lesions in our study population (77%) was similar to that observed among 175 male sexual partners of women with CIN from the Netherlands (72%) (51). Flat penile lesions were strongly associated with high-risk HPV infection and high HPV16/18/31 viral load especially in the glans, supporting findings from previous studies that HPV might play an important role in the etiology of these lesions (51;54;55;80). Flat penile lesions could also be a useful parameter for evaluating efficacy of prophylactic HPV16/18 vaccines, due to their common occurrence and strong associations with high-risk HPV and HPV16/18/31 viral load.

Circumcision was associated with increased odds of papular lesions and pearly penile papules. Papular lesions and pearly penile papules are likely not sexually transmitted and have not been found to be associated with HPV infection in previous reports (51;52;59). The association found between circumcision and papular lesions and pearly penile papules might be due to small mechanical traumas during intercourse or a tissue reaction following the circumcision procedure (81).

To our knowledge, this is the first study to investigate multiple risk factors, including HPV, for penile lesions among men from sub-Saharan Africa, where the burden of ICC is among the highest worldwide. Study advantages also include the

use of a sensitive PCR assay to detect a broad range of individual HPV types and the collection of data on numerous potential risk factors. Additionally, all photos taken of penile lesions were double read in Amsterdam without knowledge of HPV status for quality control to reduce misclassification of penile lesions.

A study limitation is that penile lesions were diagnosed by colposcopy. Taking biopsies of the ascertained lesions was considered too invasive for this study population, which was participating in a RCT. Therefore, HPV infection could not be detected directly from biopsy specimens and histological diagnosis of penile lesions was not possible. Previous reports of flat penile lesions indicate that these lesions usually represent hyperplasia or low grade penile intraepithelial neoplasia, although a minority of lesions may be high grade (10;51). Only three of the large number of HPV types were assessed for HPV DNA viral load in this study. Men categorized as having low HPV16/18/31 viral load may have had a high viral load for an HPV type that was not assessed. Our sample was too small to restrict the analysis to men with single HPV type infections. Thus, viral load analyses should be interpreted with caution.

Compared to all other RCT participants, men who participated in this study were similar in respect to their baseline risk factors except that VIA participants were more likely to have two or more sexual partners in the previous 12 months, live with their female partner and be randomized to the intervention arm of the trial. While selection bias appeared to be minimal, our findings should be interpreted with caution as this was a relatively small subset of men from the RCT. The prevalence of STIs other than HPV infection was also low in our study population, and hence

important associations between penile lesions and other STIs might have been missed. Future studies are needed to determine if there are associations between laboratory-diagnosed STIs and penile lesions.

Male circumcision was strongly associated with reduced odds of flat penile lesions. Because flat penile lesions were also strongly associated with high-risk HPV infection and high HPV16/18/31 viral load, circumcision may also reduce male to female high-risk HPV transmission. Additionally, since prophylactic HPV vaccines may not be readily available to men in many less developed countries and current HPV vaccines do not include all high-risk HPV types, circumcision may provide a useful intervention to prevent HPV-associated penile lesions and ultimately invasive cervical cancer in developing countries.

Table 5.1 Comparison of baseline characteristics for men enrolled in the randomized controlled trial of male circumcision who did and did not participate in the visual inspection with acetic acid sub-study

