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Recurrence of cervical intraepithelial neoplasia at six months post cryotherapy or loop electro-surgical excision procedure at Mbabane government hospital, Swaziland

by

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EXECUTIVE SUMMARY

Introduction

Cervical cancer is the most common female cancer in sub-Sahara Africa. In 2018, Swaziland was rated with the highest burden globally. In 2014, 21% female deaths in Swaziland were due to cancer and almost half (40%) were due to cervical cancer. Although, the World Health Organization guidelines for cervical intraepithelial neoplasia (CIN) screening and treating were adapted in 2011, the recurrence of CIN post treatment is noted in practice, but the burden remains unknown. The study assessed the prevalence and factors associated with CIN recurrence among women treated with cryotherapy or loop electrosurgical excision procedure (LEEP) at 6-months treatment follow-up.

Methods

A retrospective cohort analysis of women screened for CIN between January 2014 and December 2016 at Mbabane government hospital, was conducted. The study participants included all women treated with either cryotherapy or LEEP and reviewed at six months post treatment. The statistical methods used included descriptive statistics and logistic regression. Permission for the study was obtained from the relevant authorities.

Results

The 602 study participants enrolled for the study had a mean age of 30.9 years (SD: 5.7). Few (39/602; 6.5%) had recurrence of CIN. The adjusted OR for recurrence for positive HIV status was 2.47 (95%CI 1.01-6.02; p=0.022) and 2.46 (95%CI 1.01-6.03; p=0.048) for low CD4 cell count. Anti-retroviral therapy (ART) reduced the odds for CIN recurrence by 70% (AOR 0.31; 95%CI 0.13-0.71; p=0.006).

Conclusion:

A positive HIV status and low CD4 count increased the odds of recurrence, while ART was found to be protective. The treatment modality had no effect. HIV infected individuals need ART which significantly improves CIN treatment outcomes.

Recommendation:

HIV infected individuals need ART which significantly improves CIN treatment outcomes

KEY WORDS: Recurrence, Cervical Intraepithelial Neoplasia, Human Papilloma Virus, Cryotherapy, LEEP

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ABBREVIATIONS AND ACRONYMS

AIDS : Acquired immuno-deficiency syndrome

AOR : Adjusted Odds Ratio

ART : Anti-retroviral Therapy

CI : Confidence Interval

CIN : Cervical Intraepithelial Neoplasia

EC : Ethics Committee

HAART: High Active Anti-retroviral Therapy

HIV : Human immunodeficieny Virus

HPV : Human Papilloma Virus

LEEP : Loop Electrosurgical Excision Procedure

MGH : Mbabane Government Hospital

MICS : Multiple Indicator Cluster Survey

OC : Oral Contraceptives

OR : Odds Ratio

PHU : Public Health Unit

REC : Research Ethics Committee

SIL : Squamo Intraepithelial Lesion

STI : Sexually transmitted infection

VIA : Visual Inspection using Acetic acid

WHO : World Health Organization

ZAR : South African Rand

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My Lord the God of Heaven, without Him, I would not have made it this far.

DECLARATION OF ORIGINAL AUTHORSHIP

"I declare that the dissertation/thesis, which I hereby submit for the degree Master of Science (Epidemiology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another University."

Date of Approval from Research Ethics Committee

University of Pretoria: 20 October 2016

Reference number

University of Pretoria: 389/2016

Student Signature  **Date** 27 November 2018

Supervisor Signature *N. Ledibane* **Date** 28 November 2018

Co-supervisor Signature  **Date** 28 November 2018

Name of Journal for Proposed Submission

South African Medical Journal (*SAMJ*)

