Wound Healing and Resumption of Heterosexual intercourse Following Voluntary Medical Circumcision of Adult Males in Kisumu City, Kenya

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PHD THESIS

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other university

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ABSTRACT

Background

Although male circumcision reduces men's risk of acquiring HIV heterosexually, the procedure may increase HIV transmission if sex is resumed before the wound is fully healed. World Health Organization therefore recommends 42 days post-circumcision sexual abstinence to allow for complete healing. Unfortunately, the exact time to complete healing and its determinants are not fully understood and some men are reported to engage in sex before completing 42 days of abstinence. This study evaluated post-circumcision wound healing and its predictors in order to determine whether the 42-day post-circumcision sexual abstinence recommended by WHO is optimal. Compliance with guidance on abstinence and condom use at resumption of sex were also assessed. The effect of circumcision on penile viral shedding among HIV-positive men was examined to gauge the risk of HIV transmission following circumcision of HIV-positive men.

Methods

A total of 215 HIV-negative and 108 HIV-positive men were circumcised by the forceps guided method and their post-circumcision wounds assessed for healing at weekly intervals for seven weeks and at 12 weeks. The wound was certified healed if dry with edges completely apposed all round and no gaps, disruptions, scabs or stitch sinuses.
Each participant was asked at every follow-up visit if he had engaged in sex since circumcision and whether he used a condom at the first sex following surgery. Reported intercourse was recorded in terms of the number of post-operative days that had elapsed before it occurred; and classified as early sex if it occurred before 42 days. Plasma and penile lavage samples for viral shedding studies were collected from HIV-positive men before circumcision and at each follow-up visit.

Results

Cumulative proportions of men healed were 64.7% at week 4, 83.1% at week 5, and 94.1% at week 6. There was no difference in the hazard of healing between 108 HIV-positive and 108 age-matched HIV-negative men (HR 0.91 95%CI 0.70-1.20). Post-operative infection was associated with delayed healing in both HIV-positive and HIV-negative men (HR 0.48 95%CI 0.23-1.00). Overall 37.7% (120/318) of men reported sex before 42 days and 18.8% (60/319) reported sex before complete healing. Only 7% of the men had unprotected sex before complete wound healing. Risk factors for sex before healing included being married or having two or more sex partners in the past one year and consistent alcohol consumption
Circumcision of HIV-positive men was followed by a significant rise in penile HIV viral shedding, which declined to undetectable levels by 6 weeks after surgery (MANOVA; $p<0.0001$). In 96.6% of HIV-positive men there was no viral shedding after certification of wound healing.

Conclusions and recommendations

Given that 94.0 % of men are healed within 42 days after circumcision regardless of their HIV status and there is virtually no viral shedding after healing, the WHO guidance of 6 weeks sexual abstinence after circumcision should be maintained and applied to HIV-negative and HIV-positive men. Because 5.9% of the men remained unhealed at 42-days post-circumcision, condom use at resumption of sex should be emphasized for all men. Post-circumcision wounds should however be examined on the 7^{th} post-surgery day to exclude post-operative infection and tight sutures which are both associated with longer time to healing. Adherence to the 42-day abstinence period and condom use at every sexual intercourse within three months following circumcision should minimize the risk of HIV spread in the immediate post-circumcision healing period.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
BMGF	Bill and Melinda Gates Foundation
CDC	United States Centers for Disease Control and Prevention
EIA	Enzyme Immunoassay
GOK	Government of Kenya
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
ERC	Ethical Review Committee
MC	Male Circumcision
MOH	Ministry of Health
STI	Sexually Transmitted Infections
VMMC	Voluntary Medical Male Circumcision
UNAID	Joint United Nations Program on AIDS
UNITID)	University of Nairobi Institute of Tropical and Infectious Diseases
HSV	Herpes Simplex Virus
ANOVA	Analysis of Variance
HB	Hemoglobin
PBS	Phosphate Buffered Saline
RNA	Ribonucleic Acid
JHPIEGO	Johns Hopkins Program for International Education in Gynecology and Obstetrics
WHO	World Health Organization

DEFINITION OF TERMS

Circumcision-	Complete or partial surgical removal of the foreskin.
Complete Wound Healing-	A wound was certified healed if there was complete apposition of edges, no gaps, no stitch sinuses and dry.
Early Sex after circumcision-	Sex before completing 42 days of abstinence.
Sex before Healing-	Sex before certification of wound healing by a clinician.
Penile Viral Shedding-	Detectable viral load in penile coronal lavage sample.

CHAPTER 1: INTRODUCTION

1.1 Background information

Three randomized controlled trials conducted at Rakai in Uganda, Orange Farm in South Africa and Kisumu in Kenya between 2000 and 2006 conclusively demonstrated that medical circumcision reduces men's risk of acquiring HIV heterosexually by about 60%.[1,2,3,4] Consequently, World Health Organization (WHO) and the Joint United Nations Program on AIDS (UNAIDS) endorsed medical male circumcision (MC) in March 2007 as an additional HIV prevention measure for regions with high prevalence of heterosexually acquired HIV and low circumcision rates such as Eastern and Southern Africa.[5] Mathematical models have predicted that routine male circumcision across sub-Saharan Africa could prevent up to six million new HIV infections and three million deaths within two decades.[6,7] Although modeling studies have shown that implementation of large-scale Voluntary Medical Male Circumcision (VMMC) programs can significantly reduce the population level transmission of HIV and are cost-effective, the timely realization of the predicted public health benefits depends on rapid scale up of MC programs, high uptake of MC by sexually active HIV-negative men and minimizing HIV related risk behaviors and risk compensation after MC.[8, 9,10,11] In Kenya the largest impact of scaled-up MC programs was projected for Nyanza Province, where HIV prevalence is highest and MC rates are lowest.[12,13] Therefore in 2008, the country launched the first phase of MC program in Nyanza province with an objective of increasing the proportion of men aged 15-49 years who are circumcised from 84% to 94% by 2013. The overall strategy was to roll out MC for HIV prevention in three overlapping phases.

The first phase was to focus on communities that do not traditionally circumcise and to prioritize MC for sexually active men with the goal of achieving high coverage as quickly as possible. The second phase was intended to focus on improving safety and correct incorporation of HIV prevention messaging among traditionally circumcising communities while maintaining high coverage in non-circumcising communities. The third phase was designed to incorporate Early Infant Male Circumcision (EIMC) as a component of the national MC program for sustained high population coverage.

Kenya's MC services are offered in line with the WHO guiding principles which include recommendation of six weeks sexual abstinence after circumcision to allow for complete wound healing before initiation or resumption of sex and allows provision of MC to HIV-positive men who seek the services and are clinically eligible.[14,15]MC is provided as a package of services consisting of HIV testing and counseling (HTC), screening and treatment of Sexually Transmitted Infections (STI), provision and promotion of condoms, counseling on risk reduction and safe sex, and surgical excision of foreskin under local anesthesia using forceps guided, dorsal slit or sleeve resection method.[14] Men are counseled to abstain from sex during the first 42 days following circumcision because intercourse during this time is likely to occur before complete wound healing and may be associated with increased risk of coital injury and of transmitting or acquiring HIV.[16,17,18]

1.2 Problem Statement

Since 2008 when the roll out of MC programs for HIV prevention started, several published studies assessing sexual behavior in the immediate post-circumcision period have reported that resumption of sex before the recommended 42-day abstinence period is common among adult men.[16,18,19,20,] Reported proportion of circumcised men engaging in sex before completing the recommended abstinence period of 42 days varies widely from 3.9% to 30.7 %. But the risk it poses for HIV transmission or acquisition has not been evaluated adequately because published studies did not accurately record the timing of first post-circumcision sex relative to complete wound healing and whether a condom was used in such instances. The 42-day post-circumcision sexual abstinence recommended by WHO is based on the assumption that virtually all men will be healed within 42 days after surgery but not on any published studies of wound healing after circumcision. Even though MC programs have adopted the 42-day post-circumcision abstinence, it is important to validate this guidance through systematic investigation to determine how long it actually takes for post-circumcision wounds to heal including its predictors.

Based on the recommendation of 42-day post circumcision abstinence, previous publications on sexual behavior in the post-circumcision period defined any sexual intercourse occurring before 42 day post-surgery as ***"early sex"***.[16, 20,19] But given that the wound in some men may be fully healed before 42 days, whereas others may not be fully healed within this period, the term ***"sex before wound healing"*** is introduced in this thesis to describe instances of sexual intercourse before certification of wound healing by a clinician, regardless of whether they occur before or after 42 days. This distinction between early sex and sex before healing is maintained throughout this thesis.

Focusing on the risk of HIV transmission in the immediate post-circumcision period, sex before wound healing should be the primary concern rather than early sex because some men are completely healed before 42 days and instances of early sex in such cases may be relatively safe. Conversely, men with delayed wound healing past 42 days may be at an increased risk of HIV acquisition or transmission even if they abstain for 42 days as recommended. Therefore abstinence for 42 days does not by itself guarantee reduced risk of HIV infection unless the wound is fully healed within this period and condoms are used if complete healing is not ascertained. Sex before complete wound healing could increase the risk of HIV acquisition by men because the unhealed wound provides an easy portal of entry for the virus. It can also increase the risk of HIV transmission from HIV-positive men to their sex partners through increased viral shedding. Both the risk of HIV acquisition and transmission can be mitigated through condom use. Therefore, the issue of greatest concern here should be unprotected sex before complete wound healing. Thus, if unprotected sex before complete wound healing is common, it may increase the overall risk of HIV spread after circumcision and erode the HIV prevention benefits of the intervention.[11,19,21]

Though high rates of early sex have been reported, to date no study has assessed the post-circumcision wound healing and resumption of sexual activity at intervals frequent enough to establish the temporal relationship between complete wound healing and resumption of sex.[19,20]Furthermore, differences among the published studies in the definitions, frequency of observations for ascertainment of wound healing and in methodologies for collecting data on resumption of sex make it difficult to determine the real extent of the problem of early sex in relation to complete wound healing and the factors that contribute to it.

Herman-Roloff et al., (2012) interviewed 1,344 newly circumcised adult males in Kisumu, Kenya at 28–45 days post-surgery to determine how long they abstained before resuming sex and conducted genital exam to ascertain status of wound healing at the time of interview.[20] About 30.7% of the men in that study reported engaging in sex before 42 days after MC and self-reported condom use among them was 44%. Participants in that study were interviewed about resumption of sex and examined to ascertain wound healing only once between day 28 and day 45 post-surgery. This may have resulted in underestimation of the proportion of men resuming sex before 42 days because participants who resumed sex before 42 days but after the interview were missed in the count for early sex. And since status of wound healing was assessed only once at the time of interview, it was not possible to determine whether the wound healing occurred before or after resumption of sex in those who were already healed and had engaged in sex by the date of interview. It was also not possible to determine this temporal relationship for those who were not yet healed and had not resumed sex by the time of interview.

A study published by Hewett et al., (2012) used observational data collected as part of Zambia's male circumcision program to measure sexual behavior in the post-surgical wound healing period and found that 24% of circumcised men engaged in sex early before 42 days and 82% reported at least one instance of unprotected sex.[19] Men were interviewed about resumption of sex only once at 42 days. The temporal sequence between healing and resumption of sex could not be determined because the study did not include wound assessment to certify healing.

A widely cited study by Wawer et al., (2009) among serodiscordant couples in Uganda found that HIV-positive men who resumed sex before complete wound healing were at a higher risk [relative risk (RR) = 2.92; 95% confidence interval (CI) = 1.01 to 8.46] of infecting their female partners than those who waited for complete healing.[18] Participants were followed up between 24 and 48 hours, 5 and 9 days, 4 and 6 weeks, and 6, 12, and 24 months post-surgery. They were asked if they had resumed sex and their wounds were inspected for healing at each visit. Such a follow-up schedule is not sufficiently frequent to adequately assess the relative timing of complete healing and resumption of sex.

Wound healing was only certified at scheduled post-operative visits so the long intervals between visits made it difficult to accurately determine the timing of complete healing. Sex before healing was therefore prone to misclassification.

An analysis of pooled data from three randomized controlled trials of male circumcision to reduce incidence of HIV showed that the proportion of circumcised men reporting sex before 6 weeks varied widely in research settings from 3.9% in Kisumu, 5.4% in Rakai and 22.5% in Orange farm.[16]Assessment for resumption of sex and certification of wound healing following circumcision were conducted from week 4 to week 6 and at 3 months depending on the study site. Accurate classification of participants who had sex before complete wound healing was not possible because of infrequent assessments for certification of healing.

To address the shortcomings of previous studies, this study assessed post circumcision wound healing and resumption of sex at weekly intervals from date of circumcision till complete healing and at 12 weeks. In this way, it was possible to determine the temporal sequence between complete wound healing and resumption of sex for each participant and to determine whether the WHO-recommendation of 42 days post-circumcision sexual abstinence is optimal. In summary, this study assessed how long it takes for circumcision wounds to heal and the level of compliance with current guidance on post-circumcision sexual abstinence with a shift of emphasis from early sex to sex before healing, which is more relevant for programs.

Another important concern relating to risk of HIV transmission in the post-circumcision period is the inevitable circumcision of HIV-positive men in program context. As MC program is rolled out, HIV-positive men will inevitably be circumcised with unknown HIV status. Furthermore some HIV-infected men aware of their status may choose circumcision for non-HIV prevention benefits such as improved hygiene, reduced risk of phimosis, paraphimosis, genital ulcer disease, HSV-2 and HPV.[22,23,24]

While the HIV testing rate among men seeking MC in Kenya is approximately 92%, the remaining 8% who are circumcised with unknown HIV status will become more significant as the program is rolled out.[25] Additionally, according to WHO and Kenya national guidelines for MC, clinically eligible men who test positive for HIV should not be denied circumcision.[14,15]For example, during Kenya's first rapid results initiative for jump starting MC program in 2009, about 79% of the men who tested HIV-positive and knew their test results opted for circumcision and underwent surgery.[26]

Overall, there is limited data on the effect of MC on plasma viral load or penile viral shedding, both of which may influence the transmissibility of the virus from HIV-infected men to their sex partners.[27,28,29,30]In addition to breaching dermal integrity, MC causes inflammation and stress, which may lead to increased penile viral shedding and amplify the risk of HIV transmission in the post-circumcision period.[31,32,33,34] Wawer et al., (2009) reported a significant increase in plasma viral load (+0.20 log10 copies/mL, p=0.002) within 4 weeks post-circumcision among ART-naive men.[18] It is possible that the post circumcision increase in plasma viral load could lead to increased viral shedding and higher infectivity, but no published study has reported the effect of circumcision on penile HIV viral shedding.[35, 36, 37]This study therefore assessed changes in CD4+T-cell counts, HIV viral load and penile viral shedding after medical circumcision of HIV-positive men which may have a bearing on their risk of transmitting HIV during the healing period.

1.3 Objectives

General objective

To assess time to resumption or initiation of sex following medical circumcision of adult males and to validate the optimum duration of post-circumcision sexual abstinence based on how long it takes for post circumcision wounds to heal.

Specific objectives

1. To establish how long it takes for post-circumcision wounds to heal in HIV-negative and HIV-positive men aged 18 –35 years and identify risk factors for delayed healing.
2. To determine the level of compliance with instructions on post-circumcision sexual abstinence among adult men and identify factors that influence resumption or initiation of sex within the first three months after medical circumcision.
3. To determine if time to wound healing in HIV-positive men varies as a function of CD4 + T-cell counts at circumcision.
4. To establish if circumcision of HIV-positive men is associated with significant change in penile viral shedding.

1.4 Research questions

a) Is the 42-day post circumcision sexual abstinence recommended by WHO and adopted by MC programs adequate for complete wound healing among both HIV-negative and HIV-positive men?
b) What are the predictors of delayed post-circumcision wound healing and of early sex and sex before complete wound healing among adult men?

1.5 Hypotheses

H_{O1}

There is no difference between rate of early sex and sex before healing among newly circumcised men in Kisumu. (Rejected)

H_{A1}

There is a difference between rate of early sex and sex before healing among newly circumcised men in Kisumu

H_{O2}

There is no difference in proportion of men healed by weekly intervals from circumcision among HIV-negative compared to HIV-positive men. (Accepted)

H_{A2}

There is a difference in proportion of men healed by weekly intervals from circumcision among HIV-negative compared to HIV-positive men

H_{O3}

There is no difference in viral shedding among ART naïve HIV-positive men from before to after circumcision. (Rejected)

H_{A3}

There is a difference in viral shedding among ART naïve HIV-positive men from before to after circumcision.

1.6 Justification

This study was justified on the following grounds:

- It would provide data for validating the WHO recommendation of 42 days sexual abstinence after circumcision
- It would provide more accurate information on how long it takes for post-circumcision wounds to heal in HIV –positive and HIV –negative men through more frequent assessments than in previous published studies
- It would generate detailed behavioral and biomedical data for assessing risk associated with sex during the first 3 months

CHAPTER 2: LITERATURE REVIEW

2.1 Prevalence of Male circumcision

Male circumcision is the complete or partial surgical removal of foreskin from the penis.[38] The term "circumcision" is derived from the Latin word circumcidere (meaning "to cut around"). It is one of the oldest and most common surgical procedures worldwide, and is undertaken for religious, cultural, social and medical reasons.[39,40] According to WHO and UNAIDS report on global trends and determinants of MC prevalence in 2007, approximately 30% of males in the world are estimated to be circumcised and two thirds of those circumcised are Muslims. The report also identifies religion, ethnicity, perceived health and sexual benefits, and the desire to conform to social norms as the common determinants of circumcision. Religion is not cited as a major determinant of male circumcision except in Islam and Judaism, while other religions including Hinduism, Buddhism and modern Christianity appear to have a neutral stance towards the practice. In the same report, neonatal circumcision is reported to be common in Israel, the United States of America, Canada, Australia and New Zealand, and in much of the Middle East, Central Asia and West Africa, but is uncommon in East and Southern Africa, where median age at circumcision varies from boyhood to the late teens or twenties. It concludes that, in several countries, prevalence of non-religious circumcision has undergone rapid increases and decreases, reflecting cultural mixing and changing perceptions of health and sexual benefits. [41]

In Eastern and Southern Africa, the prevalence of MC has mainly been determined by ethnicity and there is a belt across 13 countries with mainly Bantu speaking groups that do not traditionally practice MC and constitutes the zone of highest HIV prevalence in Africa.[42] These 13 countries were the first to be prioritized for roll out of male circumcision for HIV prevention in Africa (Figure 1).

The countries are Botswana, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe. The Gambella province in Ethiopia was subsequently added, making Ethiopia the 14th VMMC priority country.