Risk Factor	Participated in VIA sub-study (n=267) n (%)*	Did not participate in VIA sub-study (n=2517) n (%)*	p-value†
Age (years)			0.24
≥22	69 (25.8)	776 (30.8)	
20-21	112 (42.0)	973 (38.7)	
≤19	86 (32.2)	768 (30.5)	
Randomization Assignment			0.06
Uncircumcised	119 (44.6)	1274 (50.6)	
Circumcised	148 (55.4)	1243 (49.4)	
HPV DNA positivity‡			
HR-positive	95 (39.4)	685 (36.3)	0.62
LR-positive	26 (10.8)	220 (11.6)	
Negative	120 (49.8)	984 (52.1)	
Education			0.33
Secondary or tertiary	169 (63.3)	1668 (66.3)	
Primary or none	98 (36.7)	849 (33.7)	
Employment status			0.68
No Income	92 (34.5)	899 (35.7)	
Income	175 (65.5)	1618 (64.3)	
Condom use last 6 months			
>50%	85 (34.7)	787 (36.5)	0.85
≤50%	95 (38.8)	806 (37.4)	
Never	65 (26.5)	561 (26.0)	
Marital Status			
Not living with partner	243 (91.0)	2374 (94.7)	0.01
Living with partner	24 (9.0)	132 (5.3)	
Age at first intercourse (years)			
16-21	129 (50.0)	1239 (51.4)	0.66
8-15	129 (50.0)	1170 (48.6)	
Partners in last 12 months			
0-1	71 (26.8)	1012 (40.6)	<0.001
≥2	194 (73.2)	1481 (59.4)	
Lifetime # of female partners			
1-4	117 (48.3)	1282 (54.5)	0.18
5-7	61 (25.2)	535 (22.8)	
≥8	64 (26.5)	534 (22.7)	
Years of sexual activity			
0-3	77 (30.0)	789 (29.0)	0.46
4-5	74 (28.8)	587 (24.3)	
6-7	58 (22.6)	566 (23.4)	
8-14	48 (18.7)	473 (19.6)	

Table 5.1 continued

Risk Factor	Participated in VIA sub-study (n=267) n (%)*	Did not participate in VIA sub-study (n=2517) n (%)*	p-value†
HSV-2-seropositive			
No	182 (71.4)	1748 (72.3)	0.76
Yes	73 (28.6)	670 (27.3)	
N. gonorrhea			
Positive	6 (2.3)	48 (1.9)	0.64**
Negative	254 (97.7)	2436 (98.1)	
C. trachomatis			
Positive	16 (6.2)	108 (4.4)	0.21**
Negative	244 (93.8)	2375 (95.6)	
Self-reported STI††			
No	248 (97.2)	2015 (98.1)	0.49**
Yes	7 (2.8)	42 (2.0)	

VIA: visual inspection with acetic acid; HPV: human papillomavirus; HR: high-risk; LR: low-risk; HSV-2: herpes simplex virus type 2; STI: sexually transmitted infection

*Percentages do not include missing values

†P-value comparing men who did and did not participate in the VIA sub-study using Pearson's chi-square test unless otherwise noted.

‡Men with HPVX infections were excluded from high and low-risk HPV categorizations unless a high-risk HPV type was detected.

**Fisher's exact test

††Sexually transmitted infection (current or within the last 6 months)

Table 5.2 Comparison of risk factors assessed at the 24-month follow-up visit between circumcised versus uncircumcised men participating in the visual inspection with acetic acid sub-study

		Circumcised (n=143)		Uncircumcised (n=124)		
Risk Factor	N	n	%*	N	%*	P-value†
Age (years)						
≥24	70	37	25.9	33	26.6	0.96
22-23	115	61	42.7	54	43.6	
≤21	82	45	31.5	37	29.8	
HPV DNA positivity‡						
HR-positive	77	30	22.7	47	39.8	0.01
LR-positive	28	17	12.9	11	9.3	
Negative	145	85	64.4	60	50.9	
Education**						
Secondary or tertiary	169	87	60.8	82	66.1	0.37
Primary or none	98	56	39.2	42	33.9	
Employment status**						
No Income	175	97	67.8	78	62.9	0.40
Income	92	46	32.2	46	37.1	
Condom use last 6 months						
>50%	108	53	41.7	55	53.4	0.07
≤50%	66	44	34.6	22	21.4	
Never	56	30	23.6	26	25.2	
Marital Status						
Not living with partner	197	100	69.9	97	78.2	0.12
Living with partner	70	43	30.1	27	21.8	
Age at first intercourse (years)						
16-21	91	47	35.6	44	36.4	0.90
8-15	162	85	63.6	77	63.6	
Partners in last 12 months						
0-1	121	56	39.7	65	52.4	0.04
≥2	144	85	60.2	59	47.6	
HSV-2-seropositive						
No	158	89	86.4	69	84.2	0.67
Yes	27	14	13.6	13	15.8	
Lifetime # of female partners						
1-4	87	45	37.2	42	37.2	0.42
5-7	62	36	29.7	26	23.0	
≥8	85	40	33.1	45	39.8	