GLOSSARY

- *Adolescent*: An adolescent is defined as any person between the ages of 10 and 19 years.[1]
- *Cervical intraepithelial neoplasia (CIN)*: dysplastic changes beginning at the squamo-columnar junction in the uterine cervix that may be precursors of squamous cell carcinoma: grade 1 (CIN 1), mild dysplasia involving the lower one third or less of the epithelial thickness; grade 2 (CIN 2), moderate dysplasia with one third to two thirds involvement; grade 3 (CIN 3), severe dysplasia or carcinoma in situ, with two thirds to full-thickness involvement.[2]
- *Cervicography*: Technique, equivalent to colposcopy, for photographing part or all of the uterine cervix.[2]
- *Colposcopy*: A procedure that allows a physician to take a closer look at a woman's cervix and vagina using a special instrument called a colposcope. It is used to check for precancerous or abnormal areas. The colposcope can magnify the area between 10 and 40 times.[53]
- *Cryotherapy*: a procedure used to destroy tissue of both benign and malignant lesions by the freezing and re-thawing process. Liquid nitrogen or nitrous oxide gas is the most commonly used freezing source.[3]
- *Dysplasia*: abnormal growth or development of cells, tissue, bone, or an organ. [2]
- *Early sexual debut*: Ffirst sexual encounter before or at the age of 16.[2]
- *Loop electrosurgical excision procedure (LEEP):* partial excision of a uterine cervix with dysplasia using a specially designed wire loop under local anaesthesia; LEEP loops deliver high-frequency, low-voltage, alternating electric current, minimizing thermal damage, while preserving haemostasis.[3]
- *Menopause*: The time in a woman's life when menstrual periods permanently stop; it is also called the "change of life."[4]
- *Oncogenic*: causing or tending to cause the formation and development of tumors.[4]
- *Parity*: The number of children borne by a woman.[4]
- *Post-menopause*: The period after the menopause. The post-menopause is formally defined as the time after which a woman has experienced twelve (12) consecutive months of amenorrhea (lack of menstruation) without a period.[4]
- *Pre-menopause*: It begins when a girl has her first period and ends with the first typical signs of menopause.[4]

- *Sexual debut*: The age when an individual experiences their first sexual encounter.[3]
- *VIA screening*: Inspection of the surface of the uterine cervix after 3 – 5% acetic acid has been applied to it. It is a test sometimes to determine whether the cervix is infected with human papilloma virus or whether irregularities seen on the cervix may be cancerous or precancerous.[2]

CHAPTER 1: INTRODUCTION

Cervical cancer is a growing public health challenge. According to the World Health Organization (WHO) 528,000 new cases of cervical cancer were diagnosed worldwide in 2012. In the same year, approximately 270,000 women died of cervical cancer worldwide; more than 85% of these occurring in low to middle income countries. In sub-Sahara Africa, 31.5 new cases of cervical cancer are diagnosed per 100,000 women annually; and 22.5 per 100,000 women die from the disease.[5]

Swaziland is no exception to this global plight, where the problem is fueled further by the high Human Immunodeficiency Virus (HIV) prevalence of 31% among adults (18-49 years), which is one of the highest in the world.[6] The 2018 cancer statistics from the World Cancer Research Fund, revealed that Swaziland ranked the highest globally with an age standardized rate of 75.3 per 100,000.[7] A report by the national cancer registry office documented cervical cancer as the commonest cancer in Swaziland accounting for 31.1% of all cancers.[8]

1.1 BACKGROUND

Swaziland situated in Southern Africa, is a landlocked country bordered by South Africa and Mozambique and it measures 17,364 square kilometers (km^2), making it the smallest country in Southern Africa. It is divided into four regions of Manzini, Hhohho, Lubombo, and Shiselweni (Figure 1). The national population and household census of 2007 stated the total population of the country was 1.018,449. Over half (53%) of the country comprises females and 52% of these are below the age of 20 years. Women of the reproductive age (15-49 years) account for half (49%) of the national female population. The majority of Swaziland's population (77.9%) resides in the rural areas where there are more men (52%) than women (48%).[9]