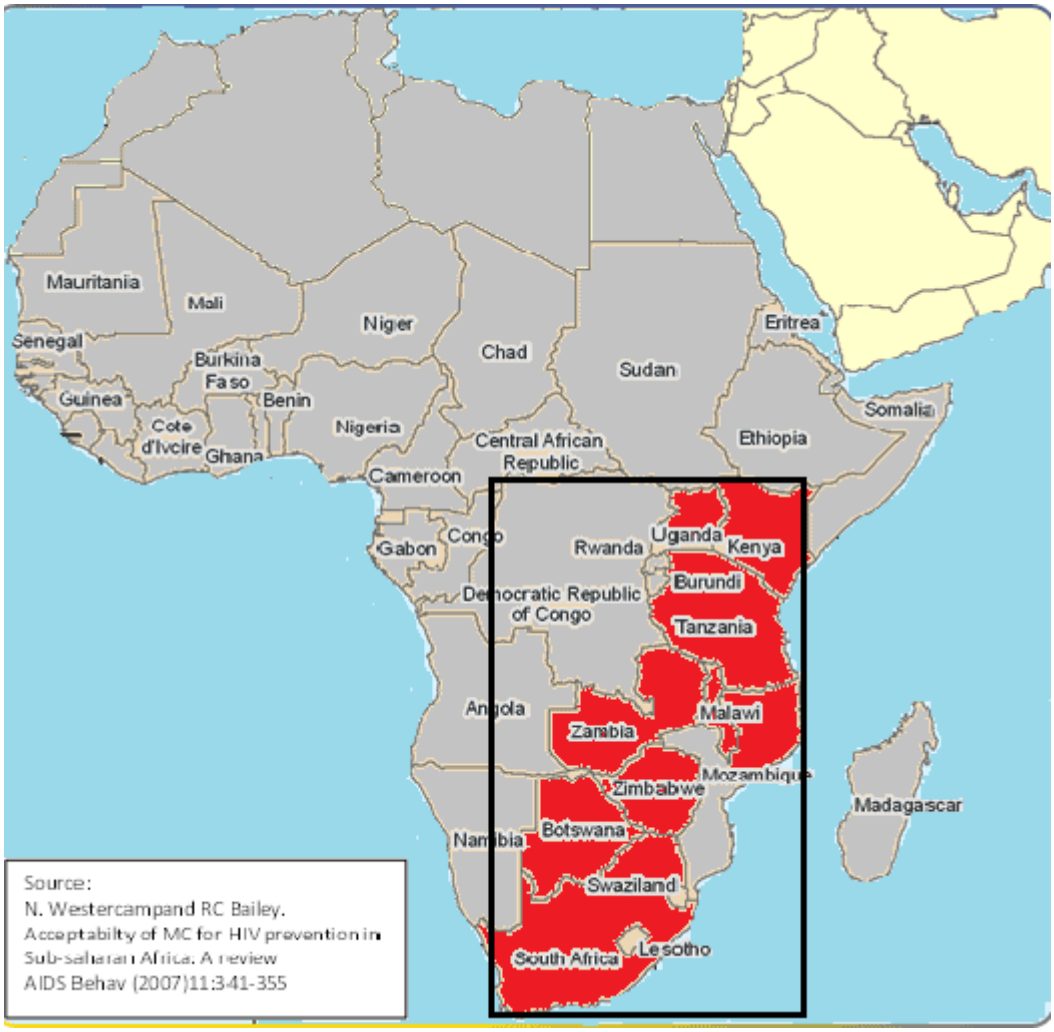


Figure 1: Fourteen African countries prioritized for male circumcision program roll out

Many international and local agencies have supported the role out of VMMC for HIV prevention in these fourteen priority countries. The President's Emergency Fund for AIDS Relief (PEPFAR) and the Bill and Melinda Gates Foundation (BMGF) have both provided significant financial support. United Nations agencies (WHO/UNAIDS) have supported these countries in the scale up of VMMC by helping to develop normative guidance, tools and policy advice such as clinical guidelines and the Joint Strategic Action Framework to Accelerate the Scale up of VMMC for HIV prevention in Eastern and Southern Africa 2012-2016.[43, 44]

In Kenya, the first AIDS indicator survey (KAIS, 2007) reported that an estimated 85% of the males aged 15-64 years were circumcised.[12] KAIS (2007) also showed that the national prevalence of HIV among circumcised men aged 15-64 years was 3.9% (95%CI: 3.3-4.5) compared to 13.2% (95%CI: 10.8-15.7) among uncircumcised men in the same age bracket. Nearly half of all uncircumcised men were Luos who live in Nyanza region, where circumcision prevalence was lowest (46.4%) and HIV prevalence was highest (14.9%). In addition to the Luos, other traditionally non-circumcising ethnic groups in the country include the Turkana and Teso. Areas with the highest concentration of traditionally non-circumcising populations were the highest priority in Kenya's first national strategic plan for VMMC roll out from 2008 to 2013.[45]

In the majority of traditionally circumcising ethnic groups in Kenya, circumcision is an integral part of a rite of passage from boyhood to manhood, although originally it may have been a test of bravery and endurance.[40] In some of the communities, circumcision under no anaethesia in the local context is also associated with factors such as masculinity, self-identity and promotes social cohesion for boys of the same age who are circumcised at the same time.[46] The overall national plan for the traditionally circumcising communities was to influence practice of circumcision in these setting to embrace safety and to incorporate age-appropriate HIV prevention messages.

Based on comparison of KAIS data in 2007 and 2012, the proportion of men aged 15-64 in Kenya who reported being circumcised increased from 85% to 91%. The proportion of circumcised men increased across all regions of the country, with the highest increase of 18.1 and 9.0 percentage points in the VMMC priority regions of Nairobi and Nyanza respectively.[47] With over 750,000 males circumcised and over 90% of the country VMMC target for the first five years achieved, Kenya is now transitioning to the second phase of VMMC program roll out for a five year period from 2014 to 2019. During this second phase, emphasis will be on maintaining high prevalence of circumcision among men aged 15 -49, introduction of EIMC for long term sustainability and interventions to make MC safer in traditionally circumcising communities.[48]

2.2 Male circumcision for HIV prevention

Fink (1986) observed that male circumcision could reduce the risk of HIV transmission. [49] Three years later, Cameron et al., (1989) found more than 8-fold risk of HIV acquisition among uncircumcised male clients of female sex workers in Nairobi, Kenya.[50] Subsequently ecological data revealed a correlation between lack of male circumcision and higher prevalence of HIV in many populations.[6,51,52,53,54,55]

A prospective study of discordant couples conducted by Quinn et al., (2000) in Rakai, Uganda, reported no seroconversions among 50 circumcised male partners of HIV-positive women, compared with an incidence of 17 per 100 person-years among 137 couples where the male partner was uncircumcised.[56]But since confounding effects in these studies were not fully excluded, randomized controlled trials of MC to prevent HIV infection were recommended. [57,58]

Three randomized controlled trials of male circumcision for HIV prevention were therefore conducted at Orange farm in South Africa, Rakai in Uganda and Kisumu, Kenya between 2001 and 2006.[1,2,3]Each study randomized uncircumcised heterosexual HIV-negative males into two groups, one of which was then circumcised. The two groups were similar in all other aspects and were followed up for 21-24 months. The incidence of HIV was significantly lower among the circumcised men with a relative risk of infection corresponding to a protective effect of approximately 60 percent. In the Kisumu trial, the protective effective effect of circumcision against HIV acquisition was sustained at 58% at 72 months, similar to overall findings of other trials under conditions of randomization.[59]

Possible biological explanations for this protective effect include removal of high concentration of cells susceptible to HIV infection in the foreskin, increased keratinization of exposed skin which may be protective against viral infection and reduction in STIs and less likelihood of micro tears and trauma to the foreskin during intercourse.[60,61]

Following review of published results of observational studies and three randomized controlled trial of MC for HIV prevention which showed that the intervention reduces men's risk of acquiring HIV heterosexually, WHO and UNAIDS endorsed MC in 2007 as an additional HIV prevention strategy for settings with high prevalence of heterosexually acquired HIV and low rates of MC.[15]Subsequently, 14 African countries including Kenya were prioritized for roll out of VMMC programs for HIV prevention. [62] Mathematical models at the beginning of MC program roll out predicted that routine male circumcision across sub-Saharan Africa could prevent up to 6 million new HIV infections and 3 million deaths within two decades.[6,7] Since then, there has been a progressive change in the pattern of MC prevalence as countries initiate VMMC programs. Over 1 million men were circumcised in the priority VMMC countries by 2012 with most notable success recorded in Kenya's Nyanza region.[63]

All the 14 countries prioritized by WHO for male circumcision program roll out are at different stages of program implementation with Kenya taking an early lead. Based on WHO guidelines, VMMC is delivered as a package of services that includes screening and treatment of STIs; HIV counseling and testing; risk reduction counseling focused on increasing the correct and consistent use of both male and female condoms, decreasing the number of multiple and concurrent sexual partnerships, and promoting other positive behaviors relevant to HIV prevention; and active referrals of HIV-positive men to care and treatment programs. Service guidelines also recommend abstinence from sex for at least six weeks following circumcision to allow time for complete healing before resuming or initiating sex.

2.3 Wound Healing following medical circumcision in adults

The foreskin which is the primary target for circumcision procedure is attached to the glans penis at the frenulum which is a highly vascularized tissue. In the past, medical circumcision of adults was often performed therapeutically as treatment for a number of conditions including phimosis and paraphimosis. But following conclusive evidence that MC reduces the risk of heterosexual HIV transmission from females to males, a large proportion of circumcisions are now done for HIV prevention.

Currently, MC of adults and adolescents is mainly performed using one of the three conventional surgical methods as detailed in the WHO/UNAIDS/JHPIEGO manual. [64] These are the forceps-guided method, the dorsal slit method and the sleeve resection method. All these methods involve excision of the foreskin under local anaesthesia followed by suturing to control bleeding and to bring the wound edges together. Each method has advantages and disadvantages. For example, the sleeve resection method produces a good cosmetic result but requires higher-level surgical skill and may take longer to perform. Circumcision by the forceps-guided method is relatively easier to perform than the sleeve resection and is more suitable where the procedure is performed by mid-level providers, but the amount of foreskin removed may vary from surgeon to surgeon. Kenya's program of male circumcision uses forceps guided method as the preferred technique of MC.[62]

Recently, innovations in male circumcision device technologies have emerged that have the potential to simplify and reduce the duration of MC procedure; and possibly enhance uptake of services. The devices for adult MC include Shang Ring and PrePex that are currently being evaluated as possible better alternatives to the conventional surgical methods.[65,66]

The MC devices are classified into 3 broad categories depending on their mechanism of action. Clamp devices act by a rapid, tight compression of the foreskin between hard surfaces to achieve haemostasis. Compression achieved in this manner is sufficient to prevent slippage of tissue so that the foreskin can be removed at the same time, or soon after placement of the device. Part of or the entire device is left in situ for a period of time to prevent bleeding. Because the device crushes the foreskin upon placement, and live tissue is excised immediately after device placement, injection of local anaesthesia is required for pain control. On the other hand, elastic collar compression devices achieve slow compression of the foreskin between an elastic ring and a hard surface that is sufficient to occlude circulation and produce tissue ischaemia and progressive necrosis. Part of or the entire device and the foreskin are left in position after device placement until the foreskin is fully necrosed and can be excised. This type of device can be applied without injected local anaesthetic. The third category of in-situ devices use the principle of ligature compression which achieves rapid compression of the foreskin by holding it tightly between a ring placed under the foreskin and a non-rigid ligature tied around the outside of the foreskin. Compression in this manner is sufficient to achieve haemostasis and prevent slippage of tissue so that the foreskin can be removed at the time of, or soon after, device application. Part of or the entire device is left in situ. Because the device crushes the foreskin, and live tissue is excised immediately after placement, local anaesthesia is required.[63]

The Shang ring is a collar clamp device that is applied under local anaesthesia and consists of two locking rings applied to foreskin as high up as possible before the distal portion is excised.[67] It achieves haemostasis by compressing the foreskin between its two non-elastic locking rings and it remains in place for one week.

PrePex is an elastic collar compression device placed under the foreskin just below the corona and achieves circumcision through ring controlled radial compression that causes foreskin necrosis over one week, after which it is removed along with necrotic foreskin. [68] Application of PrePex is done under no injectable anesthesia.

Surgical excision of the foreskin through forceps guided, dorsal slit or sleeve resection method results in a circumferential full-thickness incisional wound near the junction of the glans and shaft of penis. Any significant bleeding points in the fresh wound are ligated before the skin is closed. The wound finally remains as a circumferential incision line whose edges are held together by sutures. Usually the wound heals through repair by first intention unless there is a complication. Healing takes place through three basic stages of inflammation, tissue formation, and tissue remodeling that overlap in time.[69,70,71] The first two stages bridge the gap between the wound edges through formation of a blood clot and inflammation followed by proliferation and migration of dermal and epidermal cells, and matrix synthesis leading to re-establishment of the skin barrier.[72,73,74]The final stage involves tissue remodeling and differentiation that enables full recovery of the skin tissue and restoration of skin integrity including aesthetics.[75]

Immediately after circumcision, the incision site presents an area of compromised dermal integrity and an easy portal of entry to the HIV target cells. Therefore, men who engage in sex before complete healing may be at increased risk of transmitting or acquiring HIV.[76] Additionally, HIV-positive men with unhealed wounds may have high viral shedding, placing their sex partners at high risk of infection. WHO therefore recommends 42 days of sexual abstinence after circumcision to allow for complete wound healing before resumption or initiation of sexual intercourse.

Based on intuition, factors that may affect how long it takes for wounds to healing after circumcision include the method of circumcision, biological and behavioral characteristics such as age, nutritional status, immune status and presence or absence of chronic diseases.[77,78,79,80,81] Consequently, average time to complete wound healing may vary from one region to another depending on distribution of its determinants in the population and the method of circumcision used.

Progression of post circumcision wound healing can be assessed based on histological or clinical criteria. Histological assessment of wound healing considers distinctive immunohistochemical parameters including re-epithelialization, epidermal differentiation, cell migration, proliferation, inflammatory response as well as dermal closure, matrix distribution, and tissue remodeling which are not applicable in routine clinical settings. Clinical assessment of healing after circumcision applied in this study involved examination for approximation of edges, absence of gap or stitch sinuses, and maturation of any scar tissue present.

2.4 Resumption of sexual intercourse following medical circumcision

The WHO recommendation of 42 days post-circumcision sexual abstinence is intended to minimize the risk of resuming sex before the post-circumcision wound is fully healed. But studies have shown that married and cohabiting men tend to resume sex earlier than the recommended 6 weeks abstinence and with a good chance of intercourse before complete healing.[18,19] It is possible that the recommendation of 6 weeks sexual abstinence after circumcision may discourage some men, particularly those who are married, from seeking circumcision services.

On the other hand, sex before complete wound healing could increase the risk of HIV acquisition by providing a portal of entry for the virus, or of HIV transmission through increased viral shedding. It is conceivable that consistent and appropriate use of condoms may reduce the risk of HIV transmission even in cases of sex before complete healing. However if sex without condoms before complete wound healing is common, it may transiently increase the risk of HIV spread and erode the HIV prevention benefits of VMMC.[21] Most published studies on post-circumcision sexual behavior have defined "early sex" as resumption of sex before 42 days post-surgery and have shown that its prevalence ranges from 3.9% to 30.7 %.[16,19,20]

A study by Herman-Roloff et al., (2011) in Kisumu found that 30.7% resumed sex before 6 weeks post-surgery.[20] Participants were interviewed once any time from 28-45 days post-surgery to determine if they had resumed sex or not. They were also examined at the time of interview to ascertain if their wounds were fully healed.

Being married or cohabitating was the strongest predictor of engaging in early sex. In Zambia, Hewett et al., (2012) found that 24% of circumcised men initiated sex before six weeks and 82% reported at least one unprotected sexual event.[19]Early sex was more likely among men reporting risky sexual behaviors before circumcision. In that study, participants were interviewed at six weeks after circumcision to get self-report on whether they had resumed sex or not. The study did not include wound assessments to ascertain status of healing but recorded time lapse before resumption of sex thereby providing useful insights regarding the risk of sex before healing in the study population.

A study by Wawer et al., (2009) among serodiscordant couples in Uganda found that HIV-positive men who resumed sex before complete wound healing were at a higher risk of infecting their female partners than those who waited for complete healing (RR 2.92; 96%CI 1.01-8.46).[18]

Sex before 42 days post-surgery is discouraged due to its likelihood of occurring before complete wound healing thereby increasing the risk of HIV transmission or acquisition through compromised dermal barrier. Intercourse before 42 days post-surgery is therefore classified as early sex to emphasize the heightened risk of HIV transmission if sex occurs during this period of wound healing.[16,19,20] Although there is no proof that sex before wound healing is associated with increased risk of HIV transmission or that visible healing is sufficient to mitigate this risk, it is logical to expect lower hazard of HIV transmission among men who are fully healed even if they engage in sex before abstaining for 42 days.

Men who are fully healed before 42 days may therefore be deemed not to present an increased hazard of HIV acquisition or transmission. Conversely men with delayed healing may be at increased risk of acquiring or transmitting HIV despite abstaining for the recommended period. For these men, abstaining for the recommended 42 days may still be associated with increased risk of HIV infection if sex occurs without a condom. In summary, long abstinence does not by itself guarantee reduced risk of HIV infection unless the wound is fully healed and condoms are used if complete healing is uncertain. This study examined time to first sex after circumcision in relation to complete healing through weekly interviews and wound examination to ascertain the temporal relationship between these two events regardless of whether resumption of sex occurred before or after 6 weeks.

Two operational definitions of early sex are therefore applied to distinguish between 1) Early sex before the recommended 42 days of abstinence and 2) Sex before complete wound healing which may be before or past 42 days depending on the time taken to achieve complete healing.

2.5 Risk of HIV transmission or acquisition following male circumcision

Although male circumcision reduces men's risk of acquiring HIV heterosexually by 60%, its public health benefits may be mitigated if HIV-positive men engage in sexual intercourse before complete healing of the post-circumcision wound.

Although any sex before completing the recommended period of abstinence may be associated with increased risk of risk of HIV transmission, HIV-positive men who engage in sex before complete healing are of particular interest because they may have increased viral shedding, which may heighten the risk of HIV transmission to their partners.[76,18]

With ongoing rapid scale-up of male circumcision, many HIV-infected men are likely to be circumcised without knowledge of their HIV status. In Kenya, the rate of HIV testing among men seeking circumcision is approximately 92% but all programs may not be that successful. [25] Additionally, according to WHO guidelines, clinically eligible men who test positive for HIV are not denied circumcision.[14] Some HIV-infected men may opt for circumcision for real or perceived health benefits other than HIV prevention, such as improved hygiene, better sexual performance, or to reduce the risk of phimosis, paraphimosis, genital ulcer disease, HSV-2 and HPV.[82,83,84] For example, during Kenya's first rapid results initiative for jumpstarting VMMC in 2009, about 79% of the men who tested HIV-positive opted for circumcision.[26]

Little is known about the effect of circumcision on plasma viral load or penile viral shedding and hence the infectivity of HIV-positive men following circumcision. This poses an important pragmatic challenge since the ongoing scale up of VMMC may involve circumcision of large numbers of HIV-infected men. One study in Uganda reported a significant increase in plasma viral load at four weeks following circumcision of HIV-positive men who had never received any antiretroviral therapy. There was however no increase in viral load following circumcision of men who were already on antiretroviral therapy (ART) or had no detectable viral load in plasma at the time of circumcision. [18]

Assessing the effect of circumcision on penile viral shedding as an indicator of the risk of HIV transmission associated with circumcision of HIV-positive men is therefore critical to MC programs. Changes in plasma viral load and CD4+ t-cell count following surgery are important in such assessments as potential explanatory variables for the relationship between circumcision and penile viral shedding.

CHAPTER 3: MATERIALS AND METHODS

3.1 The study design

This was a prospective cohort study of 215 HIV-negative men in one arm and 108 HIV-positive men in another arm. Each participant was circumcised on the day of enrolment and followed up weekly for seven visits and at twelve weeks. The first participant was enrolled on March 28, 2011 and the last participant completed follow up on February 24, 2012. Men in both arms were circumcised by the forceps guided method and their post-circumcision wounds examined weekly by a clinician till complete healing and at 12 weeks.[14] A wound was certified healed if there was complete apposition of edges, no scab or opening along the incision line, no stitches or suture tracks and was dry. Healing was reported both as the proportion of men healed at each weekly time point since circumcision and as mean time to certification of healing for HIV-negative and HIV-positive men.