HPV: human papillomavirus; HR: high-risk; LR: low-risk; HSV-2: herpes simplex virus type 2
*Percentages do not include missing values
† P-value comparing circumcised versus uncircumcised men using Pearson's chi-square test
‡Men with HPVX infections were excluded from high and low-risk HPV categorizations unless a high-risk HPV type was detected.
**Assessed at the baseline visit

Table 5.3 Prevalence of flat penile lesions after 3% acetic acid application by penile site among circumcised and uncircumcised men from Kisumu, Kenya

	Overall (N=267)		Circumcised (N=143)		Uncircumcised (N=124)		P-value[†]
	n	%[*]	n	%[*]	n	%[*]	
All sites combined	33	12.4	1	0.7	32	26.0	<0.001
Foreskin[‡]	27	10.2	1	0.7	26	21.0	<0.001
Frenulum	9	3.4	0	0.0	9	7.3	<0.001
Glans	7	2.6	0	0.0	7	5.7	0.004
Missing	1				1		

*Percentage of men does not include missing values
[†]Comparing circumcised and uncircumcised men using Fisher's exact test
[‡]Foreskin remnant among circumcised men

Table 5.4 Age-adjusted prevalence odds ratios for risk factors of penile lesions among 267 Kenyan men at the 24-month visit

		Flat penile lesions* n=33; HPV%=77.4%		Papular Lesions n=129; HPV%=39.2%		Pearly Penile Papules n=183; HPV% = 41.1%	
Risk Factor	N	n	OR (95% CI) †	n	OR (95% CI) †	n	OR (95% CI) †
Age (years)							
≥24	70	9	1.0 (Reference)	31	1.0 (Reference)	41	1.0 (Reference)
22-23	115	10	0.7 (0.3, 1.7)	59	1.3 (0.7, 2.4)	87	2.2 (1.2, 4.2)
≤21	82	14	1.4 (0.6, 3.5)	39	1.1 (0.6, 2.2)	55	1.4 (0.7, 2.8)
Circumcision status							
Uncircumcised	124	32	1.0 (Reference)	43	1.0 (Reference)	76	1.0 (Reference)
Circumcised	143	1	0.02 (0.003, 0.1)	86	2.9 (1.7, 4.7)	107	1.9 (1.1, 3.2)
HPV DNA positivity‡							
Negative	145	7	1.0 (Reference)	76	1.0 (Reference)	103	1.0 (Reference)
Positive	112	24	5.5 (2.2, 13.3)	49	0.7 (0.4, 1.2)	72	0.7 (0.4, 1.3)
LR-positive	28	2	1.6 (0.3, 8.0)	17	1.4 (0.3, 3.2)	18	0.7 (0.3, 1.7)
HR-positive	77	22	8.0 (3.2, 19.9)	27	0.5 (0.3, 0.9)	48	0.7 (0.4, 1.3)
HPV DNA positivity in glans‡							
Negative	163	8	1.0 (Reference)	87	1.0 (Reference)	119	1.0 (Reference)
Positive	95	23	6.3 (2.7, 15.0)	39	0.6 (0.4, 1.0)	56	0.5 (0.3, 0.9)
LR-positive	29	3	2.3 (0.6, 9.3)	18	1.4 (0.6, 3.2)	18	0.6 (0.3, 1.3)
HR-positive	64	20	8.9 (3.6, 21.7)	20	0.4 (0.2, 0.8)	36	0.5 (0.3, 0.9)
HPV DNA positivity in shaft‡							
Negative	188	16	1.0 (Reference)	94	1.0 (Reference)	128	1.0 (Reference)
Positive	69	15	2.9 (1.3, 6.3)	31	0.8 (0.5, 1.5)	47	1.1 (0.6, 1.9)
LR-positive	25	6	3.3 (1.1, 9.6)	11	0.8 (0.3, 1.8)	15	0.7 (0.3, 1.6)
HR-positive	39	9	3.2 (1.3, 7.9)	16	0.7 (0.4, 1.5)	28	1.4 (0.6, 3.0)
HPV 16/18/31 viral load**							
HPV negative	145	7	1.0 (Reference)	76	1.0 (Reference)	103	1.0 (Reference)
Low	22	4	4.3 (1.1, 16.6)	9	0.6 (0.3, 1.6)	14	2.0 (0.9, 4.3)
High	14	4	7.6(1.8, 32.8)	4	0.4 (0.1, 1.3)	8	1.3 (0.8, 2.2)