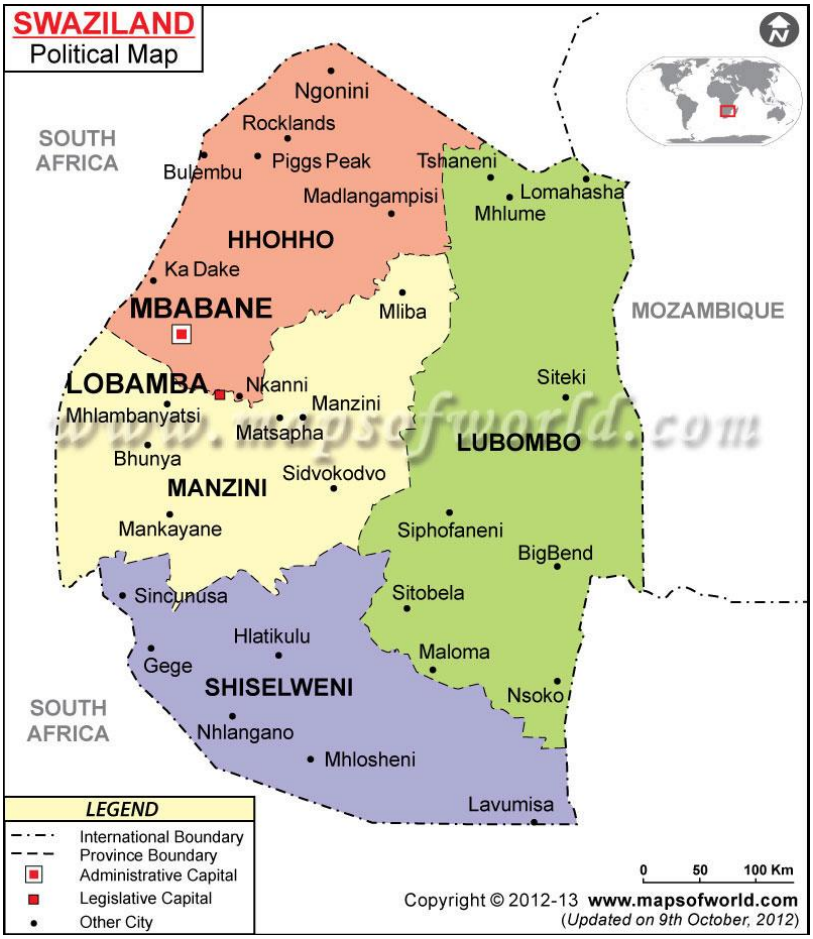


Figure 1: Political Map of Swaziland

SOURCE:https://www.google.com/search?q=map+of+swaziland&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiXjLeo_PrdAhVqtYsKHWLCAKYQ_AUIDigB&biw=1093&bih=522#imgrc=hVudPaTQhwg6vM:

The health service delivery system consists of both formal and informal sectors. The formal sector comprises the public, private-not-for-profit and the private-for-profit components. Within the public sector, the government owns 40.1% of the health facilities, whilst 22.7% are privately owned by doctors, 12.2% are owned by faith-based institutions, 10.8% by industrial facilities, 7.3% by non-governmental organisations (NGO), and 7% by nurses.[9] The informal sector consists mainly of traditional and alternative health care providers.[9] Figure 2 below outlines the structure of the health service provision within the public sector.

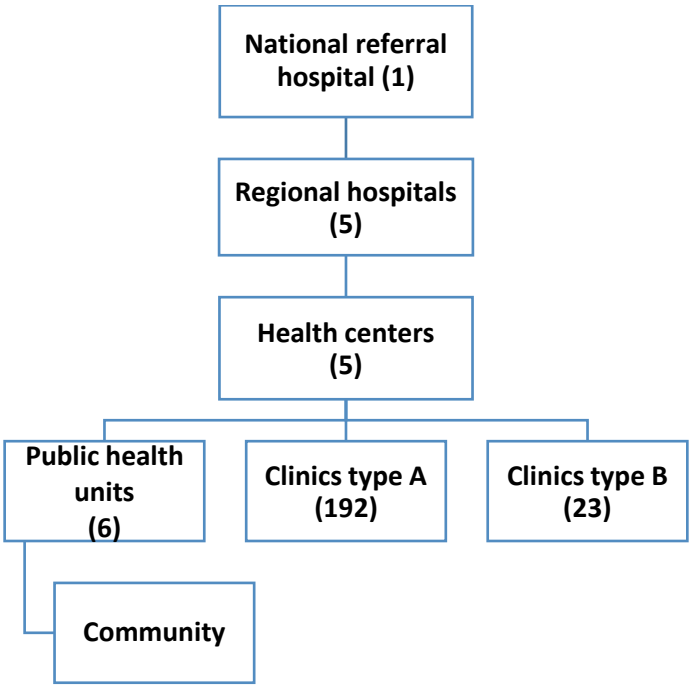


Figure 2: Structure of the Swaziland Health Care System in the Public Sector

In total, there are 287 health facilities organised within a five-tier health care system of; 1 national referral hospital (Mbabane government hospital), 2 specialised referral hospitals (TB hospital and psychiatric hospital), 5 regional hospitals, 2 specialised and 6 private hospitals, 5 health centres and 6 public health units (PHUs) which are units mainly focused on maternal and child health services including antenatal care, post-natal care, and expanded programme on immunization (EPI), 23 clinics with and 192 without maternity units, 6 specialised clinics. [9] Type A clinics do not have maternity wings whereas type B clinics include a maternity wing.[9]