Each participant was asked at every follow up if he had engaged in sexual intercourse since circumcision. Those who reported having engaged in in sexual intercourse were asked to state the date when they engaged in sex, type of partner they had sex with and whether a condom was used. Time to resumption of sex was recorded in days since circumcision and reported both as proportion of men who had resumed sex by weekly intervals since circumcision, and as mean time to resumption of sex. Temporal sequence between post-circumcision wound healing and resumption of sex was determined and reported for each participant by comparing time to resumption with time to certification of wound healing.

Blood samples were collected from each study participant for baseline biological profiling and at each follow up for HIV-positive men to monitor viral load in plasma and for CD+ T-cell counts at baseline and at two weeks post-surgery. Penile lavage samples were collected from HIV-positive men at baseline and at all follow up visits to assess changes in penile viral shedding following circumcision.

Relationship between demographic, biological and surgical covariates and the two main outcome variables namely, time to complete healing and time to resumption of sex, were examined using binomial logistic regression, odds ratio, Kaplan Meier methods and Cox proportional hazard models.

3.2 The study site

This study was conducted in Kisumu city where most residents are of Luo ethnic group that does not traditionally practice male circumcision. Kisumu is the third largest city in Kenya. It is situated in Nyanza region at the shores of Lake Victoria, an area with the highest prevalence of HIV (14.9%) and the lowest rate of circumcision in the country, reported as 46.4% in KAIS 2007 and 64.5% in KAIS 2012.[12] Kisumu was selected as one of the study sites for the earlier trials of MC for HIV prevention due to a combination of high prevalence of heterosexually acquired HIV and low prevalence of MC and was later prioritized for initial roll out of VMMC program in Kenya. Community education on MC has therefore been going on at varying levels around Kisumu for over 12 years and the region has reported the largest number of MC performed in the context of research and program in Kenya.[63] Kisumu therefore presented a perfect setting for this study. Participants were recruited among men seeking VMMC services at UNIM clinic within Lumumba Health Center in Kisumu city.

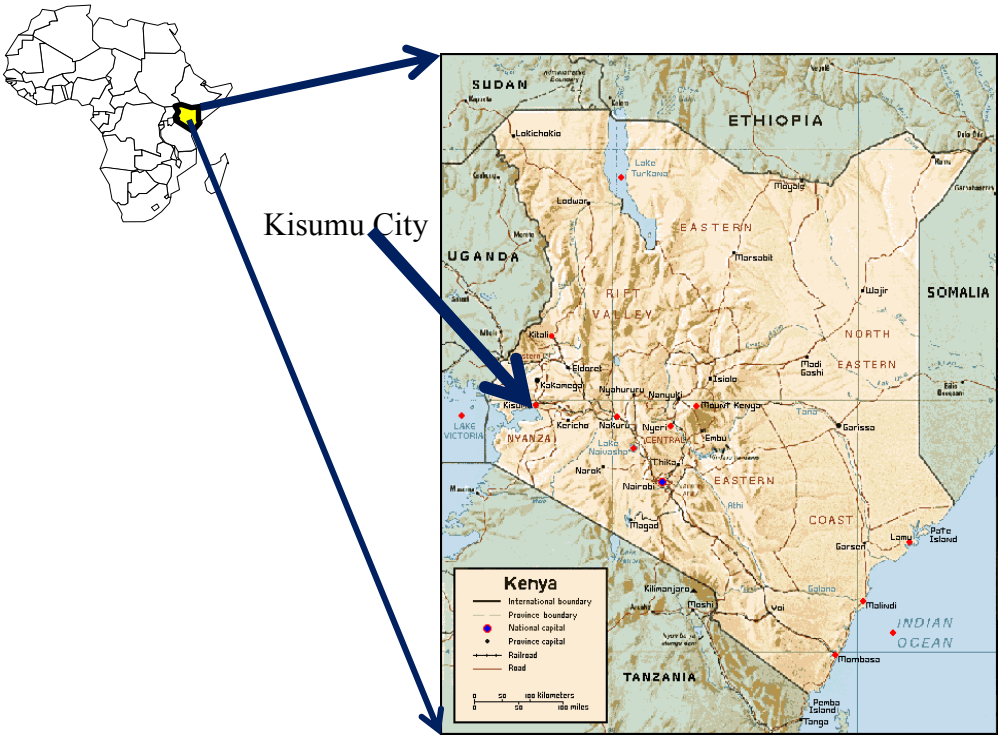


Figure 2: Map of Kenya showing location of Kisumu (Study site)

UNIM clinic provides free medical circumcision to an average of 160 men per month along with a wide range of reproductive health services including treatment of STI, counseling and testing for HIV and referral for HIV treatment and care. The clinic refers HIV-positive clients for care and treatment at a co-located treatment outlet operated by Family AIDS Care and Education Services (FACES) through PEPFAR funding.

3.3 The study participants

From 28 March to December 4, 2011, men seeking circumcision at the UNIM study clinic were verbally informed about the study and were also invited to participate through fliers. HIV-positive participants in this study were recruited among VMMC clients tested at the study clinic and through referrals from local HIV testing centers and clinics that serve HIV-positive men. Men who expressed interest in participating were screened to confirm eligibility for circumcision and for participation in the study.

Inclusion criteria:

- Male aged 18 – 35 years
- Uncircumcised (grades 1 and 2 on examination)
- Residing within or around Kisumu town
- Intending to stay in Kisumu for a period of at least 3-months following circumcision
- No contraindications against circumcision
- Willing to be tested for HIV
- Willing to be circumcised
- Able and willing to provide informed consent for participation in the study
- Able and willing to provide adequate locator information

For the purpose of inclusion in this study men were physically examined to determine the extent of pre-existing circumcision using a four-point scale to classify the foreskin from completely uncircumcised (Grade 1) to completely circumcised (Grade 4) as shown in the table below adapted from Thomas et al. (2011).[85]

Table 1: Grading of circumcision status for inclusion in study

Grade	Description	Decision
1	Foreskin covers one-half or more of the glans; completely uncircumcised	Included
2	Foreskin is past the sulcus, but covers less than one-half of the glans	Included
3	Foreskin is not past the sulcus, but can be extended past the sulcus to cover one-half of the glans without compressing the glans	Excluded
4	Foreskin is completely absent; completely circumcised	Excluded

Exclusion criteria:

- Age below 18 years or above 35 years
- Advanced HIV-infection (WHO Stage 3 or greater) excluded and referred to ART clinic
- Taking a medication that would be a contraindication for elective surgery, such as an anticoagulant or steroid
- Unconfirmed HIV test results (invalid or discrepant)
- Having a known bleeding/clotting disorder (e.g. hemophilia)
- Having an active genital infection
- Having an anatomic abnormality (e.g. hypospadia)
- Having diseases or conditions (e.g. poorly controlled diabetes, sickle cell anemia) that would make elective surgery unsafe
- Participating in another longitudinal research study

3.4 Sample size determination

The overall sample size for this study was calculated to allow point estimates of the proportion of men fully healed by day 42 and the proportion resuming sex before day 42 following circumcision. To calculate the minimum sample size for point estimation of the proportion of HIV-negative men healed by 6 weeks, the estimate of how big this proportion might be was set at 90% based on a previous published studies including one conducted in Rakai Uganda by Kigozi et al., (2008).[86] The proportion of men reporting sex before 42 days was assumed to be about 15% based on previous studies that have reported varied proportions ranging from 3-30%.[16,19,20]

The calculation was carried using the Cochran formula (1977).[87,88]

$$n = \frac{p(1-p)z^2}{d^2},$$

Where n is the sample size, z equals (1-α/2) percentile of a standard normal distribution, d is the absolute precision, p is the expected proportion.

The following assumptions were made:

A 95% confidence level resulting into z =1.96

Expected prevalence of early sex is 15% and that of healing at six weeks is 90%. Here we take p=15% since it gives the optimal sample size for the two parameters.

A precision of 5% led to confidence interval of 10 % to 20%.

Therefore $n = \frac{0.15(0.85)1.96^2}{0.05^2} = 196$

Hence n=196 rounded up to 200.

The sample size was further adjusted upwards by 15% (30) participants to cover for attrition giving a total of 230.

In order to allow for comparisons between HIV-negative and HIV-positive men through matched analysis, the recruitment strategy was designed to enroll 115 HIV-positive men matched for age with HIV-negative men (+/- 2 years).

A further upward adjustment of the sample size was made to enroll additional 100 HIV-negative men to reflect their predominance in the parent population and improve representativeness of the study population. Therefore the final calculated sample size for the study was 330, targeting 215 HIV-negative men and 115 HIV-positive men.

A sample size of 200 HIV-negative and 100 HIV-positive men would allow detection of a 14% difference in complete wound healing between the two groups at any point in time if the proportion of HIV-negative men with complete wound healing is 85%.

If the proportion of HIV-negative men with complete wound healing is 90%, there would be enough power to detect a 12.3% difference in wound healing. Due to good retention and slow recruitment of HIV –positive men, their recruitment was stopped after enrolling 108 men matched with an equal number of HIV-negative men. An additional 107 unmatched HIV-negative men were enrolled for a final sample size of 323 men (108 HIV- positive and 215 HIV-negative).

3.5 Ethical Considerations

Each participant gave a written informed consent as the first step towards enrolment in the study. Confidentiality of study participants was maintained at all times. Each participant was assigned a unique study identification number used by staff when collecting and reporting participant information. The participant's name and locator information were kept separately from his study file and locked in a cabinet with restricted access. The master list linking participants' names to their identification numbers was kept in hard copy and electronic formats in a separate confidential location with restricted access. In all study forms, participants were only identified by a unique alpha-numeric code. Data was entered in a password protected access database with restricted access. The study was approved by the Institutional Review Board of the University of Illinois at Chicago and the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee.

3.6 Study procedures

3.6.1 Enrolment

After screening, all eligible men interested in the study were given an opportunity to participate. They were taken through the consent form and given a chance to ask questions for clarification. If the study staff was satisfied that a potential participant understood the study goals and procedures, he was invited to give a written informed consent either in English, Kiswahili or Dholuo, depending on the participants' preference (Appendix 1). For each HIV-positive man enrolled, a HIV-negative match of same age plus or minus 2 years was enrolled. After enrolling 108 HIV-positive men and matched with 108 HIV negative men, an additional 107 HIV –negative participants were enrolled to provide a better age representation of men seeking VMMC services in Kisumu who tend to be younger and predominantly HIV-negative.

After enrolment, a questionnaire assessing sexual risk behavior, demographic and other characteristics that may be associated with study outcomes was administered in addition to the standard VMMC pre-operative clinical screening (see appendix 8-10). Physical examination data collected at pre-operative screening included temperature, pulse, respiration rate, blood pressure, height and weight.

3.6.2 Circumcision by the forceps guided method

Each participant in this study was circumcised by the forceps guided method.[14] The participant was asked to lie on his back in a comfortable position. After scrubbing and putting on two pairs of sterile gloves, the surgeon prepared the skin with betadine solution, ensuring that the inner surface of the prepuce and glans are clean and dry.

The outer pair of gloves was then removed. The participant was draped with two drapes and a center “O” towel. A dorsal nerve and field block was done using no more than 10 ml of 2% lignocaine without epinephrine. Anaesthetic effect of the nerve block was checked and additional infiltration done if necessary but taking care not to use more than 10ml of lignocaine. The prepuce was then held with two mosquito forceps, one on each lateral aspect, and a curved mark demarcating the intended line of incision was made approximately 1 cm proximal to the coronal sulcus. The prepuce was clamped along the mark with a Kochers clamp while retracting the glans to ensure that it remained proximal to the clamp. The prepuce was then excised along the mark using a surgical blade.

Bleeders were identified and ligated using 3/0 plain catgut. Using 3/0 chromic catgut on a taper 4/8 circle needle, a U-shaped horizontal mattress stitch was made at the frenulum to join the skin at the “V” shaped incision, which was then stitched and tagged with a mosquito forceps. Using vertical mattress stitches, four quarters were tagged, and a vertical mattress stitch added, after which a simple stitch was made centrally between every two mattress stitches (resulting in a total of 16 stitches). The wound was cleaned and dressed with Sofratulle, then with a regular dressing bandage and a strapping.

The participant was advised to rest for 30 minutes, and if stable, discharged home on mild analgesics. The participant was told to come back at any time in case of complications and given a hotline to call when necessary.

The participant was also told to remove dressing at home on day three and guided on how to do so then given an appointment to return for follow up visit on day 7 after surgery. Finally, the participant was told to abstain from sex for at least six weeks or until the wound is certified as completely healed.

3.6.3 Follow-up

After circumcision, each study participant was scheduled for weekly follow up for 7 visits and a final visit at week 12. At each follow up visit, a follow up questionnaire was administered in a face-to face interview and the wound examined for complete healing, tight sutures, infection and other adverse events (Appendix 15). Data was also collected on resumption of sexual activity and other time sensitive post- operative behaviors. An outline of study procedures at each scheduled visit is presented in Table 2.

Any visit that did not occur within two days before or after the scheduled date was classified as missed. Any information collected outside the two-day window before or after the scheduled visit date was not included in the analysis. If a participant failed to turn up on the date of scheduled appointment, tracing action was initiated to locate him and ensure his attendance within two days. Through this strategy 97.1% (2510/2584) of the scheduled visits were completed.

Table 2: Schedule of study visits and procedures

	WK0	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK12
Screening, enrolment (Day 0)									
Administer consent for MC and for participation in study (n=323)									
Administer baseline questionnaire (n=323)	X								
HIV test, HSV2, Hb, Blood Sugar, Serum albumin (n=323)	X								
Pre-operative clinical examination (n=323)	X								
Circumcision by forceps guided method (n-323)	X								
Follow-up (WK1, WK2, WK3, WK4, WK5, WK6, WK7, WK12)									
Examine wound for complete healing (n=323)		X	X	X	X	X	X	X	X
Examine examination for tight sutures(n=323)		X	X	X	X	X	X	X	X
Examine wound for infection(n=323)		X	X	X	X	X	X	X	X
Examine wound for other adverse events (n=323)		X	X	X	X	X	X	X	X
Administer follow up questionnaire (All-323)		X	X	X	X	X	X	X	X
Collection of laboratory specimens									
Blood for CD4+ t cell count (n=108)	X		X						
Blood for plasma viral Load (n=108)	X	X	X	X	X	X	X	X	X
Penile lavage for HIV shedding (n=108)	X	X	X	X	X	X	X	X	X
Finger stick sample for repeat HIV test (n=215 HIV-Ve)									x

WK = week

3.6.4 Monitoring post circumcision wound healing

Following circumcision, all participants were examined weekly for seven visits to monitor wound healing then at 3 months post-surgery. Participants whose wounds remained unhealed at week 7 post-surgery were reviewed weekly till certification of healing. Wound healing was assessed by clinicians using the following operational definition: complete apposition of edges along the incision line, dry, no scab or opening along the incision line, no stitches or suture tracks. The number of post-operative days that had elapsed at the time of certified healing was recorded for each individual.

For analysis purposes, an individual could not be considered healed until he was certified healed at all subsequent visits. Participants were censored if they were either lost-to-follow-up before certification of healing or completed the full 12 weeks of follow-up without achieving complete healing.

3.6.5 Monitoring resumption of sex following adult male circumcision

Following WHO and Kenya National guidelines for medical male circumcision under local anaesthesia, men were advised to abstain from sex for 42 days post-circumcision.[14,15] Participants were asked at each follow-up if they had engaged in sex since circumcision. Those who answered in the affirmative were prompted to provide the date of first post-surgical sexual intercourse and to provide details regarding type of partner, condom use, alcohol use and personal perception of wound healing at the time of resuming sex.

Counseling on behavioral risk reduction including post-circumcision abstinence was reinforced at each follow-up contact. Reported sex before the day of confirmed healing was classified as "sex before healing". For each reported sexual intercourse, the number of post-operative days that had elapsed since circumcision was recorded. Any reported sex that occurred before 42days after circumcision was classified as "early sex".

3.6.6 HIV testing

All participants underwent provider initiated HIV testing in accordance with the Kenya National guidelines for VMMC and national guidelines for HIV testing and counseling.[89,90] HIV testing was done through serial testing approach using Determine ® HIV 1/2 (Abbot Diagnostic Division, Hoofddorp, Netherlands) for screening and SB Bioline HIV ½, 3.0 for confirmation of positive tests results. The testing strategy included tie-breaking with Uni-gold in cases of discrepant test results between determine and Bioline. The strategy for handling discrepant results for first two tests was to use Uni-Gold ® HIV 1 Recombigen (Trinity Biotech Plc. Wicklow, Ireland) as a tie breaker and to take blood specimens for confirmatory testing by double HIV ELISA at the University of Nairobi. Both HIV-positive and HIV-negative participants were informed of their test results and received appropriate counseling before and after circumcision. All HIV-negative men underwent repeat HIV-testing at the completion of the three months follow up. Standard operating procedures for laboratory tests are included in the appendix.

3.6.7 CD4+ T cell counts

CD4+ T-cell counts were performed on blood samples collected from 108 HIV-positive men just before circumcision and at 2 weeks post-surgery, using BD FACSCount™ (Dickinson and Company, Franklin Lakes, New Jersey). Blood was collected in a 4ml EDTA vacutainer (lavender cap) from the participant immediately before circumcision and at two weeks after circumcision. Following collection the tube was gently inverted 15 times immediately to avoid clotting. Blood was transferred to the laboratory from the clinic under ambient conditions for processing within 24hrs from the time of collection.

3.6.8 Viral Load assays in Plasma and penile coronal lavage samples

Blood and penile coronal lavage samples for viral load determination were collected from 108 HIV-positive participants on the day of circumcision, at subsequent weekly follow up for seven weeks or until complete healing of the circumcision wound. The blood samples were collected in EDTA bottles and centrifuged at 1200 revolutions per minute (RPM) for 10 minutes then aliquoted in 1ml croyovials for storage at -80^{o} till assay. To collect penile lavage specimens, 5mls of phosphate buffered saline (PBS; pH7.2) was measured into a 50ml sterile cup and 2.5mls drawn in a fine tipped transfer pipette and used to irrigate the penile coronal area. This irrigation was repeated three times and the wash fluid drained back into the same 50ml cup each time.[91,92,93] The penile lavage samples were aliquoted into 1ml sterile cryotubes then stored at -80^{o} C until assay.

Viral load assay in both plasma and penile lavage was done using COBAS TaqMan/Ampliprep assay version 2.0. (Roche Diagnostics Systems, Branchburg, New Jersey, USA). At assay, after thawing, plasma samples were vortexed and 1ml was ultra-centrifuged for 1 hour at 16,400 rpm at 4° C to pellet the virus. Viral pellets were re-suspended using 1,100µL of the 1X PBS and tested using CobasAmpliprep/CobasTaqman (CAP/CTM) version 2.0 with lower threshold for detection of 20 copies per mL. HIV-1 viral RNA was quantified using HIV-1 QS which compensates for effects of inhibition and controls the preparation and amplification processes. Plasma samples less than 1ml were diluted 1 in 2 with 1x PBS and ultra-centrifuged to pellet the virus. Results were multiplied by the dilution factor. Results were reported in copies per ml. Penile coronal lavage samples were allowed to thaw at room temperature before processing. Samples were ultra-centrifuged at 16400rpm for 1 hour at 4 degrees to pellet the virus and to remove the supernatant (causes inhibition during PCR).

The viral pellets were re-suspended in 1000ul of RNAase free water/DEPC treated water vortexed for 5 seconds and used for viral load testing (the rest of the procedure similar to plasma viral load). No dilution was done (to avoid further dilution on the samples due to low viral load detection rate in lavage).