Table 5.4 continued

Risk Factor	N	Flat penile lesions* n=33; HPV%=77.4%		Papular Lesions n=129; HPV%=39.2%		Pearly Penile Papules n=183; HPV% = 41.1%	
		n	OR (95% CI)†	n	OR (95% CI)†	n	OR (95% CI)†
Education††							
Secondary or tertiary	169	17	1.0 (Reference)	80	1.0 (Reference)	113	1.0 (Reference)
Primary or none	98	16	1.6 (0.8, 3.5)	49	1.1 (0.7, 1.8)	70	1.2 (0.7, 2.1)
Employment status††							
No Income	175	20	1.0 (Reference)	85	1.0 (Reference)	122	1.0 (Reference)
Income	92	13	1.4 (0.7, 3.2)	44	1.0 (0.6, 1.6)	61	0.9 (0.5, 1.5)
Condom use last 6 months							
>50%	108	13	1.0 (Reference)	50	1.0 (Reference)	77	1.0 (Reference)
≤50%	66	4	0.5 (0.1, 1.5)	32	1.1 (0.6, 2.0)	46	1.1 (0.5, 2.1)
Never	56	11	1.7 (0.7, 4.2)	26	1.0 (0.5, 2.0)	35	0.8 (0.4, 1.6)
Marital Status							
Not living with partner	197	26	1.0 (Reference)	99	1.0 (Reference)	141	1.0 (Reference)
Living with partner	70	7	0.8 (0.3, 1.9)	30	0.7 (0.4, 1.3)	42	0.6 (0.3, 1.1)
Age at first intercourse (years)							
16-21	91	11	1.0 (Reference)	48	1.0 (Reference)	62	1.0 (Reference)
8-15	162	22	1.1 (0.5, 2.4)	76	0.8 (0.5, 1.3)	111	1.0 (0.6, 1.8)
Partners in last 12 months							
0-1	121	20	1.0 (Reference)	60	1.0 (Reference)	79	1.0 (Reference)
≥2	144	13	0.5 (0.2, 1.0)	68	0.9 (0.6, 1.5)	103	1.4 (0.8, 2.3)
HSV-2-seropositive							
No	158	21	1.0 (Reference)	82	1.0 (Reference)	111	1.0 (Reference)
Yes	27	2	0.5 (0.1, 2.3)	12	0.7 (0.3, 1.6)	19	0.9 (0.4, 2.3)
Lifetime # of female partners							
1-4	87	14	1.0 (Reference)	37	1.0 (Reference)	54	1.0 (Reference)
5-7	62	4	0.4 (0.1, 1.1)	36	1.9 (1.0, 3.6)	44	1.5 (0.7, 3.1)
≥8	85	14	1.0 (0.4, 2.2)	38	1.2 (0.6, 2.1)	60	1.7 (0.9, 3.2)

Table 5.4 continued

Risk Factor	N	Flat penile lesions* n=33; HPV%=77.4% n	OR (95% CI)†	Papular Lesions n=129; HPV%=39.2% n	OR (95% CI)†	Pearly Penile Papules n=183; HPV% = 41.1% n	OR (95% CI)†
Years of sexual activity							
0-3	63	7	1.0 (Reference)	33	1.0 (Reference)	46	1.0 (Reference)
4-5	64	12	1.9 (0.7, 5.2)	28	0.7 (0.4, 1.5)	41	0.7 (0.3, 1.5)
6-7	73	7	0.9 (0.3, 2.9)	31	0.7 (0.4, 1.4)	52	1.0 (0.4, 2.0)
8-14	52	7	1.3 (0.4, 4.6)	31	1.7 (0.7, 3.8)	33	0.8 (0.4, 2.1)