The referral system mandates a patient to first consult at a clinic before being referred to the health centre, then to the regional hospital or the national referral hospital. The PHUs regularly have community service outreaches. Where clinical competencies are deficient, patients are referred to the neighbouring countries.

In 2016, cervical cancer screening was offered at 71 health facilities which included all the hospitals, all health centres and some of the clinics.[10] Treatment of pre-cancer was offered at all the hospitals, all health centres and 3 clinics.

"Pre-cancer", also referred to as cervical intraepithelial neoplasia (CIN), can be diagnosed using screening methods such as a HPV-DNA testing, Papanicolaou smear test (Pap smear) and visual inspection using acetic acid (VIA). Treatment modalities for pre-cancer include cryotherapy, cold knife conization, and loop electrosurgical excision procedure (LEEP).[11] These treatment modalities are available in Swaziland thus rendering the pre-cancer stage the best time to intervention with treatment.[12] Beyond this stage, all overt cancer cases in Swaziland are referred outside the country for treatment which is often a challenge. Hence, the importance of early intervention during the pre-cancer stage.

1.2 RESEARCH PROBLEM

Cervical cancer is the most common HPV-related cancer in Swaziland accounting for over half of all female cancers.[8] The current treatment protocol for cervical pre-cancerous lesions entails a thorough clinical assessment at initial consultation. The mode of treatment is determined by extent of the lesion. If it is mild dysplasia involving the lower (or less) one third of the epithelial thickness (CIN 1/ grade 1), cryotherapy is the treatment of choice.

However, if it is between grade 2 (CIN 2) and grade 3 (CIN 3); that is, covering more than 75% of the endo-cervix or encroaches onto the endo-cervical canal, the loop electrosurgical excision procedure (LEEP) is performed.[12] If a grade 1 lesion has an unfavourable response after 6 months, LEEP follows.[12]

It is apparent that some women present with recurrence after cryotherapy (particularly those who are HIV infected). The quantifiable burden of cervical intraepithelial neoplasia recurrence after initial treatment in Swaziland remains unknown.

1.3 AIM AND OBJECTIVES

The aim of the study was to determine the burden of cervical intraepithelial neoplasia recurrence at six months post treatment with either cryotherapy or LEEP between January 2014 and December 2016 at Mbabane government hospital, Swaziland.

The specific objectives were:

1. To describe the profile of study participants enrolled in the CIN screening programme
2. To estimate the proportion of CIN recurrence at 6 months among participants screened with VIA and stratified by HIV status
3. To determine CIN recurrence rates by treatment modality: Cryotherapy versus LEEP

CHAPTER 2: LITERATURE REVIEW

2.1 PATHOPHYSIOLOGY OF CIN AND CERVICAL CANCER

Cervical intraepithelial neoplasia, which can progress to invasive cervical cancer (ICC) is mainly caused by the human papilloma virus (HPV). HPV is categorized into low risk non-oncogenic types, mainly implicated in chondylomata; and the high risk (hrHPV) oncogenic types, implicated in cancer and causes over 95% of all cervical cancer cases.[13] Infection of the cervix with HPV does not always lead to cervical cancer as there must be favourable host factors (including genetic susceptibility) to allow the integration of the viral DNA into the host DNA resulting in cervical cell dysplasia.[14]

Upon infection with HPV; depending on certain patient, genetic or environmental factors; the disease can progress over months to years before becoming invasive or it can regress to normal epithelium.[15] The higher the CIN grade, the less likely the regression of disease.[15] Figure 3 below demonstrates the progression of CIN from HPV infection until it becomes invasive cancer.