Since plasma viral load did not change from baseline to week 6 post-circumcision in the first 19 men, additional assays were not run. Viral load assays in penile lavage were done for the same 19 men and for the next 10 enrolled ARV-naïve men. Viral load assays were done at CDC HIV-Research laboratory in Kisumu which is ISO 15189 accredited and whose viral load platform is certified by the Virology Quality Assurance Program (VQA) and College of American Pathologist (CAP). At assay, after thawing, plasma samples were vortexed and 1ml was ultra-centrifuged for 1 hour at 16,400 rpm at 4° C to pellet the virus. Viral pellets were re-suspended using 1,100µL of the 1X PBS and tested using Cobas Ampliprep/CobasTaqman (CAP/CTM) version 2.0 with lower threshold for detection of 20 copies per mL. HIV-1 viral RNA was quantified using HIV-1 QS which compensates for effects of inhibition and controls the preparation and amplification processes. Samples less than 1,000µL were diluted 1 in 2 with 1x PBS and ultra-centrifuged to pellet the virus. Results were multiplied by the dilution factor. Results were reported in copies per ml (see Table 3).

Table 3: Viral load scoring criteria

Result	Interpretation
Target not detected	Report as viral load undetectable
<2.00E+01 cp/ml	Report as viral load undetectable
≥2.00E+01 cp/ml	Report viral copies /ml
>1.00E+07cp/ml	Dilute sample to determine actual viral copies or report as >10,000,000 cp/ml
Invalid flags	An invalid result- repeat the run

3.6.9 Baseline laboratory profiling

In addition to HIV testing according to the Kenya National HIV testing and counseling guidelines, all participants had baseline hemoglobin, serum albumin and blood sugar measurements and were also tested for HSV-2 using Kalon HSV-2 IgG ELISA (Kalon Biological Limited, Andershot, United Kingdom). Serum albumin estimation was done using Roche Cobas c 501 analyzer. Baseline blood sugar, serum albumin and hemoglobin levels were estimated using standard operating procedures provided by the manufactures (Appendix 16)

3.6.10 Data Collection

Male circumcision program data was collected on each study participant using the Ministry of Health-approved national data forms (see Appendix 10). Each participant was interviewed at enrollment by a trained nurse counselor using a standard enrollment questionnaire with a series of questions about their socio-demographic characteristics and sexual behavior.

Laboratory data at baseline and at follow up visits was extracted from source documents and summarized on a standard form completed at relevant visits. A standard follow up questionnaire was administered at visit to collect time-sensitive and behavioral information from week one post-surgery to the end of follow up (Appendix 15). Data analysis was conducted using SAS version 9.2 (SAS Institute, Cary, NC) and intercooled STATA 9.0 (StataCorp, Texas).

3.6.11 Quality assurance

Accuracy of data collection was assured through standardized training of study clinicians and intense pretesting of data collection tools. In order to control for experience of the surgeon, a possibly influential covariate with time to healing, [94] each of the surgeons engaged in this study had previously performed over 500 hundred circumcisions either under the Kenya Ministry of health VMMC program or as part of previous studies at the UNIM clinic. A standard operating procedure was developed for each study activity and compiled into a single manual made available and used by all study personnel. Data quality checks were performed on all source documents at the end of each day by a designated quality assurance officer before data entry. Visits checklists were used to ensure that all expected study procedures were completed before a visit is concluded. All laboratory assays were performed at UNIM, RCTP and CDC HIV-R laboratories all of which are registered by the Kenya Medical Laboratory and Technicians Board and have well documented internal and external quality assurance systems.

3.6.12 Statistical methods

Baseline demographic and behavioral characteristics were summarized using counts and percentages. Analyses examined the relationship between HIV status and time to complete healing in an age-matched cohort of 108 HIV-positive and 108 HIV-negative men and time to healing in 215 HIV-negative men. Kaplan-Meier plots were used to assess time to complete wound healing in the age-matched cohort stratified by HIV status. Cox proportional hazard models were used in both series to examine the relationship between a priori identified potentially important demographic, biological, behavioral, and surgical covariates with the hazard of healing at any time point. Demographic characteristics considered were age and marital status. Biological characteristics were: HIV-status, HSV-2 status, baseline random blood sugar, hemoglobin, serum albumin, body mass index (BMI), and the presence of a post-operative infection. Behavioral characteristics were: baseline alcohol consumption (days/week), physical activity in the first week following surgery (riding a bicycle, digging, and walking long distances), and timing of sexual activity. Surgical covariates considered were: amount of dermis exposed at week 1 (in mm), evidence of tight sutures as clinically assessed at week 1; duration of surgery as a proxy for difficult circumcision, and surgeon cadre (clinical officer vs. nurse).

A priori identified covariates including age, marital status, personal perception of healing, HIV status, education, number of sex partners in the past year and alcohol use were examined for association with sex before healing and early sex. Relative risks and 95% confidence intervals were used to estimate the crude relationship between these covariates and the two main outcome variables.

Log binomial regression was used to estimate adjusted risks and 95% confidence intervals for the outcomes, adjusted for age, marital status, HIV status and number of sexual partners in the last year.

Change in CD4+ t- cell count from baseline to two weeks after circumcision was analyzed using mixed-effects regression so as to account for missing data at baseline and at follow up. Multivariate Analysis of Variance (MANOVA) was used to assess the change in HIV viral load in plasma and in penile lavage following circumcision through six-weeks of follow-up. A correlation analysis explored the relationship between plasma HIV viral load and penile viral shedding. X^2 test was done for association between baseline CD4+ T cell counts (categorized as low or high using a cut-off of 350 cells/µL)and ART treatment and viral shedding, respectively.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter starts with an overview of baseline demographic, biological and behavioral characteristics of the entire study population of 215 HIV-positive and 108 HIV-negative aged 18 to 35 years. Each of the 323 participants was scheduled for weekly post-circumcision visits till week 7 and at week 12; giving a total of 2,584 expected visits, of which 2,510 (97.1%) were completed (Table 4). The expected visits at each weekly time point was 323 and was the denominator for calculating the proportion of completed each week.

Table 4: Number and proportion (%) of completed visits

Week	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**Wk 6**	**Wk 7**	**Wk 12**	**Total**
Completed visits No.	321	316	317	315	316	313	310	302	2510/2584
completed visits %	99.4%	97.8%	98.1%	97.5%	97.8%	96.9%	96.0%	93.5%	97.1%

Participant characteristics are summarized using counts and percentages then presented in Tables 5, 6 and 7. Detailed results are arranged under three broad subheadings corresponding to the main study objectives focusing sequentially on wound healing after circumcision, resumption of sex after circumcision and penile viral shedding in the post-circumcision healing period. Statistical significance is reported only where the *p*-value is less than 0.05 and confidence interval for all reported estimates was set at 95%. Data on healing is presented as cumulative proportion of participants healed by 7 day intervals since circumcision and reflects the frequency of assessments for wound healing.

Data on resumption of sex is reported in days elapsed since circumcision and is reported as cumulative proportion of participants who have resumed sex by 7 day intervals since circumcision. Viral shedding was recorded as viral copies per mL of penile lavage and reported as the proportion of HIV-positive men with detectable viral shedding from the penis by 7 day intervals since circumcision. Mean time to healing and to resumption of sex was reported in days since circumcision. Mean viral loads in penile lavage and plasma are reported by 7 day intervals from date of circumcision till six weeks.

4.2 Screening and enrolment

From March 28 to December 4, 2011, a total of 1,331 men seeking VMMC at UNIM clinic were screened for the study. Out of these, 323 men were enrolled into the study, while the remaining 1,008 were found ineligible for enrollment. The reasons for ineligibility included being aged below 18 years or above 35 years, having a medical condition that met a criterion for exclusion or being a non-resident of Kisumu city. Of the 323 men enrolled into the study, 215 were HIV-negative with the remaining 108 being HIV-positive. Figure 3 shows the participants screening and enrollment scheme.

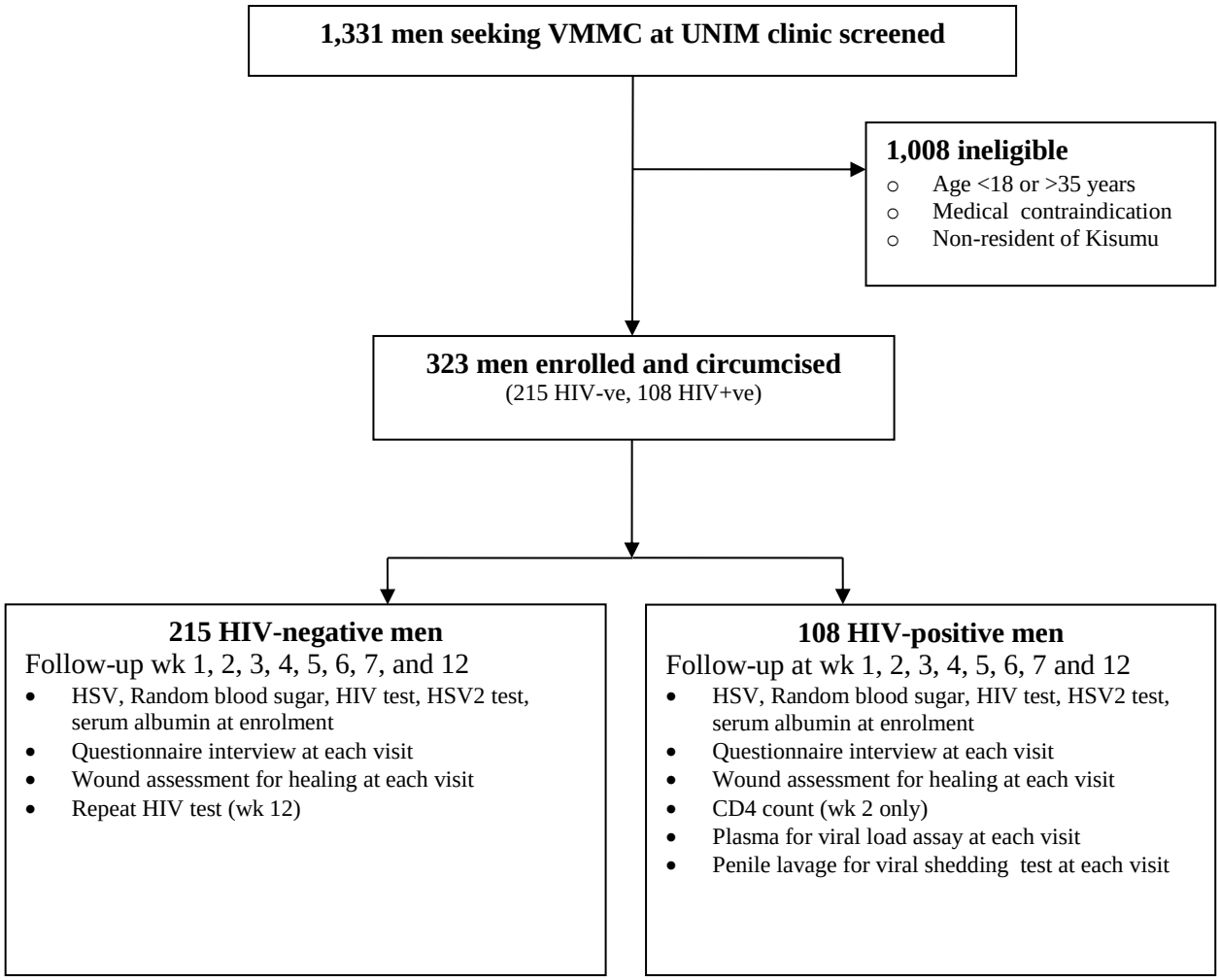


Figure 3: Participants screening and enrolment scheme

4.2.1 Baseline characteristics for entire study population

Table 5: Baseline demographic and behavioral characteristics of all participants (N=323)

Baseline characteristics	n (%)
Reported age at enrollment (years)	
18-24	111(34.4%)
25-29	127(39.3%)
30+	85(26.3%)
Marital status	
Single	174(53.9%)
Married or cohabiting	149(46.1%)
HIV status	
HIV-Negative	215(66.6%)
HIV-Positive	108(33.4%)
Education	
None	3(0.9%)
Primary	151(46.8%)
Secondary	113(35.0%)
Post-secondary	56(17.3)
Ever had sex	
Yes	319(98.8%)
No	4(1.2%)
Ever used a condom	
Yes	267 (83.7%)
No	52(16.3%)
Condom use at last sex	
Yes	117/267 (43.8%)
No	150/267 (56.2%)
Sexual partners in past year	
None	10(3.1%)
One	141(43.6%)
Two	91(28.2%)
Three or more	81(25.1)
Current extramarital partnerships	
Yes	71(47.7%)
No	78(52.3%)
Alcohol use (days per week)	
None	179(55.4%)
Less than one	32(9.9%)
One to two	65(20.1%)
Three or more	47(14.6%)

The median age of the study participants was 26 years (IQR 23-30) and nearly all the participants (319/323) reported ever having had sex. Of those who reported ever having had sex, 84% (267/319) reported having ever used a condom and 44% (117/267) reported using a condom at the last sexual intercourse. None of the four men who had never had sex before circumcision initiated sex during follow up.

Forty six percent (149/323) of the men were married or cohabiting. About half of the married or cohabiting men (71/149) reported an ongoing extramarital sexual partnership. Fifteen percent (47/323) reported consistent alcohol use of three days or more per week. Almost half of the study participants had one or more sex partners in the past one year. Majority of the men (52.3%) had secondary or post-secondary education whereas 46.8% had primary education and only 0.9% had no education.

4.2.2 Baseline biological and behavioural characteristics by HIV status

Out of the 323 men enrolled in the study, 108 were HIV-positive and 215 were HIV-negative. None of the HIV-negative men seroconverted during the three months of post-circumcision follow up. At baseline, there was no difference between HIV-positive and HIV-negative men with respect to the number of sex partners in the past year, alcohol intake, random blood sugar level and serum albumin. HIV-positive men were however more likely to be married, older, have lower haemoglobin level and had more than twice higher rate of HSV-2 compared to HIV-negative men.

Overall, 58.3% of HIV-positive men were married, 56% reported having two or more sex partners in the past one year and 31.5% reported consistent alcohol intake at least once per week. About 38% were already on ART and 25% had reached CD4+ T-cell counts threshold for initiation of ART but were not yet on treatment (see Table 6).

Table 6: Baseline characteristics of study participants by HIV status

	HIV+ (n=108)	**HIV- matches (n=108)**	**p-value (matched analysis)**	**All HIV- (n=215)**
Enrollment age (years)				
18-24	22 (20.4%)	22 (20.4%)	--	89 (41.4%)
25+	86 (79.6%)	86 (79.6%)		126 (58.6%)
Marital status				
Single	45 (41.7%)	50 (46.3%)	p=0.49	128 (59.5%)
Married	63 (58.3%)	58 (53.7%)		87 (40.5%)
Sexual partners in past year				
None	5 (4.7%)	0 (0.0%)	p=0.06	4 (1.9%)
One	42 (39.3%)	45 (42.4%)		96 (45.3%)
Two	27 (25.2%)	36 (34.0%)		64 (30.2%)
Three or more	33 (30.8%)	25 (23.6%)		48 (22.6%)
Alcohol use (days per week)				
None	63 (58.3%)	51 (47.2%)	p=0.18	116 (53.9%)
Less than one	11 (10.2%)	8 (7.4%)		21 (9.8%)
One to two	20 (18.5%)	25 (23.2%)		45 (20.9%)
Three or more	14 (13.0%)	24 (22.2%)		33 (15.4%)
HSV-2 serology				
Positive	81 (75.0%)	50 (46.3%)	p<0.0001	78 (36.3%)
Negative	27 (25.0%)	58 (53.7%)		137 (63.7%)
Random blood sugar (mg/dL)				
< 70	0 (0.0%)	3(2.8%)	p=0.22	4 (1.9%)
70 – 139	103 (95.4%)	102 (94.4%)		203 (94.4%)
140 – 200	4 (3.7%)	2 (1.9%)		6 (2.8%)
>200	1 (0.9%)	1 (0.9%)		2 (0.9%)
Hemoglobin (g/dL)				
<13.0	20 (18.5%)	4 (3.7%)	p<0.001	10 (4.7%)
≥13.0	88 (82.5%)	104 (96.3%)		205 (95.3%)
Serum albumin (g/dL)				
<3.40	10 (9.3%)	1 (0.9%)	p<0.01	5 (2.3%)
3.40 – 5.40	97 (89.8%)	105 (97.2%)		208 (96.7%)
>5.40	1 (0.9%)	2 (1.9%)		2 (0.9%)
CD4 count at baseline (cells/µL)	61 (57.0%)	--	--	--
>350	46 (43.0%)			
≤350				
ART at baseline				
Yes	41 (38.0%)	--	--	--
No	67 (62.0%)			

Table 7 shows baseline characteristics of 108 HIV-positive men whose CD4+ T-cell counts were determined at baseline and at two weeks post-circumcision and of the sub-sample of 29 men whose penile viral shedding was assessed weekly for six weeks. The median CD4+ T-cell count for HIV-positive men was 385 cells/µL (IQR=237.0 - 536.5) and 43% (46/108) had CD4+ T-cell counts below 350 cells/µL. HSV-2 serology was positive in 75% of HIV-positive men compared to 36% in HIV-negative men.

This difference was statistically significant ($p<0.0001$). Even though the overall Haemoglobin and serum albumin levels were essentially within normal range for the entire study population, HIV-positive men had significantly lower Haemoglobin levels ($p>0.001$) and lower serum albumin ($p>0.01$) than HIV –negative men.

Table 7: Baseline characteristics of 108 HIV-positive men and the subsample of 29 men assessed for viral shedding

	HIV+(n=108)	Men assessed for viral shedding (n=29)	X^2 (p-value)+
Age (years)			
18-24	22 (20.4%)	6 (20.7%)	0.02 (0.99)
25-29	44 (40.7%)	12 (41.4%)	
30+	42 (38.9%)	11 (37.9%)	
Marital status			
Single	45 (41.7%)	14 (48.3%)	0.39 (0.53)
Married	63 (58.3%)	15 (51.7%)	
CD4 Count (cells/µL)*			
≤350	46(43%)	14 (48.3%)	0.31 (0.58)
>350	61(57%)	15 (51.7%)	
ART at enrollment			
No	67(62.0%)	29(100.0%)	Not applicable
Yes	41(38.0%)	0	
HSV-2 serology			
Positive	81 (75.0%)	23(79.3%)	0.14 (0.71)
Negative	27 (25.0%)	6 (20.7%)	
Sexual partners in past year ¶			
None or one	47 (44.0%)	14 (48.3%)	1.35 (0.51)
Two	27 (25.2%)	5 (17.2%)	
Three or more	33 (30.8%)	10 (34.5%)	
Alcohol use (days per week)			
None	63 (58.3%)	15 (51.7%)	0.52 (0.92)
Less than one	11 (10.2%)	4 (13.8%)	
One to two	20 (18.5%)	6 (20.7%)	
Three or more	14 (13.0%)	4 (13.8%)	

+X^2 values apply to the difference between the 29 study men and the 79 men not included in assessment for viral shedding

***** One man had invalid CD4 T-cell count results at baseline hence n=107

¶ Missing data for sexual partners for one participant hence n=107

** Men not on antiretroviral treatment at baseline were selected for viral shedding analysis

The subsample of 29 men whose penile viral shedding data were available was similar to the entire population of HIV-positive men.

4.3 Post-circumcision wound healing

The results presented in this subsection address the first specific objective of this study which was intended to establish how long it takes for post-circumcision wounds to heal in HIV-negative and HIV-positive men aged 18 –35 years and identify risk factors for delayed healing.