OR: Odds ratio; CI: confidence interval; HPV: human papillomavirus; HR: high-risk; LR: low-risk; HSV-2: herpes simplex virus type 2
*Visible after the application of 3% acetic acid
†Odds ratios adjusted for age categorized as ≥24, 22-23 and ≤21 years
‡Men with HPVX infections were excluded from high and low-risk HPV categorizations unless a high-risk HPV type was detected.
**High HPV viral load defined as HPV 16, 18 and/or 31 >250 copies/scrape in the glans or shaft HPV specimens. All other specimens containing HPV 16, 18 or 31 were considered to have low HPV16/18/31 viral load.
††Assessed at the baseline visit

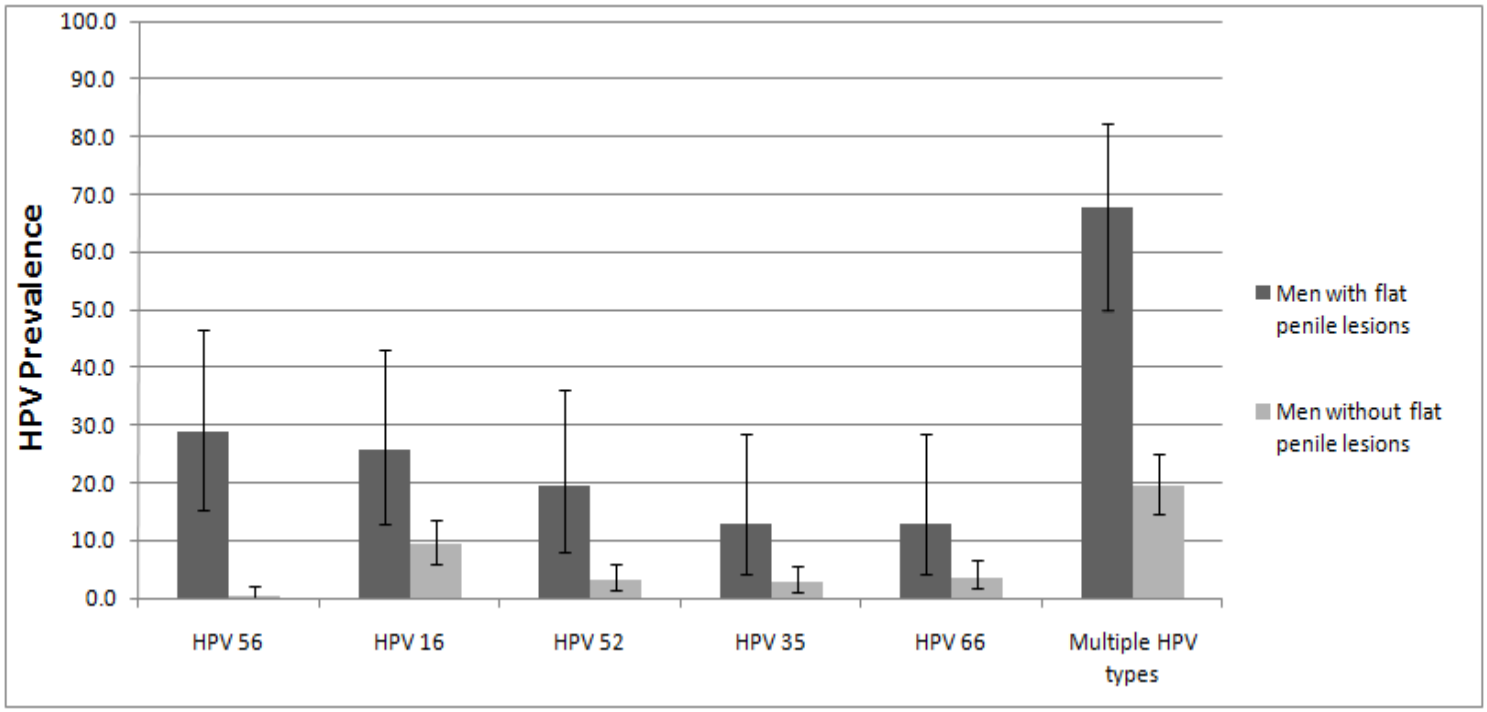

Figure 5.1: Human papillomavirus (HPV) prevalence and 95% confidence intervals for the five most common HPV types among men with flat penile lesions compared to men without flat penile lesions.

CHAPTER 6.
CONCLUSIONS

Summary of Findings and Public Health Significance

The first aim of this dissertation was to investigate the natural history of HPV infection in a cohort of uncircumcised Kenyan men aged 17-24. The IR for the first HPV type detected was 24.3/1,000 person-months. As hypothesized, this was within the range of previously reported IR estimates (18-35/1,000 person-months) (36;37;39). The natural history of HPV infection among men may be different than that previously described for women, as men appear to have a higher incidence of HPV infection compared to women. Unlike women, who generally show lower rates of clearance for high versus low-risk HPV types, men in our study showed a similar pattern of high and low-risk types.

HPV16 was the most prevalent HPV type (9.2%) in this population and also had the highest incidence with a rate of 5.5/1,000 person-months. The high rate of acquisition of HPV16 has a clear implication for cancer risk among men and their sexual partners as HPV16 is the most common HPV type found in penile cancer among men (2), cervical, vulvar and vaginal cancers among women (1;19) and anal and oropharyngeal cancers among both sexes (3;4). IRs for penile cancer in less developed countries including those in Latin America and sub-Saharan Africa are among the highest worldwide (82).

The second aim of this dissertation was to determine the association between penile lesions and HPV DNA positivity, HPV viral load and socio-demographic risk factors of ascertained HPV-associated penile lesions. As hypothesized, lack of male circumcision and high HPV16/18/31 viral load were strongly associated with increased odds of HPV-associated flat penile lesions. A higher number of sexual