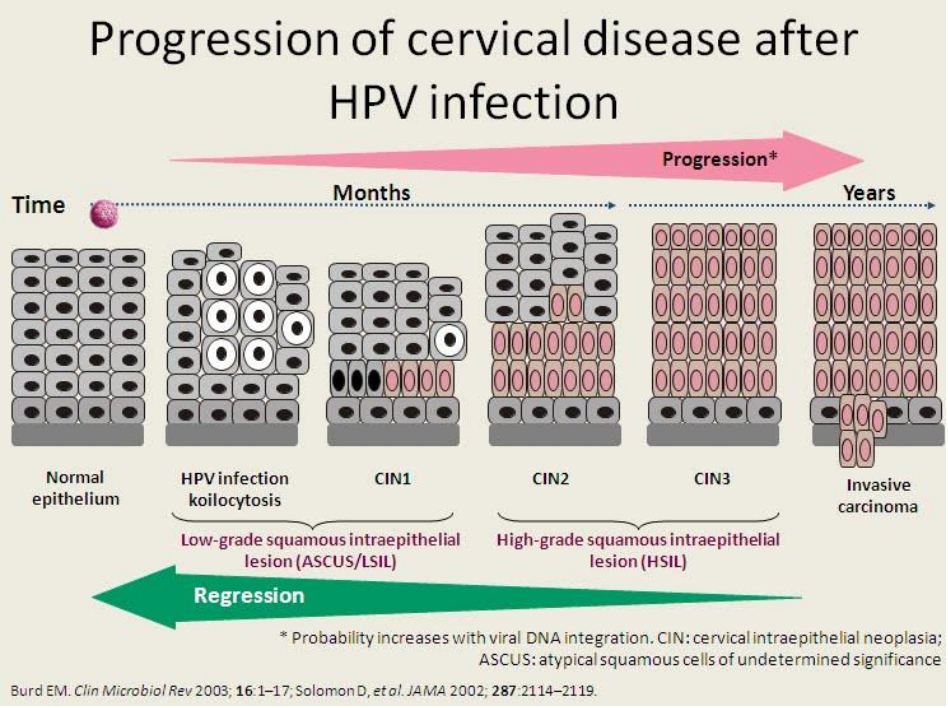


Figure 3: Progression of cervical disease after HPV infection
SOURCE:
https://slideplayer.com/slide/9231686/27/images/15/HPV+cervical+disease%3A+cervical+carcinogenesis+%28Progression+%26+regression%29.jpg

2.2 RISK FACTORS AND PROGRESSION OF CERVICAL CANCER

There are several risk factors associated with the development of cervical cancer which include but are not limited to immunosuppression, multiple sexual partners, oral contraception use, smoking, and early sexual debut. Chronic infection with and the presence of the oncogenic type of the sexually-transmitted HPV is the causative agent in all cases of cervical cancer.[16-19]

Immune suppression is also a well-documented risk factor for the development of cervical cancer. HIV infected individuals are at higher risk of HPV infection and are infected by a broader range of HPV types[1]. It takes about 15 to 20 years for cervical cancer to develop in women with competent immune systems.[1] However, in patients with impaired immune systems the progression from infection with HPV to cervical intraepithelial neoplasia to cancer may be more rapid (5-10 years). [1,12]

The risk of squamous intra-epithelial lesions (SIL), also known as cervical intraepithelial neoplasia (CIN), is higher in HIV infected women; and with increased life expectancy due to highly active anti-retroviral therapy (HAART), their risk of cervical cancer may inadvertently increase.[20] Literature has shown a synergetic effect of HIV infection and HPV (implicated in all cervical dysplasia and cancer cases).[20-22]

Kafuruki et al. have shown that other factors associated with CIN include multiple sexual partners, a history of sexually transmitted infections, and a low baseline CD4 cell count.[23] Sun et al. assert that the association between oral contraceptive use and combined contraceptive risk is due to oestrogens and progestogens which interact with hormone receptors (mainly progesterone) within the cervical tissue and modulate the natural history of HPV infection. Pregnancy causes elevated progesterone level, which in turn increases HPV gene expression, and thus more viral copies and multiplication of virus-transformed cells.[24, 25]

Conversely, oestrogen also plays a critical role in the genesis of cervical cancer, as well as its persistence and continued development.[24] During the first trimester, there is a transient low immune response to HPV, and a resultant viral persistence. However, recovery ensues at the beginning of the third trimester with reinforcement during the postpartum period where there is eventual regression of the infection.[18] Other implicated risk factors include smoking and early sexual debut.[25, 26]