4.3.1 Percentage of men healed by weekly intervals since circumcision

Out of the 323 men enrolled 301(93.2%) completed 12 weeks of follow-up, and 18 men who did not complete follow-up were certified fully healed during follow-up thereby yielding an overall denominator of 319 for estimation of the proportion who had of sex before healing. The cumulative proportion of men healed was zero at week 1, 1.6% at week 2, 23.0% at week 3, 64.7% at week 4, 83.1% at week 5, 94.1% at week 6, 96.6% at week 7 and 99.7% at week 12 (Table 7). Of the 19 (5.9%) men who remained unhealed at 6 weeks, 12 were healed during the 7th week and 7(2.2%) remained unhealed beyond week 7. Of the 7 who remained unhealed beyond week 7, one man was certified healed at 8 weeks, 5 men missed all visits between weeks 7 and 12 but got healed at some point during this large gap between visits and were certified healed at week 12. Only one man who suffered a severe adverse event of sub-dermal haematoma with wound dehiscence at week one remained unhealed until week 13. This was the only severe adverse event observed among the 323 study participants (0.3%).

Table 8: Cumulative proportion of men healed at weekly time points by HIV status

	HIV-Positive (n=108)		HIV-Negative matches (n=108)		All HIV-Negative(n=215)	
	n returning for visit (%)	Cumulative % healed	n returning for visit (%)	Cumulative % healed	n returning for visit (%)	Cumulative % healed
Week 1	108 (100)	0.0	107 (99.1)	0.0	213 (99.1)	0.0
Week 2	104 (96.3)	1.9	106 (98.1)	0.9	212 (98.6)	1.4
Week 3	105 (97.2)	21.5	107 (99.1)	25.0	212 (98.6)	23.8
Week 4	103 (95.3)	59.3	108 (100)	70.4	212 (98.6)	67.3
Week 5	104 (96.3)	81.1	107 (99.1)	82.4	212 (98.6)	84.1
Week 6	101 (93.5)	93.4	107 (99.1)	92.6	212 (98.6)	94.4
Week 7	100 (92.6)	94.3	105 (97.2)	96.3	210 (97.7)	97.7
Week 12	97 (89.8)	100.0	103 (95.3)	99.1	205 (94.0)	99.5

The proportion of healed men was compared between HIV-negative and HIV-positive men at weekly intervals till week 7 and at week 12. The hazard ratio (HR) for healing between the 108 HIV-positive and 108 age-matched HIV-negative men was 0.91 (95%CI 0.70-1.20) indicating that there was no difference between the two groups. At week 4, 59.3% of HIV-positive men and 70.4% of age-matched HIV-negative men were healed. At week 6, the proportion healed rose to 93.4% in HIV-positive men and 92.6% in HIV-negative men.

The largest difference in proportions healed between HIV-positive and HIV-negative men was observed at week 4, but it was not statistically significant (p= 0.09). Similarly there was no significant difference in mean time to complete healing for HIV-positive men (33 days) and their HIV-negative matches (31 days); p=0.20. Median time to complete healing was 28 days for both groups.

Figure 4 shows a Kaplan-Meier plot of time to complete healing for an age-matched cohort of HIV-negative and HIV-positive men. It shows no difference in time to complete healing by HIV-status for 108 age matched pairs of HIV-positive and HIV-negative men (log-rank test = 0.69, p = 0.41).

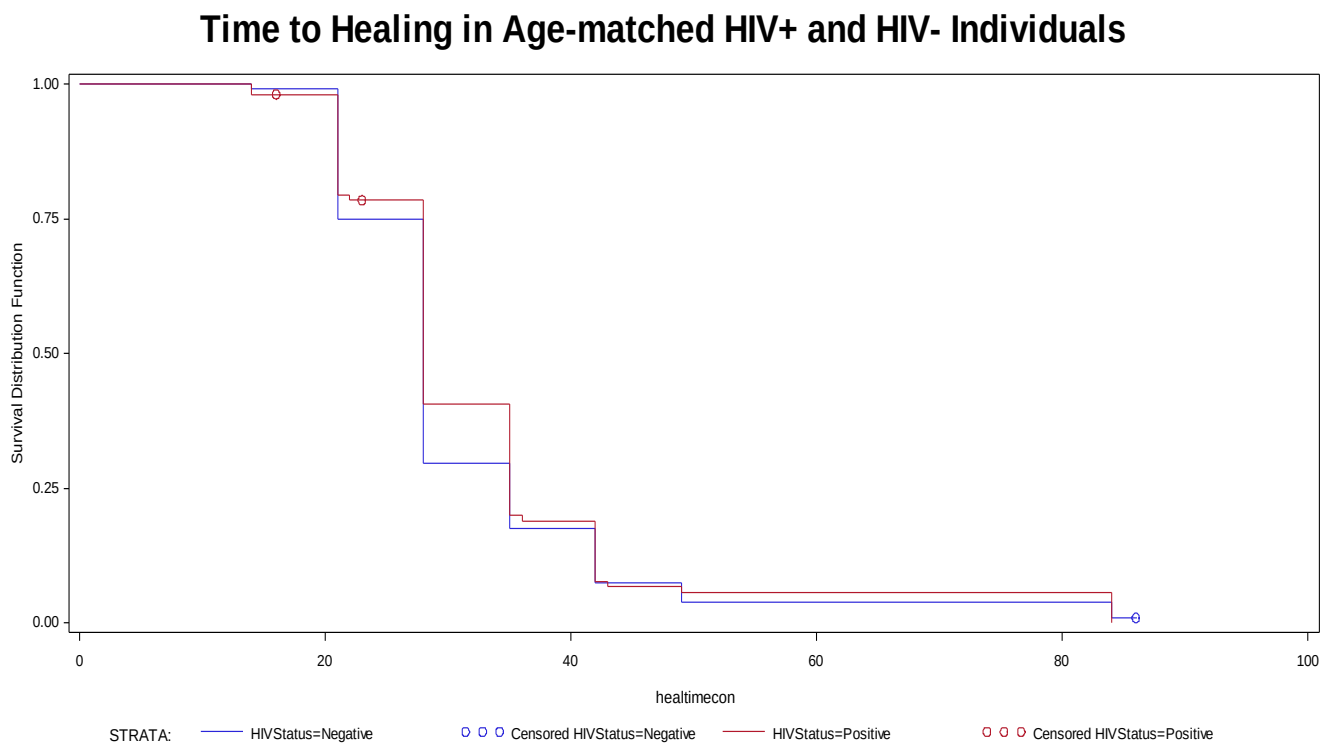


Figure 4: Time to complete wound healing by HIV status (Kaplan Meir plot)

Among 67 ART-naı̈ve HIV-positive men, the mean time to complete healing was 31.1 days for those with baseline CD4+ t-cell count greater than 350 cells/mL compared to 37.1 days in for those with baseline CD4+ t-cell count less than or equal to 350 cells/mL (p = 0.04). There was however no difference in proportions healed at week 6 between those with CD4+ t-cell count above 350 cells/mL versus those with lower counts (94.7% vs 88.9%; p = 0.64).

4.3.2 Factors associated with delayed wound healing

In the age-matched cohort of HIV-positive and HIV-negative men, mean time to complete healing was 45.9 days in individuals who had post-operative infection versus 30.9 days in men who did not (p<0.001). In univariate analysis, post-operative infection resulted in a significantly lower rate of complete healing (HR 0.53, 95%CI 0.32-0.89); see Table 9.

Table 9: Univariate Cox proportional hazard models for complete healing

	Age Matched Analysis for 108 HIV-ve and 108 HIV+ve men H.R. (95% C.I.)	All HIV-negative Participants (n = 214) H.R. (95% C.I.)
Demographic covariates		
Age 25+	--	0.91 (0.69-1.19)*
Married	0.94 (0.71-1.23)	0.86 (0.66-1.14)
Biological covariates		
HIV-Positive	0.92 (0.70-1.20)*	--
HSV2-Positive	0.99 (0.75-1.31)	1.06 (0.80-1.41)
Blood sugar $\geq$ 140 mg/dl	0.77 (0.38-1.55)	0.63 (0.31-1.28)
Hemoglobin $\geq$ 13.0 g/dl	0.95 (0.62-1.45)	0.70 (0.37-1.32)
Serum albumin $\leq$ 3.39 g/dl	0.92 (0.50-1.69)	0.95 (0.39-2.31)
Post-operative infection	0.53 (0.32-0.89)*	0.48 (0.23-1.00)*
Behavioral covariates		
Alcohol consumption $\geq$ 3 days / week	0.79 (0.56-1.13)	0.87 (0.60-1.26)
Physical activity at week 1	0.94 (0.64-1.40)	1.15 (0.76-1.74)
Sexual activity before week 3	0.92 (0.53-1.62)	1.10 (0.58-2.08)
Surgical covariates		
Dermis exposed at week 1 $\geq$ 5.0 mm	0.60 (0.33-1.12)	0.86 (0.35-2.12)
Tight sutures at week 1	0.65 (0.42-0.996)*	0.80 (0.53-1.22)
Surgical time (continuous)	1.02 (1.00-1.03)	1.01 (0.99-1.03)
Nurse vs. clinical officer	1.01 (0.73-1.40)	1.20 (0.88-1.64)

*****Indicates variables included in multivariate models due either to being a primary exposure of interest or a covariate significantly associated with time to healing at the $p<0.05$ level

Tight suture, along with post-operative infection, were included in multivariate modeling. HIV-status, although not significantly associated with time to healing, was also included in the multivariate model. In the multivariate Cox proportional hazard model shown in Table 10, post-operative infection was associated with a reduction in the rate of healing (HR 0.52 95%CI 0.31-0.87) and men with evidence of tight sutures also experienced reduced rates of healing (HR 0.63 95%CI 0.41-0.98). At six weeks post-circumcision, 72.2% of men with post-operative infections were healed compared to 94.9% among men without infection ($p<0.001$). Men presenting with evidence of tight sutures at week 1 also had reduced rates of healing (HR 0.65 95%CI 0.42-0.996), but at six-weeks post-circumcision the difference in proportions healed between men without and with evidence of tight sutures was non-significant (94.2% vs. 83.3% $p=0.07$).

Table 10: Results of multivariate Cox Proportional Hazard Models

	Age Matched Analysis (n = 108 HIV+ and 108 HIV-) H.R. (95% C.I.)	**All HIV- Participants (n = 214) H.R. (95% C.I.)**
Age 25+	--	0.91 (0.69-1.19)
HIV-Positive	0.91 (0.70-1.20)	--
Post-operative infection	0.52 (0.31-0.87)	0.48 (0.23-1.00)
Tight sutures at week 1	0.63 (0.41-0.98)	--

**Models adjusted for other significant variables from univariate analysis ($p \leq 0.05$)*

4.3.3 Men's personal perception of their healing after circumcision

In addition to clinicians' classification of wounds at each visit as healed or not, each participant was also asked whether he perceived his wound as healed or not. Participants' self-perception of their healing and clinician's report on status of the wound were compared at each of the 8 follow up visits and showed agreement only 65% of the time. Overall, in 26 (8.4%) of the 310 cases included in this analysis, the participant reported a perception of full healing before certification by a clinician. In 74(23.9%) of the 310 cases the participant reported healing at the same time as the clinician. In the remaining 210(67.7%) of cases the participant reported a perception of healing later than the clinician. Compared to clinicians, participants tended to believe that wound healing took longer.

4.4 Resumption of sexual intercourse after circumcision

Results presented in this subsection address the second specific objective of the study intended to determine the level of compliance with instructions on post-circumcision sexual abstinence among adult men and identify factors that influence resumption or initiation of sex within the first three months after medical circumcision.

4.4.1 Early sex (before 42 days)

A total of 313 (96.9%) men completed 6 weeks of follow-up and five men who did not complete 6 weeks of follow up resumed sex before exiting; hence the denominator for estimating the proportion of men who had sex before 42 days was 318. None of the four participants who had never had sex before circumcision initiated sex during follow-up. Of the 318 men who either completed 42 days of follow-up or initiated sex before 42 days, 37.7% (120/318) had early sex (Figure 5).

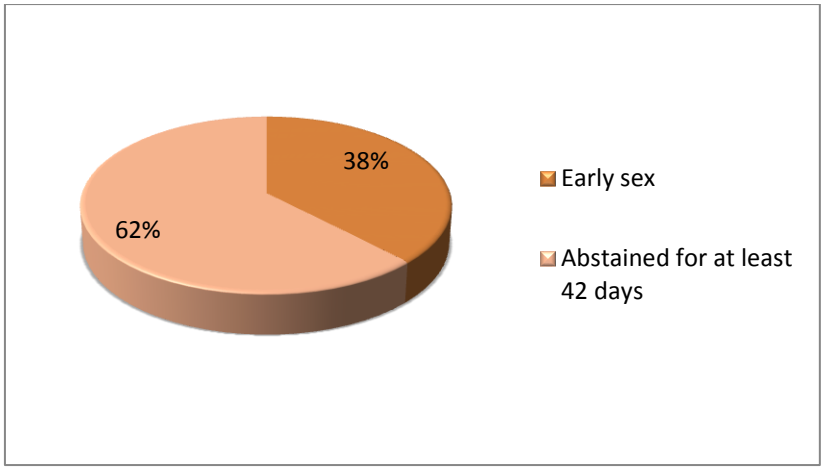


Figure 5: Self-reported early sex among 318 circumcised adults

Of the men who resumed sex early, 50.1% (61/120) were already healed, 39.2% (47/120) resumed sex with a non-regular partner and 15.0% (18/120) consumed alcohol at resumption of sex. The cumulative percentage engaged in sex since circumcision increased from 0.6% at week 2 through 12.5% at week 4 to 37.7% at week 6. Of all the men who resumed sex early, 84.1% (101/120) engaged in sex after abstaining for at least 21 days while 60% (72/120) abstained for at least 28 days. The mean time to resumption of sex following circumcision was 51 days (std error 1.2) while the median was 46 days (IQR 34-74). The proportion of men resuming sex by weekly intervals since circumcision is shown in Table 11.

Table 11: Percentage of men healed or resumed sex and respective condom use by weekly intervals from circumcision

Week since MC	Cumulative % resumed sex	Cumulative % healed	Cumulative % Condom use at resumption of sex(all men)	Cumulative % Condom use by incompletely healed men
WK0	0.0%	0.0%	--	--
WK1	0.0%	0.0%	--	--
WK2	0.6%	1.6%	50.0%	40.0%
WK3	5.0%	23.1%	50.0%	58.8%
WK4	12.5%	64.7%	57.5%	61.9%
WK5	25.3%	83.1%	64.2%	53.9%
WK6	37.7%	94.1%	68.3%	50.0%

4.4.2 Sex before complete wound healing

Of the 319 men who completed 12 weeks follow-up or were certified healed during follow up, 18.8% (60/319) resumed sex before complete wound healing. Fifty four (90%) out of the 60 men who resumed sex before complete healing had sex before 42 days post-surgery and 37 (61.7%) were married. Five of the 60 men classified as having sex before healing had delayed wound healing beyond seven weeks but missed all visits between weeks 7 and 12 and resumed sex at some point during this large gap between the two last visits. It is possible that some or all of them were already healed at the time of resuming sex; four of them reported using a condom at resumption of sex. One man with wound healing delayed past week 7 completed later weekly follow-ups and reported sex before healing, but with a condom.

4.4.3 Condom use at Resumption of sex

Among the 120 men who reported sex before 42 days, 80 (66.7%) used a condom (Table 12). Of the 60 men who resumed sex before complete healing, 37 (61.7%) used a condom. Of the 37 married men who resumed sex before complete healing 23 (62.2%) used a condom and two (5.4%) had first sex after circumcision with an extra-marital partner. There was no difference in condom use rate at resumption of sex among men who resumed before 42 days compared to those who resumed sex before healing (x^2test; p=0.50).

Table 12: Condom use among men who had sex before healing or sex before 42 days

	Early sex (n=318)	Sex before healing (n=319)
Proportion	37.7%	18.8%
Condom use	66.7%	61.7%

While 79.2% (95/120) of the men reporting early sex perceived themselves as already healed, only 63.2% of them were already certified as healed by the study clinicians. Overall, 81.7% (98/120) of the men who resumed sex before 42 days were either already healed (55.0%) or reported condom use at first sex following surgery (66.7%), or both (40.0%).

4.4.4 Men's personal perception of healing at resumption of sex

Since man's perception of their personal healing was recorded at each follow up, the proportion of men who resumed sex while believing that they were not fully healed was determined. About 79% (95/120) of the men who resumed sex early believed that they were fully healed. The remaining 25 men who resumed sex early did so while believing that they were not fully healed.

4.4.5 Risk factors for sex before complete healing and early sex

Univariate analysis of sex before 42 days and sex before healing by baseline characteristics is presented in Table 13. Married men were more likely to engage in sex before complete wound healing (RR=1.84; 95%CI 1.15-2.94), as were older men (RR=2.86; 95%CI 1.46-5.59), HIV-positive men (RR=1.64; 95%CI 1.05-2.58), those who consumed alcohol three or more times per week (RR=2.10; 95%CI 1.30- 3.41) and those who had two or more sex partners in the past year (RR=2.25; 95%CI 1.34-3.67).

Table 13: Univariate analysis of sex before 42 days and sex before healing by baseline characteristics

Baseline characteristics	Sex before 42 days % by category (n=318)	Relative Risk (95% CI)	Sex before healed % by category (n=319)	Relative Risk (95% CI)
Age in years				
18-24	27/108 (25.0%)	Ref.	9/107 (8.4%)	Ref.
25+	93/210 (44.3%)	1.77 (1.24-2.54)	51/212 (24.1%)	2.86(1.46-5.59)
Marital status				
Single	46/171 (26.9%)	Ref.	23/170 (13.5%)	Ref.
Married or cohabiting	74/147 (50.3%)	1.87(1.39-2.51)	37/149 (24.8%)	1.84(1.15-2.94)
HIV Status				
HIV-negative	78/214 (36.5%)	Ref.	33/213 (15.5%)	Ref.
HIV-positive	42/104 (40.4%)	1.11(0.83-1.49)	27/106 (25.5%)	1.64(1.05-2.58)
Sexual partners in past year				
None or one	47/150 (31.3%)	Ref.	17/150 (11.3%)	Ref.
Two or more	73/168 (43.4%)	1.39 (1.03-1.86)	43/169 (25.4%)	2.25(1.34-3.67)
Alcohol use (days/week)				
2 or fewer	95/271 (35.1%)	Ref.	44/272 (16.2%)	Ref.
3 or more	25/47 (53.2%)	1.52 (1.11-2.08)	16/47 (34.0%)	2.10(1.30-3.41)
Education				
Primary or less	68/150 (45.3%)	1.21(0.83-1.77)	34/150 (22.7%)	1.59(0.73-3.22)
Secondary	31/112 (27.7%)	0.74(0.47-1.16)	18/113 (15.9%)	1.12(0.52-2.41)
Post-secondary	21/56 (37.5%)	Ref.	8/56 (14.3%)	Ref.
Ever used a condom				
Yes	104/262 (39.7%)	Ref.	48/264 (18.2%)	Ref.
No	16/56 (28.6%)	0.72(0.46-1.12)	12/55 (21.8%)	1.20(0.68-2.10)
Condom use at last sex				
Yes	50/147 (34.0%)	Ref.	20/148 (13.5%)	Ref.
No	54/115 (47.0%)	1.38(1.03-1.86)	28/116 (24.1%)	1.79(1.06-3.00)
Current extramarital partnership	38/70 (54.3%)	1.16(0.84-1.60)	22/71 (31.0%)	1.61(0.91-2.86)
Yes	36/77 (46.8%)	Ref.	15/78 (19.2%)	Ref.
No				

The level of education was not significantly associated with resumption of sex before wound healing. Early sex was significantly associated with older age (RR=1.77; 95%CI 1.24-2.54), being married (RR=1.87 95%CI 1.39-2.51), having two or more sex partners in the past year (RR=1.39; 95%CI 1.03-1.86) and using alcohol three days or more per week (RR=1.52; 95%CI 1.11-2.08). Level of education and HIV status were not associated with early sex.