partners in the previous 12 months was also associated with higher odds of flat penile lesions in age-adjusted univariate analyses but was not significant after controlling for age, circumcision and HPV infection. Although condom use was associated with flat penile lesion regression in a previous study (57), condom use was not associated with the prevalence of flat penile lesions in this study.

Flat penile lesions could be a useful parameter for evaluating efficacy of prophylactic HPV16/18 vaccines, given their strong association with high-risk HPV and HPV16/18/31 viral load. Additionally, because prophylactic HPV vaccines may not be readily available to men in developing countries and current HPV vaccines do not include all high-risk HPV types, circumcision may provide a useful intervention to prevent against HPV-associated penile lesions and ultimately ICC in developing countries. Male circumcision was associated with higher odds of papular lesions and pearly penile papules, which might be due to a tissue reaction after the circumcision procedure (81).

Three RCTs of male circumcision and HIV acquisition have been conducted in sub-Saharan Africa to date with results indicating that male circumcision reduces the risk of HIV acquisition by approximately 60% (6;83;84). Male circumcision has also been found to significantly reduce the acquisition of HSV-2 infection and the

prevalence, acquisition and clearance of HPV infection (43;77). Because male circumcision has been found to have a strong reduction in the acquisition of HIV and other STIs, its reduced association with HPV-associated penile lesion prevalence further emphasizes the utility of male circumcision as an intervention for HIV/STI prevention in developing countries.

A systematic review of the acceptability of male circumcision in sub-Saharan Africa among men aged 13-80 years by Westercamp and Bailey in 2006 indicated that more than half of uncircumcised men in most studies would be willing to become circumcised if it was safe and effective against HIV incidence (85). Circumcising men around puberty or as infants were the preferred ages for circumcision. There are many remaining challenges regarding the implementation of male circumcision in developing countries however, including the need for extensive training and resources to ensure safety (86). Because male circumcision is not 100% effective against HIV/STI acquisition and HPV-associated flat lesions, comprehensive intervention programs are also needed to prevent men from increasing their sexual risk behaviors after circumcision (87).