There is a confirmed interaction between passive tobacco exposure and active smoking for hr-HPV infection and high grade CIN.[25] Feng et al. found that active smoking increased the risk of overall hr-HPV infection and higher CIN grade (CIN 2 and above); and that passive smoking mildly increased the risk of HPV infection although it did not affect the dysplastic progression.[26]

Early sexual debut has also been identified as a significant risk factor for cervical cancer. Louie et al. found that the risk for invasive cervical carcinoma was 2.4-fold among those who reported early age at first sexual intercourse (AFSI) and age at first pregnancy (AFP) at 16 years or younger, compared to those with AFSI and AFP at 21 years or older.[26]

Other described risk factors for cervical intraepithelial neoplasia include sexually transmitted infections (STI). Some studies have shown that a history of STIs tend to have a progression effect on cervical intraepithelial neoplasia.[23, 27]

Lifetime sexual partners is known to be a risk factor for human papilloma virus – the causative agent for CIN. A study by Chan et al. found that significantly the regression of CIN was far less in women who had had more than five sexual partners.[28] Kafuruki et al. concluded that the problem of CIN is worsened by a history of multiple sexual partners, a history of genital warts, a history STI and a low baseline CD4 T-cell lymphocyte.[23]

2.3 HPV VACCINATION EFFECTS ON CERVICAL INTRAEPITHELIAL NEOPLASIA

The human papilloma virus (HPV) vaccine was developed to target both the high risk (hr-HPV: 16, 18) strains implicated in causing dysplasia and cancer; as well as the low risk type strains (6, 11) known to cause genital warts. There are currently two licensed HPV vaccines. The Bivalent type, which contains recombinant virus-like particles of only HPV types 16 and 18 (which account for approximately 70% of cervical cancer). Another vaccine, the Quadrivalent type, also protects against HPV types 6 and 11.[29,30,31]

The newer vaccine (Nine-valent) type protects against 9 different subtypes of HPV, namely 6, 11, 16, 18, 31, 33, 45, 52, and 58.[32, 33] Denny et al. found that in sub-Saharan Africa, the most common HPV subtypes were HPV 16, 18, 45 and 35; and that HIV infected women were more prone to

invasive cervical cancer and HPV infections.[34] The vaccines available in the market are targeted against some of the HPV strains common in sub-Sahara Africa. However, the Nine-valent vaccine would fare better as it would cover against all these common subtypes. A study by Capra et al. has shown that switching from first generation HPV vaccines to a Nine-valent vaccines would increase the prevention of diseases caused by the nine HPV strains including up to 90% of cervical high grade lesions.[32, 33]

A recent study conducted in Swaziland by Ginindza et al. indicate the significant association of high hr-HPV infection prevalence and HIV among sexually active women.[35] Kang et al. have concluded that, vaccination with the Quadrivalent HPV vaccine after treatment should be considered to prevent recurrence of CIN 2-3 in those already infected with HPV.[36] The vaccine can therefore be considered beyond the sexually naïve individuals as it can prove beneficial among the sexually active.

The HPV vaccine is not yet available in Swaziland. A feasibility study conducted by the Ministry of Health with the support of WHO was introduced in 2014. Pending the adoption of that report, processes for its introduction are on-going but there is no stipulated time for its roll-out.

2.4 DIAGNOSIS AND MANAGEMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA

The screening methods available in Swaziland are VIA and Pap smear. HPV-DNA testing is not yet available. The procedure for management is cryotherapy. If a VIA positive lesion is not eligible for cryotherapy, then the patient is offered LEEP. The cryotherapy eligibility criteria is a positive screening test for cervical cancer. Lesions must be small enough to be covered by the cryoprobe and should be no more than 2mm, with all edges of the lesion fully visible without extension into the endo-cervix.[12]

Post cryotherapy a patient is reviewed after 6 months. The possible result at review could be, the absence of cervical intraepithelial neoplasia lesion, the stagnant lesion, or a progressed lesion. In practice, the majority of CIN recurrences occur in HIV infected women. [12] However, this has not been quantified.