Because the effect of marital status is modified by age, a separate multivariable model for single and married men was generated (table 14). Among single men, the largest risk factor for sex before complete wound healing was age. Those aged 25 years and older were at three times greater risk of engaging in sex before healing than younger men (RR=3.36; 95%CI 1.31-8.62). As would be expected, this factor was not significant in married men (RR=0.96 95%CI 0.39-2.32). Drinking alcohol three or more days per week was also significantly associated with sex before wound healing in single men (RR=2.32; 95%CI 1.14-4.75), but not in married men (RR=1.33 95%CI 0.68-2.53). Having sex with two or more partners in the last year was significant in both single men (RR=2.96; 95%CI 1.06-8.29) and married men (RR=1.93 95%CI 1.06-3.49). Multivariate analysis also revealed that being married (RR=1.62; 95%CI 1.16-2.24) and having two or more sex partners (RR=1.34; 95%CI 1.01-1.78) remained significantly associated with early sex after adjusting for important covariates. In summary, those who engaged in sex before the WHO recommended 42-day abstinence were more likely to be married and to have multiple sex partners in the past year.

Table 14: Multivariate analysis of sex before 42 days and sex before complete healing

	Sex before 42 days	**Sex before complete healing**	
Baseline characteristic	Multivariate	Multivariate Single men only	Multivariate Married men only
Age in years			
18-24	Ref.	Ref.	Ref.
25+	1.33 (0.89-1.99)	3.36(1.31-8.62)	0.96(0.39-2.32)
Marital status			
Single	Ref.	--	--
Married or cohabiting	1.62 (1.16-2.24)		
HIV Status			
HIV-negative	--	Ref.	Ref.
HIV-positive		1.01(0.50-2.07)	1.61(0.93-2.78)
Sexual partners in past year			
None or one	Ref.	Ref.	Ref.
Two or more	1.34 (1.01-1.78)	2.96(1.06-8.29)	1.93(1.06-3.49)
Alcohol use (days / week)			
2 or fewer	Ref.	Ref.	Ref.
3 or more	1.26 (0.93-1.69)	2.32(1.14-4.75)	1.33(0.69-2.53)

Variables not significantly associated with resumption of sex at the $p<0.05$ level under univariate analysis were dropped from multivariate analysis. These variables were HIV-status, ever used a condom, and education for sex before 42-days analysis and education and ever used a condom for sex before healing analysis. Condom use at last sex, although associated with both sex before 42 days and sex before healing, was dropped from multivariate analysis as this question was only asked of individuals having reported ever using a condom (n=262 for sex before 42 days; n=264 for sex before healing).

Kaplan Meier plots of time to resumption of sex by HIV status and by marital status were generated as shown in Figure 6 and Figure 7, respectively. There was no difference in the time to resumption of sex between HIV-positive and HIV-negative men (Log rank test; $p = 0.30$). However, married men resumed sex significantly earlier than single men (log rank test; $p=0.0001$) and the mean time to resumption of sex among the married men was 58 days versus 43.5 days for single men ($p<0.0001$).

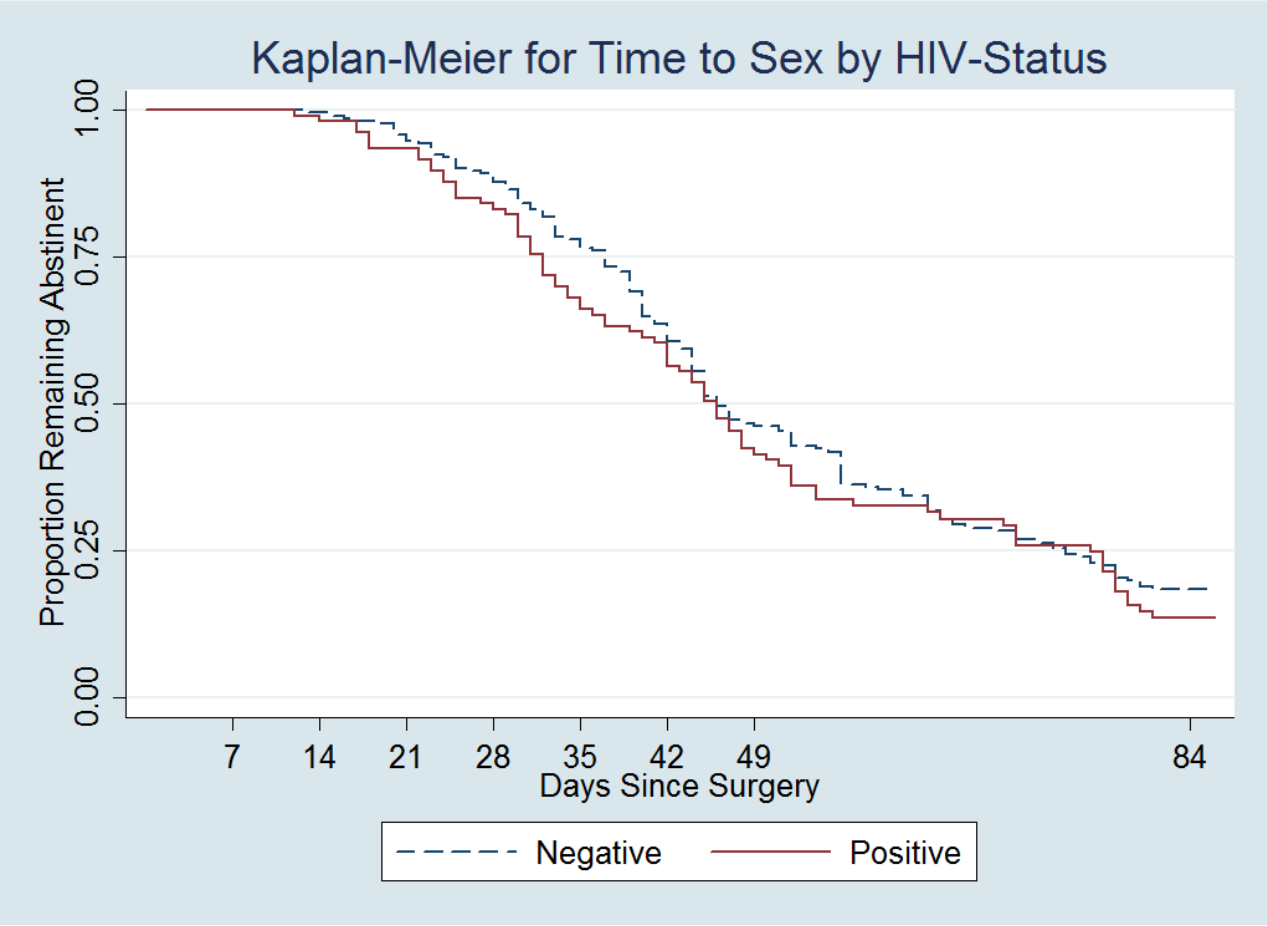


Figure 6: Kaplan Meier plot for time to sex by HIV status (Log-rank test for equality = 1.09 $p = 0.30$)

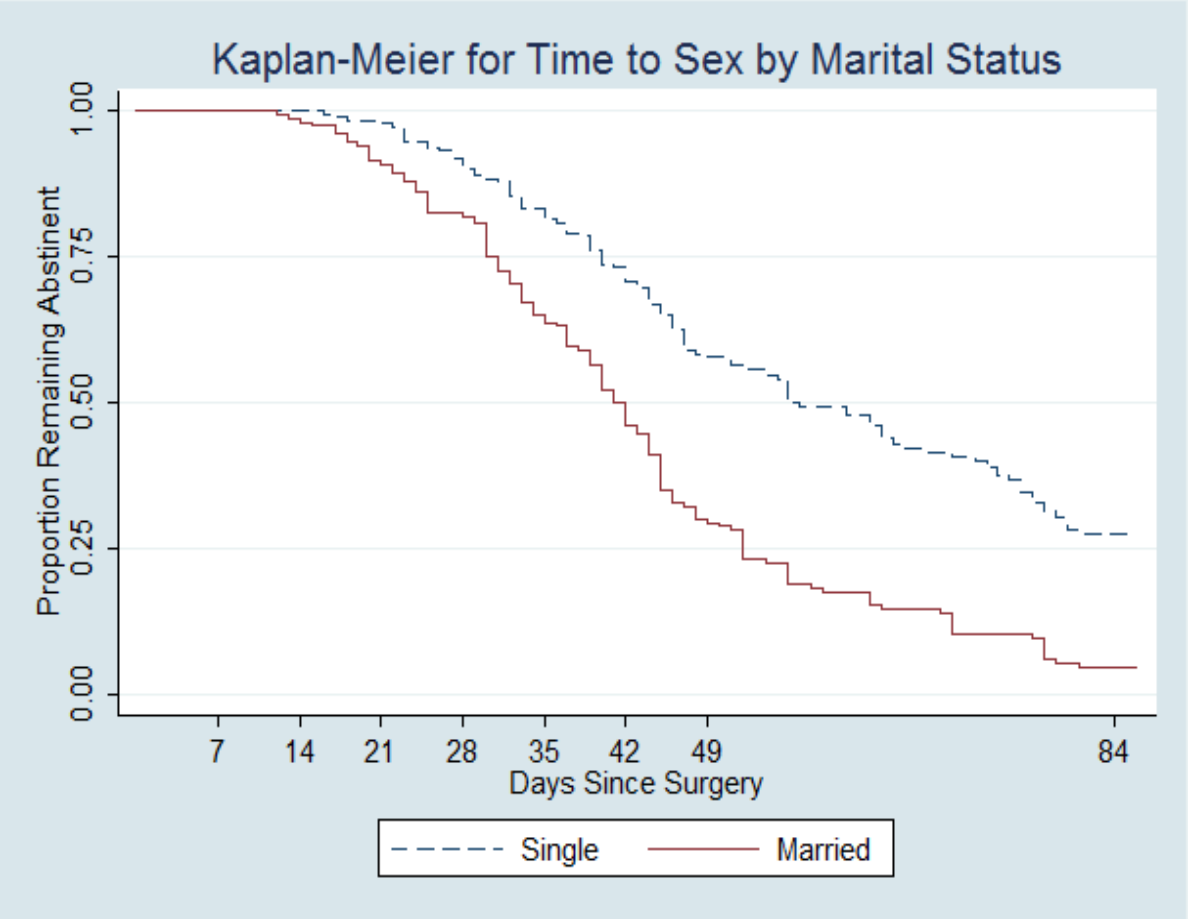


Figure 7: Kaplan Meier plot for time to sex by marital status (Log-rank test for equality = 43.9 $p< 0.0001$)

1.5 CD4+ T-cell counts, plasma viral load and viral shedding after circumcision

Results presented in this subsection address the third study objective which was to establish if circumcision of HIV-positive men is associated with significant change in penile viral shedding, viral load and CD+ T-cell counts. Results of serial measurements of CD4+ T-cell counts, plasma viral load and penile viral shedding at baseline and after circumcision are presented in Tables 16-20.

4.5.1 CD4+ T-cell counts

Out of 108 HIV- positive men enrolled in the study, CD4+ T-cell counts results were available for 107 men at baseline and 103 men at week 2 post-surgery. There was one invalid test result at baseline and five missing samples at week 2. Therefore, analysis for change in CD4+ T-cell counts is based on data from 102 individuals who had laboratory results for this variable at baseline and at two weeks following circumcision. Results are disaggregated by antiretroviral treatment status; 37 men were already on ART and 65 were not yet on ART. The results of mixed effect regression for change in CD4+ T-cells are presented for the two subgroups in Table 16.

Table 15: Change in CD4+ T-cell counts for 102 HIV positive men

Change in CD4+ T cell counts from baseline to 2 weeks for men on ART				
	Observations	Mean CD4+ T-cell counts	[95% CI]	
Week 0	37	393	320-466	
Week 2	37	396	323-470	***p*=0.81**
Change in CD4+ T cell counts from baseline to 2 weeks among ART naïve men				
Week 0	Observations	Mean CD4 count	[95% CI]	
Week 2	65	417	367-467	
	65	456	399-513	***p*=0.04**

For the 37 men already on ART, the mean CD4+ T-cell counts remained unchanged from baseline (393 cells/mm^3) to 2 weeks post-circumcision (396 cells/mm^3); p=0.81.

Among the remaining 65 ART-naïve men, CD4+ T-cell counts increased from 417 cells/ mm^3 at baseline to 456 cells/ mm^3 at week 2 post-surgery (paired t-test; p= 0.04).

4.5.2 Change in plasma HIV viral load following circumcision

Figure 8 is a line graph of plasma viral load results for 19 ART-naïve men just before circumcision and at weekly intervals thereafter, for six weeks. The overall picture suggests that plama viral load did not change from before to after circumcision. One man stood out as an outlier with a progressive decline in plasma viral load throughout the period of follow-up. His CD4+ t-cell count rose from 300 cells/mm^3 at baseline to 409 cells/mm^3 after 2 weeks. This is notable because he did not report initiating antiretroviral treatment before or during follow up.

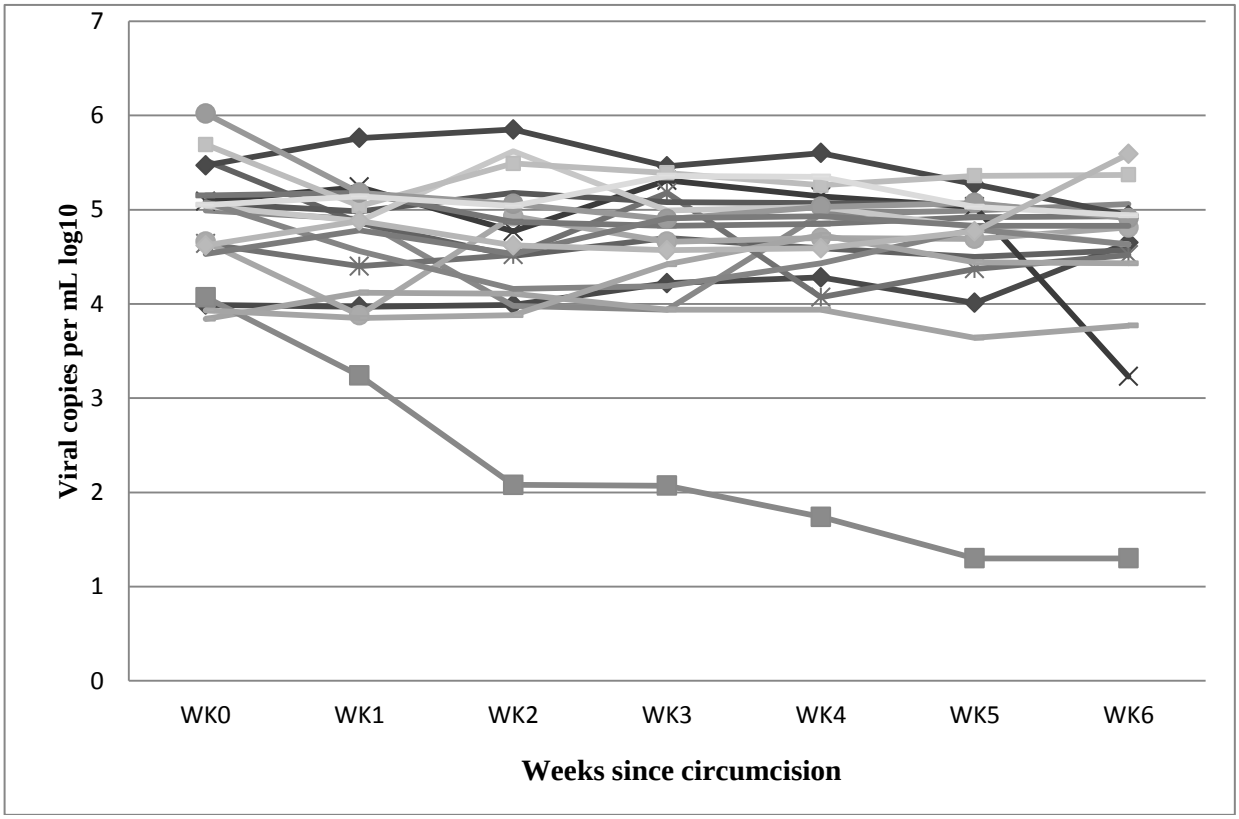


Figure 8: Weekly plasma viral load from baseline to week 6 after MC (n=19)

Table 16 shows repeated measures ANOVA results for mean change in plasma viral load from before to six weeks after circumcision for 19 ART Naïve men. There was no significant change in from baseline (4.86 $\log_{10}$ copies/mL) to week 6 post-circumcision (4.65 $\log_{10}$ copies/mL); p=0.36.

Table 16: Repeated measures ANOVA for change in plasma viral load for 19 ART naive men

Change in plasma viral load following circumcision				
Week	Observations	Mean viral load	[95% CI]	
0	19	4.86	4.57-5.16	
1	19	4.66	4.38-4.97	
2	19	4.59	4.19-4.99	
3	19	4.64	4.36-5.01	
4	19	4.65	4.25-5.04	
5	19	4.57	4.14-5.00	
6	19	4.53	4.08-4.99	***p*=0.36**

4.5.3 Penile coronal HIV viral shedding from before to after circumcision

Penile coronal viral shedding results by weekly intervals from the day of circumcision to 6 weeks post-surgery are presented for 29 ART-naïve men in Figure 9. Five out of the 29 men (17.2%) had detectable viral shedding from the penile corona before circumcision and 80% of these (4/5) had CD4+ T-cell counts below 350 cells/mm^3 but were not yet on ART. Three men who had no detectable viral shedding at baseline had no measurable virus at any time after circumcision.

The remaining 26 men had an upsurge in penile viral shedding which peaked at 1 week post-circumcision then declined to undetectable levels within 6 weeks in all but one individual, whose viral shedding became undetectable at week 7 (not shown in Figure 9).

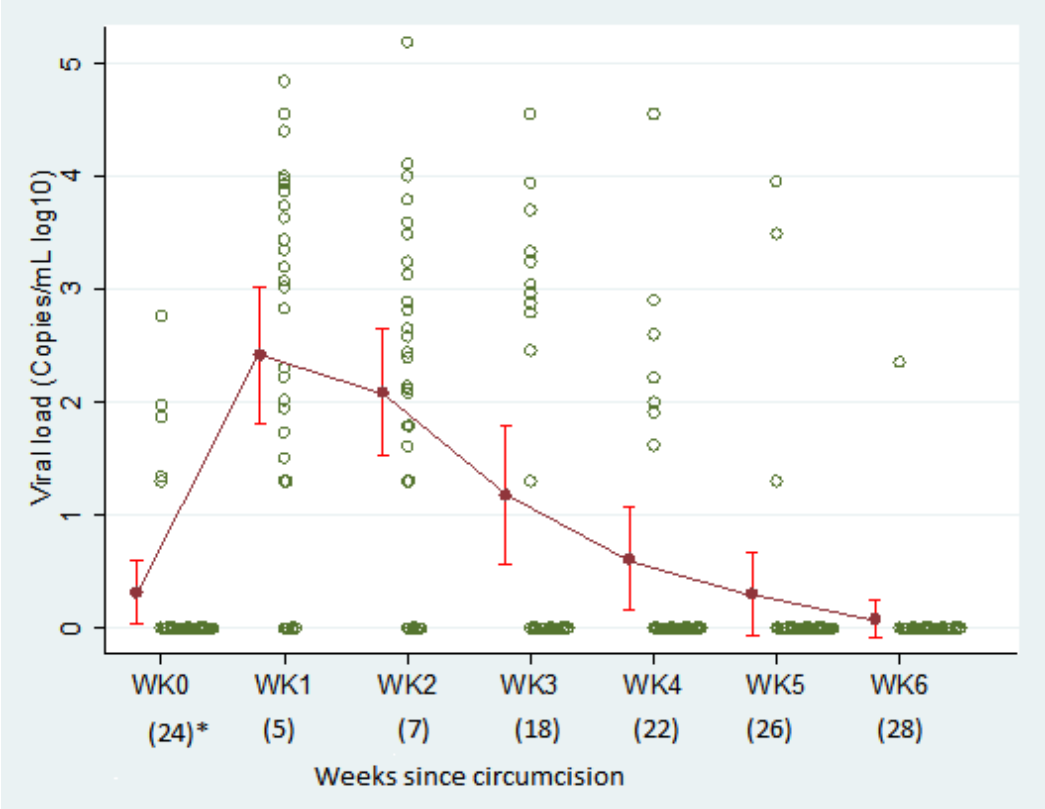


Figure 9: Penile coronal viral shedding detected at weekly intervals (n=29).