Strengths and Limitations

Strengths

The findings in this dissertation are the first to characterize the natural history of low-risk HPV infections among men from sub-Saharan Africa. Few prospective studies have described the natural history of HPV infection among men (Table 2.1), and to my knowledge, this is the largest study among men to date. A sensitive

GP5+/6+ PCR assay was used to detect a wide range of HPV types in a central laboratory allowing the comparisons of natural history studies among women. Separate HPV testing for glans and shaft specimens also allowed for stratified analyses by anatomical site.

The results of the second aim of this dissertation, to my knowledge, are the first to investigate the association between circumcision and HPV-associated flat penile lesions, and the only study to investigate risk factors of HPV-associated penile lesions among men from Africa. Study advantages also included the inclusion of data on numerous potential risk factors, a sensitive PCR assay to detect a broad range of HPV types and a real-time PCR assay to detect viral loads from three high-risk HPV types. Penile lesions are often diverse in their presentation, which can influence the accuracy of peniscopy (51). All photos taken of penile lesions were double read in Amsterdam for quality control in order to prevent the misclassification of penile lesions.

Limitations

The 12-month interval between each visit was a limitation of the natural history analyses. Participants could have acquired a new HPV infection and cleared it before the subsequent follow-up visit. Incident HPV infections could have also occurred and persisted until they had the opportunity to be detected at the 12 or 24-month follow-up visits. Thus, IRs for the first HPV type detected likely underestimate the actual IR of HPV infection. We were also unable to determine if a participant had a persistent HPV infection, or cleared and re-acquired another HPV infection of the

same type within the 12-month interval. This may have led to an overestimation of HPV persistence over a 12-month period in our study as a participant who cleared an infection and re-acquired an HPV infection with the same HPV type during the 12 month period also met the definition for having a persistent infection detected. A reliable median duration of clearance could also not be estimated due to the length of the interval between study visits, as the median time to clearance in two previous studies was 3-6 months.

There was a potential lack of complete ascertainment of penile HPV infection since this study used HPV DNA results from sampling two anatomical sites (the shaft and the glans/coronal sulcus). A validation study among a subset of men from this study population indicated that sampling from the urethra did not increase the sensitivity of HPV detection, but samples from the scrotum were not included in this study (34). Sampling from the scrotum however, has not been shown to substantially increase the sensitivity in HPV detection over that found in the shaft and glans/coronal sulcus, especially among uncircumcised men (33). Sampling variability in penile exfoliated cell collection was another potential limitation in this study. All clinical officers were trained and followed a standard protocol for HPV sample collection in order to reduce any sampling variability both between and within subjects.

A limitation of all natural history studies of HPV infection among men, including this study, is the lack of complete ascertainment of HPV-associated penile lesions. Because flat penile lesions are associated with higher HPV viral loads, screening for these lesions in natural history studies among men could help

differentiate a positive HPV result with a productive HPV infection from one with a low HPV copy number or HPV contamination from a sexual partner (10). However, HPV-associated flat lesions are difficult to detect since they are not visible without the application of acetic acid and given the low percentage of participation in this study, implementing VIA exams in order to screen for these lesions can also be difficult.

The loss to follow-up and non-adherence to circumcision assignment were also potential limitations of the natural history analyses. Compared to all other men with a baseline HPV result (n=780), men missing 12 or 24-month HPV results or circumcised before their 24-month visit (n=322) were similar in respect to their baseline characteristics except that they had a slightly lower prevalence of *C. trachomatis* infection (1.9 vs. 4.8%, respectively; p=0.03).

Beta-globin positivity was relatively low in this study population, especially in the shaft specimens. Beta-globin may not be the optimal control for HPV DNA testing in men as penile exfoliated cells, especially those from the shaft, are more keratinized and may contain less human DNA compared to cervical cells (18). Results in this dissertation did not differ substantially when analyses were restricted to beta-globin positive samples, which is consistent with the idea that HPV copies amplified by PCR techniques can often be higher than those of the beta-globin gene (18).

The generalizability of our results may be limited given that participants in this study were a subset of men from one region in Kenya who met criteria to participate in a RCT that entailed circumcision (e.g. aged 18-24, uncircumcised and

HIV seronegative at enrolment). Compared to circumcised men, uncircumcised men may be more likely to acquire (43) and less likely to clear HPV infections (42;43), thus the findings from the first aim of this dissertation should be compared to those of circumcised populations with caution. Additionally, the relatively small age range of included participants limited the assessment of HPV clearance by age.

There was also a low participation rate (19%) for the VIA exam. Men in the VIA exam were more likely to have ≥2 sexual partners in the past 12 months compared to all other men enrolled in the RCT. However, men in the VIA sub-study did not have a higher baseline prevalence of HPV or other STIs and the number of sexual partners between the 12 and 24-month visits was not associated with penile lesions in this study after controlling for age and circumcision status. Although selection bias appeared to be minimal, our findings should be interpreted with caution as this was a relatively small subset of men from the main RCT. The small sample size of men who participated in the VIA exam (n=267) also limited the number of ascertained penile lesions. For example, since there were only two cases of condyloma acuminata detected, a risk factor analysis for this type of lesion could not be conducted.

Penile lesions were diagnosed by colposcopy, as taking biopsies of the ascertained lesions was considered too invasive of a procedure for this study population to be nested within a randomized, controlled study design. HPV infection could not be detected directly from the biopsy specimen and the histological diagnosis of penile lesions could not be determined. Previous reports of flat penile

lesions indicate that these lesions are usually hyperplasia or low grade penile intraepithelial neoplasia, although a minority of lesions can be high grade (10;51).

Potential risk factors of penile lesions, including condom use and number of sexual partners, were obtained through interviews as part of the main RCT. Self-reported data may not be reliable if men exaggerated or failed to disclose information about important risk factors. All interviews were conducted by male counselors who were trained to be respectful and ensure confidentiality in order to help participants feel more comfortable in reporting personal information.

Only three of the large number of HPV types were assessed for viral load in this study. Participants categorized as having low HPV16/18/31 viral load may have had a high viral load for an HPV type that was not assessed as our sample was too small to restrict the analysis to men with single HPV infections. Thus, viral load analyses should be interpreted with caution.

Future Research Directions

While only a limited number of studies have investigated the natural history of HPV infection among men, even fewer studies have investigated multiple risk factors of HPV incidence and/or persistence in men (*see Chapter 2*). Future studies investigating possible risk factors of acquisition and persistence of HPV infection among men are needed from more diverse study populations. Studies are especially needed from sub-Saharan Africa where no risk factor data are currently reported, except for one RCT that investigated the effect of male circumcision on the incidence and clearance of HPV infection in Uganda (43;44). Investigators are

currently evaluating the efficacy of circumcision in male to female HPV transmission among men enrolled in the Uganda RCT and their female sex partners (43).

Based on previous cross-sectional analyses of baseline data from uncircumcised men screened for the main RCT in Kenya, laboratory-diagnosed *C. trachomatis* and *N. gonorrhea* infections were risk factors of HPV DNA prevalence (18). It remains unknown, however, whether C*. trachomatis* or *N. gonorrhea* infections increase the risk of incident HPV infections in men, and whether men with HPV and *C. trachomatis* or *N. gonorrhea* co-infections are more likely to have persistent HPV infections.

Flat penile lesions were strongly associated with high-risk HPV infection and increased HPV16/18/31 viral load, thus circumcision may also reduce male to female high-risk HPV transmission. Follow-up studies are needed to determine if there is an effect of HPV-associated penile lesions on HPV transmission between sexual partners.

Finally, future studies are needed to determine if there are associations between laboratory-diagnosed STIs and HPV-associated penile lesions. The prevalence of STIs other than HPV and HSV-2 infection were low in the VIA study population. Thus important associations between penile lesions and other STIs including *C. trachomatis* and *N. gonorrhea* might have been missed.

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