The error bars represent the 95% confidence intervals for the mean viral load in penile lavage. * Number of participants with no detectable viral shedding at each weekly time point.

Repeated measures ANOVA from baseline to six weeks showed highly significant changes in viral shedding following circumcision (Table 18).

Table 17: Change in Penile Coronal viral from baseline to 6 weeks after circumcision

Change in penile viral shedding following circumcision				
Week	Observations	Mean viral load	[95% CI]	
0	29	0.32	0.04-0.60	
1	29	2.41	1.82-3.02	
2	29	2.08	1.52-2.64	
3	29	1.18	0.56-1.79	
4	29	0.61	0.16-1.07	
5	29	0.30	-0.07-0.67	
6	29	0.08	-0.08-0.24	***p*<0.01**

4.5.3 Association between CD+ T-cell count, plasma viral load and penile coronal viral shedding

Depending on baseline CD4+ T-cell counts 29 ART naïve individuals were grouped into two categories of low CD4+ T-cell counts(<350 vs cells/mm^3) and high (≥350 cells/mm^3). Similarly the same individuals were grouped into two categories based on penile coronal viral shedding (none vs any) and based on HSV 2 status (positive vs negative). Results of test of association done across these dichotomous variables and for correlation between plasma viral load and penile coronal viral shedding are summarized in Table 19.

Table 18: Association between virological parameters in HIV positive men from

Variables	Test	p or r value
Baseline CD+ T-cell count (High vs Low) and Viral shedding (yes vs No)	Fishers exact test	p=0.33
HSV status (positive vs Negative) and Viral shedding	Chi-square test	p=0.27
plasma viral load and penil coronal viral shedding baseline (Wk0)	Correlation	r=0.53
plasma viral load and penile coronal viral shedding baseline (Wk1)	Correlation	r=0.00*
plasma viral load and penile coronal viral shedding baseline Wk 2	Correlation	r=0.00*
plasma viral load and penile coronal viral shedding baseline Wk 3	Correlation	r=0.41
plasma viral load and penile coronal viral shedding baseline Wk 4	Correlation	r=0.44
plasma viral load and penile coronal viral shedding baseline Wk 5	Correlation	r=0.71
plasma viral load and penile coronal viral shedding baseline Wk 6	Correlation	r=0.6276

Baseline CD4+-T-cell counts (<350 vs >350 cells/mm3) was not associated with penile coronal viral shedding (none vs any) (Fishers exact, p=0.33); nor was viral shedding associated with HSV-2 serostatus (Chi-square, p=0.27). Plasma viral load and penile viral shedding were correlated at week 1 (r=.60; p= 0.007) and at week 2 (r=.58; p=0.009), but not thereafter (Tables 19-20 and Figure 10).

Table 19: Correlation between plasma viral load and viral shedding

Week	Correlation	P value
0	0.1509	0.5253
1	0.7038	0.0005*
2	0.6385	0.0024*
3	0.1947	0.4109
4	0.1815	0.4439
5	0.088	0.7121
6	0.1156	0.6276

**** p value significant at 5% level***

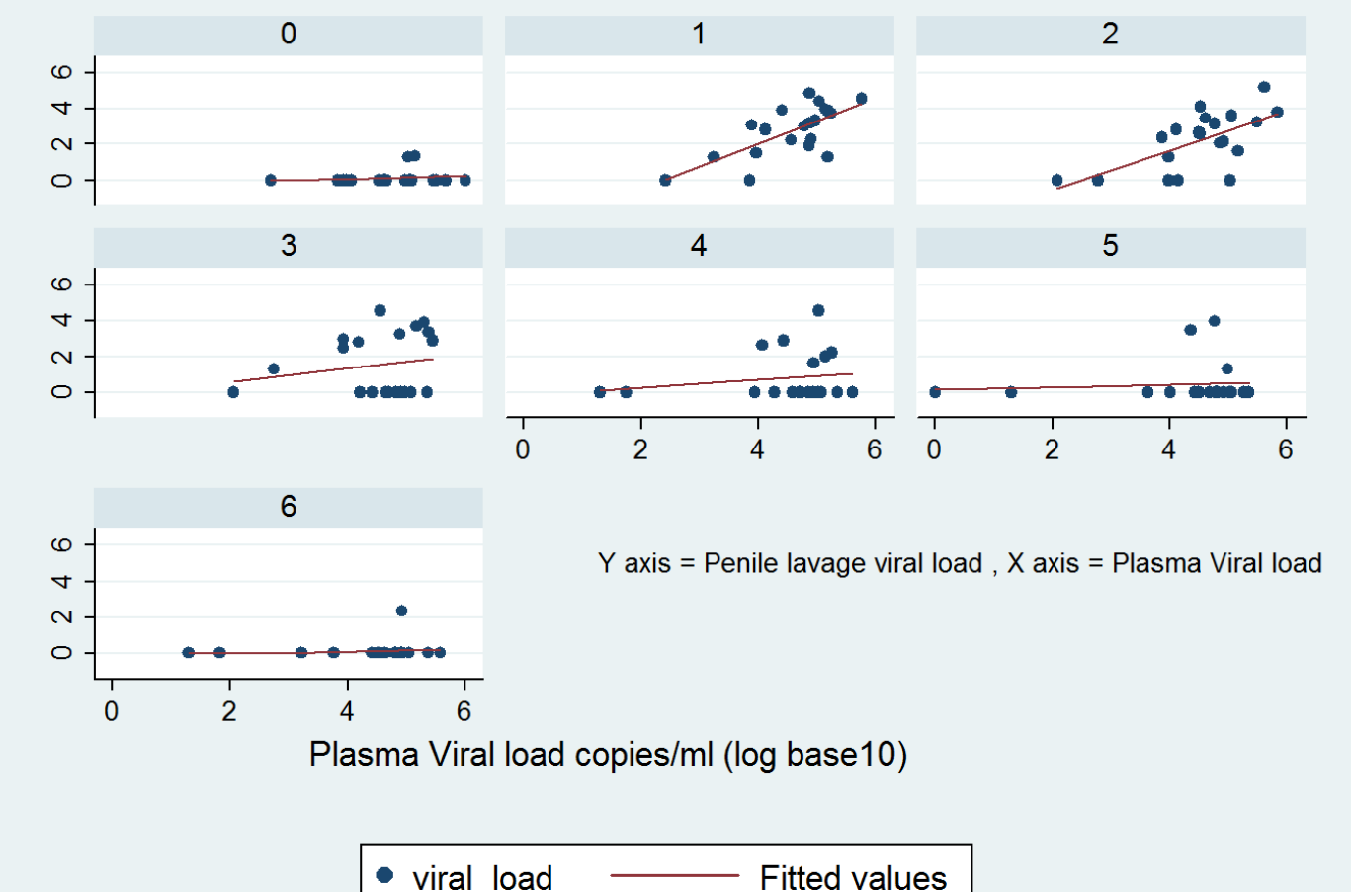
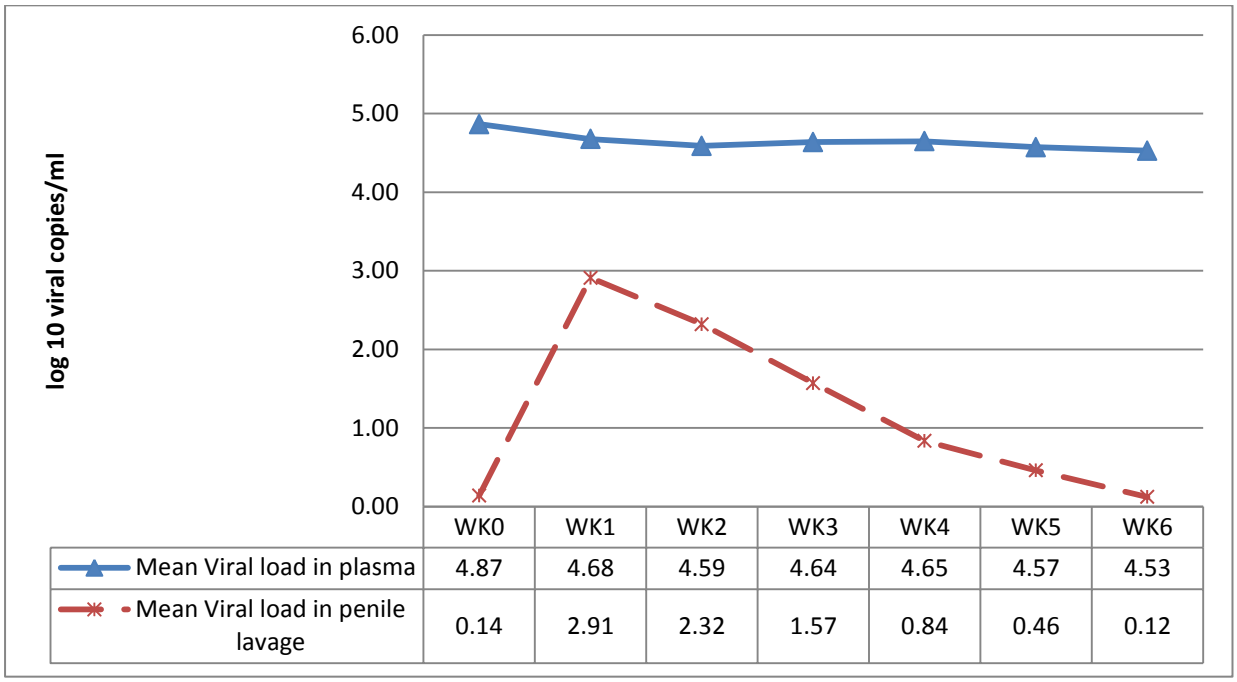


Figure 10: Correlation between plasma viral load and viral shedding from baseline to week 6. (n=19)

Figure 11 highlights the lack of correlation between viral load in plasma and penile coronal viral shedding



	WK0	WK1	WK2	WK3	WK4	WK5	WK6
Mean Viral load in plasma	4.87	4.68	4.59	4.64	4.65	4.57	4.53
Mean Viral load in penile lavage	0.14	2.91	2.32	1.57	0.84	0.46	0.12

Figure 11: Mean viral load in plasma and in penile lavage at weekly time points since circumcision

4.5.4 Sequence of cessation of viral shedding, wound healing and resumption of sex

The temporal sequence of clinical certification of wound healing, self-reported resumption of sex, and change in penile HIV viral shedding is shown in Figure 9. Viral shedding peaked at weeks 1 and 2 before any of the men resumed sex. Weeks 3-5 were characterized by a steep decline in viral shedding and sharp increases in the proportion of men certified as healed or resuming sex. By week 6, 45% of men had resumed sex and 93% of men were certified as fully healed. Most importantly, in 96.6% (28/29) of the men, no viral shedding was detected after certification of wound healing. Only one man (3.4%) still had penile viral shedding from an unhealed wound when he first resumed sex; and he reported using a condom. Neither the mean viral load nor the proportion of men shedding virus differed between those who resumed sex early compared to those who delayed sex to six weeks.

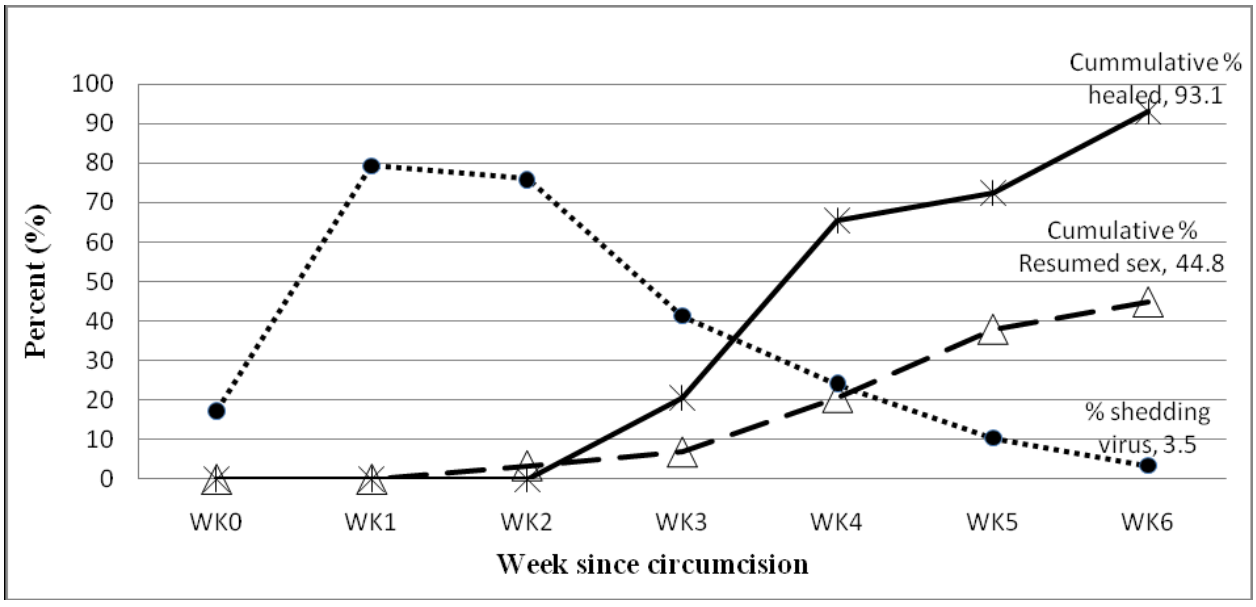


Figure 12: Proportion of HIV-positive men shedding detectable virus from penis, assessed as healed and reporting resumption of sexual intercourse by weekly intervals (n=29).

CHAPTER 5: DISCUSSION

5.1 Wound healing after circumcision

The first objective of this study was to determine how long it actually takes for adult men to heal after circumcision by the forceps guided method.

This study showed that following circumcision by the forceps guided method, 64.7% of men are healed within 4 weeks and 94% are healed by 6 weeks. While the rate of complete healing at 6 weeks was not available from the Kisumu[2] and Rakai[3] randomized controlled trials of MC for HIV prevention, week 4 healing rate of 64.7% observed in this study is much lower than 98.7% reported at 30-days in the Kisumu trial and 86.1% reported at week 4 in the Rakai trial. The Kisumu and Rakai trials did not use stringent enough protocols of follow up for assessment of wound healing. The observations classified as 30-days or 4-weeks post-surgery in the two trials varied widely in their actual timing. In the Kisumu trial, 5.3% of the wounds were assessed at or before 27 post-operative days, 89.7% were assessed between 28 and 34 days, and the remaining 5.0% were assessed beyond 35 days yet the results were reported for day 30. In the Rakai trial, 25.5% of men were assessed at or before 27 days, 69.5% at 28–34 days, and 5.1% after 35 days and the results were reported for day 28. Therefore, the lower rate of healing reported in this study is likely to be a result of more frequent examination of post-circumcision wounds at weekly intervals and the stringent condition that wound healing is re-affirmed at all repeat examinations till week 12. Additionally, the criteria for classification of wounds as completely healed as applied in this study excluded delayed complications occurring after initial certification of healing and uncertainties arising from inter-observer variations which may have been missed in the Rakai and Kisumu trials.

During analysis, a conservative approach was taken whereby an individual was only considered completely healed if he was certified healed at all subsequent follow up visits.

Although nearly all post-circumcision wounds were healed within 6 weeks regardless of HIV status, ART naïve HIV-positive men with CD4+ T-cell counts less than or equal to 350 cells/μL had a significantly longer mean time to complete healing compared to those with CD4+ T-cell counts above 350 cells/μL (37.1 days vs. 31.1 days; p=0.04). This suggests that low CD4+ T-cell counts is associated with slower progression of healing in early post-operative phases before 6 weeks. The study also showed that of all the covariates examined, only early post-operative infection and tight sutures are associated with longer time to complete healing. However, only early post-operative infection was associated with significantly lower proportion of men healed at 6 weeks. In view of these findings, the current WHO-recommendation of 42-days post-circumcision sexual abstinence should be maintained and applied for both HIV-negative and HIV-positive men. But wounds should be examined clinically on the seventh post-circumcision day to exclude early post-operative infection and signs of tight sutures which are associated with longer time to healing or lower proportions healed at 6 weeks. Men with early post-operative infection or signs of tight sutures at week 1 should be counseled to anticipate longer abstinence beyond 42 days and only resume sex after healing is certified by a trained clinician. This should particularly be emphasized for HIV-positive men whose risk of transmitting HIV may be higher due to post-circumcision upsurge in viral shedding. VMMC programs should however promote 42 days abstinence and condom use at every sexual encounter within the first 3 months after circumcision.

Although nearly 65% of the circumcised men are fully healed within 28 days after circumcision and may be at no additional risk of acquiring or transmitting disease, it would not be prudent to consider a shorter period of sexual abstinence for this subgroup because there is no clear way of identifying them before complete healing. Self-assessment by clients for complete healing before engaging in sex may be considered to justify a shorter period of sexual abstinence for men who heal early. But a comparison between men's personal perception of own healing and clinician's assessment showed an agreement rate of only 65%. Therefore promoting self-assessment for complete healing would be counterproductive because not all men can be trusted to assess themselves accurately. Objectivity may also be compromised if self-assessment is done just before intercourse, since sexual urge may compromise rational judgment.

This study showed no difference between HIV-negative and HIV-positive men with regard to proportions of individuals healed by weekly intervals since circumcision. Kigozi et al., (2008) reported a lower prevalence of healing at 6 weeks among HIV-positive men compared to HIV-negative men (92.7% vs 95.8%; $p<0.007$). [86] Although, this study showed a similar trend, the difference in prevalence of healing between HIV-positive men and HIV-negative men at week 6 was not statistically significant (93.3% vs 94.4%; $p=0.637$).

Similar post-circumcision healing rates between HIV-negative and HIV-positive men in this study may be because the program only allows circumcision of clinically healthy individuals. This approach may have been effective in excluding the HIV-positive men at high risk of delayed wound healing. These findings support the current WHO guidance of providing VMMC services to HIV-positive men who opt for the procedure and are clinically eligible.

5.2 Resumption of sexual intercourse in post-circumcision healing period

The second objective of this study was to assess men's compliance with the recommendation for them to abstain from sex for at least six weeks following circumcision and risk factors for early resumption of sex.

Although WHO recommends 42 days of sexual abstinence after circumcision to allow for complete wound healing before engaging in sex,[14] no published study has examined resumption of sex in relation to time of healing observed at regular and frequent enough intervals. This study found that 18.8% of men circumcised at UNIM clinic in Kisumu, Kenya resumed sex before clinicians judged them to be fully healed. In contrast, findings in the same study showed that 37.7% of the men resumed sex before the recommended 42 days of abstinence, irrespective of whether their wounds had healed or not. Using the WHO recommended cut-off of 42 days of sexual abstinence post-circumcision, the finding in the this study is similar to the 30.7% rate of early sex found by Herman-Roloff et al., (2012) in western Kenya but higher than the 24% estimate reported by Hewett et al., (2012) for Zambian men.[19,20] The higher rate of non-compliance with the WHO recommendations in this study may be due to the shorter (weekly) intervals between assessments of both healing and resumption of sex which reduced misclassification. In contrast, the Kenyan study by Herman-Roloff et al., (2012) asked about resumption of sex between 28 and 42 days post-surgery, which would result in underestimation of the proportion resuming sex before 42 days.

For the Zambian study, men were interviewed just once at 42 days post-surgery. The higher proportion of men engaging in early sex in this study may also be due to inclusion of HIV-positive men and recruiting HIV-negative men matched for age.

HIV-positive men in Kenya are older and more likely to be married, and both these variables are associated with early resumption of sex.[12] In contrast, the Zambian study included younger men of unknown HIV status aged 15-29 years, of whom only 7.6% were married.

Only 23 of 319 men (7.2%) had unprotected sex before complete wound healing. All but one of these 23 men resumed sex before the WHO-recommended 42-day abstinence period.

Although the results of this study reflect poor compliance (62.3%) with the WHO recommendation for 42 days of post-surgical abstinence, they indicate that few men (6.9%) are at risk of acquiring or transmitting HIV due to unprotected sex within the recommended abstinence period. Since nearly all instances of sex before healing occurred within 42 days post-surgery, these results thus suggest that the current policy of counseling men undergoing circumcision to abstain from sex for at least 42 days post-surgery and to use a condom upon resumption of sex is likely to minimize HIV transmission during the post-operative period.

Men with the highest risk of non-compliance with advise on post-circumcision abstinence, whether measured as sex before complete wound healing or as sex before 42 days, are married men and men with two or more sex partners in the year before they are circumcised. The results of this study for married men are consistent with others from Kenya and call for greater emphasis on counseling couples about male circumcision and to provide education targeted to women about the importance of abstaining from sex for a sufficient interval after their partners' circumcision.[19,20,95] Text messaging to circumcised men and their spouses should also be considered, although trial of repeated text messages in one study had limited efficacy in delaying time to resumption of sex.[96] Older single men should also be targeted for tailored counseling about the risks of early resumption of sex.

Single men aged 25 years and older were shown to be more than three times more likely to resume sex before complete wound healing than their younger counterparts.

Single men, who take alcohol more than thrice per week, are also at greater risk of resuming sexual intercourse before they are fully healed. Thus additional innovative means of counseling men to be aware of the influence of alcohol on their sexual risk behaviors would be prudent.

HIV-positive and HIV-negative men had similar levels of adherence to the WHO-recommended 42-day sexual abstinence. HIV-positive men were however one and half times more likely to have sex before complete healing, but this difference was not significant after adjusting for age and marital status.

Although the mean HIV testing rate for men seeking circumcision in Kenya is approximately 92%, [25] not all programs are this successful, and many HIV-positive men may be getting circumcised with unknown serostatus. Therefore, it is encouraging that this study showed that both HIV-positive men HIV-negative men heal within 42 days, and they are at the same risk of engaging in early sex.

A comparison between men's personal perception of own healing and clinician's assessment showed an agreement rate of only 65%, but data was not collected on how participants made judgments about the healing status of their wound. Such information could be useful for designing more effective counseling to reduce instances of early sex after circumcision. However, because instances of early sex may be driven by false personal perception of healing, VMMC programs should aggressively promote abstinence for 42 days and condom use for the first three months after circumcision regardless of the status of healing or clients' personal perception of risk.

In summary although nearly 38% of men did not adhere to the WHO recommended 42 days of post-circumcision abstinence, only half that proportion (19%) resumed sex before complete healing, and only 7% had unprotected sex before healing.

The mean time to healing was less than 6 weeks for HIV-positive and HIV-negative men and time to resumption of sex was similar for the two groups. To reduce the proportion of men who resume sex before they are healed, programs should develop innovative ways to reach and educate married men and men who have more than one sex partner.

The period of healing after circumcision is brief and the levels of unprotected sex before complete wound healing are not high enough to offset the long-term protective effects of VMMC. Furthermore none of the HIV-negative men retested at the end of follow up secoconverted. Lack of seroconversion among HIV-negative men is good news but is of little significance because secoconverters may have been in the window period. Overall, this study has provided additional information to design interventions to address to address concerns over resumption of sex after circumcision directly. More attention should be devoted to reducing sex before 42 days post-circumcision and promoting condom use in VMMC programs for HIV prevention.

5.3 Changes in CD4+ T-cell counts, plasma viral load and penile coronal viral shedding following circumcision of HIV-positive men

The third objective of this study involved examining the effect of circumcision on CD4+ T-cell counts, plasma viral load and penile coronal viral shedding in order to gauge the risk of HIV transmission in the post circumcision healing period. The underlying concern was that circumcision of HIV-positive men could increase their risk transmitting to their sexual partners if the surgical procedure and subsequent wound healing process are associated with increased plasma viral load and in penile viral shedding. [18,33] Local injury, inflammation and the stress of surgery have the potential to cause cortisol-mediated changes in virological parameters among men living with HIV. [97]

This study showed no change in CD4 T-cell counts from baseline to two weeks post-circumcision in seropositive men who were on ART but a slight increase among ART-naive men from 417 cells/µL to 456 cells/µL, p=0.04). The biological basis and significance of this selective increase in CD4 t-cell count among ART naïve men after MC is unclear and requires further investigation. In contrast to findings from Rakai, Uganda, where Wawer et al., (2009) found significant increases in plasma viral load four weeks after circumcision[18], this study found no significant change in plasma viral load at any point during the first six weeks following circumcision. Wawer at al., (2009) reported that plasma viral loads among ART-naïve men were elevated at four weeks after circumcision by a mean of 0.20 log10 copies/mL above baseline level.

While the sample size of 19 men was small, thereby limiting power to detect significant changes, the viral loads of men in this study did not rise, but rather declined, even though insignificantly, after circumcision from a mean of 4.86 log10 copies/mL at baseline to 4.65 log10 copies/mL at week 4 post-surgery. The Rakai sample was restricted to men who had CD4+ T-cell counts above 350 cells/mm3 and detectable virus at baseline. The baseline plasma viral load levels (mean 4.30 log10 copies/mL) for men in the Rakai study were lower than those for the men in this study. Restricting analysis to the 10 men, whose CD4+ T-cell counts were above 350 cells/mm^3 and had detectable plasma viral load at baseline, still yielded a mean viral load of 4.68 log10 copies/mL at baseline with a slight reduction to 4.62 log10 copies/mL at week 4 post-surgery. The reasons for the differences between the results of this study and those from Rakai are unclear and should be investigated further.

Wawer et al., (2009) speculated that increases in viral load among the ART-naive HIV-positive participants in their study could lead to greater risk of HIV transmission in the post-circumcision period [18], but this is inconsistent with the results in this study. There is no clear scientific explanation for the inconsistency between the finding in this study and the Rakai study.

Regarding penile viral shedding, five of 29 (17%) men had detectable HIV viral shedding at baseline. Viral shedding before circumcision could have been associated with scratches, abrasions or micro-lesions of the penile skin, which would be un-related to circumcision and have been found to be frequent in this population.[76,98] This study was however not designed to detect micro-tears and abrasions. There are no published reports of studies on the effect of male circumcision on penile coronal HIV viral shedding with which to compare these results.

Overall, the present study also showed a transient post-circumcision increase in penile viral shedding that peaked at one week, then declined to undetectable levels by 6 weeks after circumcision. Penile viral shedding increased dramatically to a peak mean of 2.41 log10 copies/mL one week after circumcision and then declined steadily such that only one of 29 men had any detectable penile shedding at week 6, and he had returned to undetectable levels by week 7. The transient post-circumcision increase in viral shedding observed in this study reinforces the general intuitive view of increased risk of male-to-female transmission by HIV-positive men who resume sex before the WHO-recommended period of 42 days sexual abstinence following circumcision.

Considering data on resumption of sex after circumcision, the upsurge in penile viral shedding following the procedure returns to baseline levels before most men resume sex. Herman-Roloff showed that 30.7% of recently circumcised men resume sex before 6 weeks, but few before 4 weeks. [20]But the present study showed that 38% of men resumed sex before 6 weeks, with just 15% reporting sex before 4 weeks. Among the 29 HIV-positive men included in the analysis for viral shedding, only one man (3.4%) resumed sex before his wound was healed and when he was still shedding detectable virus. Wawer et al., (2009) noted that the possible short-term increase in transmission to partners of circumcised HIV-positive men if sex is resumed early is unlikely to have a substantial effect on the HIV epidemic because the exposure period of possible increased risk is short and the number of HIV-positive men with uninfected female partners who resume sex early will generally represent a small proportion of MC program clients.[18]

These results indicate that the number of HIV transmissions likely to occur during the immediate post-circumcision period could be lower than previously considered because only a small proportion of HIV-positive men 4.9% have uninfected partners [12] and only a few men (3.3%) are likely to resume sex when they are still shedding the virus and infectivity is high.

The direct positive linear correlation between plasma viral load and viral shedding reported here during the first two weeks after circumcision has programmatic implications. Men with higher viral loads are likely to have high viral shedding in the post-circumcision period. One possible approach suggested by these data is to offer ART treatment to all HIV-positive men seeking VMMC; however, while such treatment may prevent a few new infections, if it is restricted to the period just before and after circumcision, it could induce ARV drug resistance.[99,100]

However as increasing numbers of HIV-positive men enter ART treatment, those seeking circumcision are more likely to have suppressed viral loads and less viral shedding during the immediate post-surgical period. Although penile viral shedding was correlated with plasma viral load during weeks 1 and 2 after circumcision, this correlation did not persist thereafter, indicating that plasma viral load is unlikely to be a useful predictor of penile viral shedding during the crucial period when some men may resume sex with an unhealed wound.

These findings are consistent with the many studies that have shown HIV-1 compartmentalization between blood and other tissues and they demonstrate that assessment of penile viral shedding rather than plasma viral load would be more useful for determining the risk of HIV transmission during the post-circumcision period.[101,102]

Given that in 96.6% of the circumcised HIV-positive men no viral shedding was detected after full wound healing, it is reasonable to conclude that the post-circumcision upsurge in viral shedding was due primarily to local breach in epithelial integrity.

VMMC programs should therefore promote abstinence for 42 days after circumcision and consistent condom use during the healing period as the main strategies for minimizing the risk of HIV transmission.

The finding that penile viral shedding returns essentially to baseline levels by week 6 post surgery is good news in view of the WHO guidelines recommending 42 days of sexual abstinence after medical circumcision.[15]Since 94% of men are fully healed by Week 6 post-surgery and although approximately 38% of men resume sex before 42 days post-circumcision, only 7% had unprotected sex before they were certified as fully healed, the risk of HIV transmission due early sex may be low.

Wawer et al., (2009) noted that the risk of HIV transmission due to sex before the circumcision wound is healed is brief and likely to be small in proportion to the number of new infections to be averted in the population over decades through the protective effect of male circumcision.[18]

The results of the present study support this view, and further suggest that the risks of HIV transmission to the partners of HIV-positive men are considerably less than previously considered. [11]Nevertheless, the study has shown that 24% of ART-naive HIV-positive men continue shedding virus above baseline level up to four weeks post-surgery; therefore developing effective counseling and communications strategies for both men and their female partners to avoid sex before 42 days after circumcision and to optimize condom use remains an essential component of a comprehensive MC program for HIV prevention. [26]

In summary, the results suggest that circumcision of HIV-positive men who are clinically eligible would pose no significant additional risk with respect to HIV-transmission if men adhere to the recommended abstinence period of 6 weeks. Given these results and increasing focus on global health and the fact that circumcision has benefits beyond HIV prevention, programs should consider promoting circumcision equally for HIV-positive and HIV-negative men. Same guidance of 42 day post-operative sexual abstinence and condom use should be applied to HIV-positive and HIV-negative men circumcised by the forceps guided method.

5.5 Limitations of the study

This study had several limitations. First, it did not adjust for possible influence of hygiene practices and elevation of the penis after circumcision on healing. If measured, teasing out the effect of these covariates may be helpful for further understanding of post-circumcision healing.

Another limitation is the age-matching of HIV-negative men to enrolled HIV-positive men who tend to be older and predominantly married. Thus, the age distribution of the study population may not accurately reflect the ages of men seeking VMMC, who tend to be younger, more likely to be single, and thus likely have lower rates of sex before wound healing. HIV-negative men were however oversampled by an extra 107 individuals to guard against this potential source of bias. Another limitation is that the clinicians assessing the wounds were the same ones who performed the circumcisions and were not blinded to the purposes of the study. This could potentially result in a bias for reporting healing early, despite extensive training to the contrary. However, each participant could be served by any one of the four clinicians at each visit. This potential source of bias was also addressed by an intensive training program for clinicians and continuous monitoring for adherence to the definition of complete wound healing that was piloted repeatedly before onset of the study.

A further limitation was the reinforcement of counseling on post-circumcision abstinence at weekly follow-ups, whereas men served in the national VMMC program receive similar counseling only once at surgery and possibly at 1 week follow up.

Participants may have been more motivated to properly care for their wound or to refrain from sex than those circumcised as part of the national MC program. Such frequent follow ups with counseling could also result in reporting bias by participants, who might want to please the interviewer with a "correct" answer. It is possible that, under the Kenya National VMMC program, the frequency of early onset of sex is greater than was reported in this study, because men are not so frequently counseled about post-circumcision sexual abstinence and condom use. But the overall proportion of men reporting sex before 42 days post-circumcision is very similar to findings from two previous studies conducted among clients attending the Kenyan VMMC program. [20,103]

The viral shedding component of this study had a number of limitations. Although participants were residents of Kisumu who were seeking MC, they may not have represented the full range of HIV-positive men in the community who could be served by the Kenyan MC program. All the study participants accepted HIV testing but in the Kenyan MC program the HIV testing uptake rate before MC is only 92%. The sample sizes, especially for plasma viral load and plasma viral shedding were small thereby limiting the power to detect significant changes over time. However, the changes observed were small and data are sufficient to demonstrate consistency of no change in plasma viral load after circumcision and the consistent pattern of an upsurge in viral shedding initially after circumcision with declines to undetectable levels by six weeks post-surgery.

The results of this study are specific to the forceps-guided method of circumcision. A recent study comparing Shang Ring with conventional surgical circumcision in Kenya and Zambia showed that 76% of men circumcised by Shang Ring method were healed at 42-days post-circumcision, compared to 85% of men undergoing conventional surgical circumcision.[104] A pilot study of PrePex for circumcision of adult men in Nyanza region of Kenya showed that only 50% of the men circumcised through this device are healed at 42 days.[105] Another study of the PrePex device for male circumcision in Rwanda reported that the number of days required for complete healing following removal of the device was 31 days or approximately 38 days from device placement.[106] Based on these findings, circumcision with PrePex may require a longer healing period than either the forceps-guided method or the Shang Ring. The longer healing period associated with PrePex and Shang devices is inevitable because healing following these procedures is by secondary intention.

Given that both these devices are associated with a longer time to complete healing and are currently under consideration for wide scale use, a review of guidance on duration of post-circumcision sexual abstinence should be considered before rolling out these devices.

Another important issue for consideration relates to the recommendation for maintaining the current duration of post-circumcision abstinence based on the assumption that complete healing as defined and applied in this study is sufficient to mitigate the risk of HIV transmission. This makes biological sense and may be true but it is not proven and given ethical limitations, cannot be examined in an experimental manner.

While the criteria used for visually assessing wound healing on the surface is sufficient from a clinical perspective, the method alone cannot determine complete wound healing vis-à-vis the hazard of HIV risk, particularly in the context of the stress and pressure on the wound during sexual activity. This uncertainty makes a strong case for promotion of universal condom use in the first three months post-circumcision to minimize the risk of transmission from healed wound when subjected to mechanical stress during sexual intercourse.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

The conclusions and recommendations arising from different aspects of this study are summarized in Table 21.

This is the first study to systematically assess wound healing through weekly observations following male circumcision by forceps-guided method. Based on its results, the WHO recommendation for 42-days post-circumcision sexual abstinence should be maintained and applied for HIV-positive and HIV-negative men circumcised by the forceps guided or other surgical approaches where healing is by first intention. Factors associated with delayed wound healing were identified as post-operative infection, tight sutures, and low CD4 + t-cell counts in ARV-naive HIV-positive patients; these should be taken into account in training VMMC service providers.

Although nearly 38% of men did not adhere to the WHO recommended 42 days of post-circumcision sexual abstinence, only half that proportion (19%) resumed sex before complete healing, and only 7% had unprotected sex before healing. Risk factors for early resumption of sex included being married, having multiple sex partners in the year before circumcision and consistent alcohol intake. Therefore, to reduce instances of early sex after MC, programs should develop innovative ways to reach and educate married men and men who have more than 1 sex partner. Older single men who drink should be targeted with special counseling messages because they are at especially high risk of resuming sex early. Overall, the period of healing after circumcision is brief and the levels of unprotected sex before complete wound healing are not high enough to offset the long-term protective effects of VMMC.

With regard to viral shedding, this study showed a transient upsurge in penile viral shedding immediately after circumcision which declines to baseline levels within the WHO-

recommended 42 day post-surgical abstinence period. The results reinforce the need for robust counseling and strong communications campaigns emphasizing the importance of abstinence for the full 42 days post-circumcision and consistent condom use upon the resumption of sex.

Table 20: Summary of conclusions and recommendations

Conclusions	Recommendations
94% of post-circumcision wounds are healed within 42 days regardless of HIV status.	The WHO recommendation of 42 days post-circumcision sexual abstinence should be maintained and applied for HIV-negative and HIV-positive men eligible for surgery
About 6.0% of circumcised men remained unhealed beyond the recommended 42-day period of post-circumcision sexual abstinence.	It is prudent to stress condom use within the first 3 months after circumcision for all men.
Men's personal perception of own healing and clinician's assessment showed a low agreement rate of only 65%. But compared to clinicians, study participants tended to believe that wound healing took longer.	All men should abstain from sexual intercourse for at least 42 days after circumcision regardless of their personal perception of healing status.
Post-operative infection and tight sutures are associated significantly lower proportion of men healed at 6 weeks.	Post-circumcision wounds should be inspected on the 7th post-operative day especially to identify men with early infection and tight sutures who should be counseled to abstain from sex for longer than 42 days or until healing is certified by a clinician.
The risk of HIV transmission due to early sex before 42 days is low and transient, since over 81% of the men who reported early sex either used a condom or were already healed. Only 7% of men had unprotected sex before complete healing.	Aggressively promote condom use at every sexual intercourse within 3 months post-circumcision to minimize the risk of HIV spread due to sex before complete healing.
Risk factors for sex before healing are being married or having two or more sex partners in the past year. Among single men, age above 24 years and consistent alcohol consumption were associated with sex before healing.	Emphasize on counseling couples about male circumcision and to provide education targeted to women about the importance of abstaining from sex for a sufficient interval after their partner's circumcision. Counsel men to be aware of the influence of alcohol on their sexual risk behaviors.
Circumcision of HIV-infected men is associated with an upsurge in viral shedding that declines to undetectable pre-circumcision levels within 6 weeks after surgery.	Compliance with current guidance of 42 day post circumcision sexual abstinence and consistent condom use should minimize risk of transmission following circumcision of HIV positive men.

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8. APPENDICES