2.5 CRYOTHERAPY, LEEP AND HIV INFECTION

Cryotherapy is a procedure used to destroy abnormal cells by freezing. This procedure can be as much as 90% effective in eliminating the abnormal tissue.[37] It uses a cryogun and nitrous oxide gas for freezing the abnormal cells, can be done on outpatient basis, and does not need administration of anaesthesia. In relation to HIV, there is no effect of increasing the shedding of HIV after cryotherapy.[22] Melnikow J et al. noted that cryotherapy, compared with other treatments, was associated with the highest rate of subsequent disease (AOR for cancer= 2.98).[38]

In contrast, the loop excision electro-surgical procedure (LEEP) is surgical and provides excisional treatment. This procedure minimizes blood loss by thermal cautery during excision but may cause thermal artifact that impairs the interpretability of a histology specimen.[39]

A study conducted in Kenya concluded that LEEP performed by clinical officers was well-accepted by HIV-infected women and appears safe, resulting in minimal side-effects, even among women with early resumption of intercourse.[24] LEEP is the preferred modality of treating cervical intraepithelial neoplasia if cryotherapy is not indicated.[40]

A study comparing the two modalities, found that the efficacy of cryotherapy was 88%, and 94% for LEEP; and the cure rates were 82% and 79% for the cryosurgery and LEEP, respectively.[41]

2.6 SCREEN AND TREAT PROGRAM

The ‘Screen and Treat’ program as stipulated by the WHO was adopted in 2011 at 3 Swaziland health facilities, and in 2015 expanded to 54 facilities. However the rate of screening has remained low at 13.4%.[42] The program comprises screening using visual inspection with acetic acid (VIA) and treating those who screen infected with cryotherapy or LEEP depending on eligibility.[21] With a good program, the incidence of invasive cancer can be markedly reduced.

Cervical pre-cancer is asymptomatic and thus many women only seek medical attention when the disease is often advanced and invasive with clinical symptoms. Screening using VIA is only performed in women of pre-menopausal age because the procedure demands that the provider visualizes the transformation zone (area of application of acetic acid) which cannot be visualized in post-menopausal women. The transformation zone is embedded in the endo-cervical canal, during post-menopausal age.[43]

The treatment of invasive cancer is complex and involves surgery, radiotherapy and chemotherapy.[44] The only treatment modality available in Swaziland is surgery. Patients are referred to South Africa, a neighbouring country, for radiotherapy and chemotherapy. This being the case, the strengthening of the screen and treat program is paramount in order to effectively manage those affected during the pre-cancer period.

There is a high rate of loss-to-follow-up for most patients diagnosed with cervical intraepithelial neoplasia. In 2013, 36% of the patients with a positive VIA screening test mandating an intervention (cryotherapy or LEEP) from the Mbabane government hospital cervical screening clinic, did not return for follow-up.[45] Further research is needed to investigate the reasons for the high loss-to-follow-up rate but the current assumption is lack of access, especially due to financial constraints, as most patients are from a low socio-economic background.

2.7 RECURRENCE OF CIN AFTER TREATEMENT

The magnitude of recurrence of cervical intraepithelial neoplasia after treatment has been document by a few studies. Huchko MJ et al. found that by 6 months after the treatment of CIN, 7.1% of the patients had recurrent CIN (2 and above).[46] Recurrence of CIN depends on a variety of factors which include the initial CIN grade, treatment type, and age among others.[38] There is an increased long-term risk of progression to invasive cancer among women treated for CIN, particularly those treated with cryotherapy in comparison with other treatment modalities.[38]

Infection with HIV is a documented independent risk factor for CIN recurrence.[47, 48] A study by Fruchter et al. found that CIN may recur despite multiple treatments, and chronic condylomatous changes are common in HIV infected women.[30] The risk of persistent HPV infection, premalignant lesions and cervical cancer is higher among immune suppressed women compared with immune competent women, and data further submits that cervical cancer occurs up to 10 years earlier in HIV infected women.[44,49] Furthermore, cervical cancer is at an advanced stage upon presentation and diagnosis, has more treatment-related complications and a higher tendency for recurrence and more likely to cause death in HIV infected women compared with the uninfected.[26,50-52]

If infected with HIV, the CD4 cell count is a factor to be considered for cervical disease. WHO clinical staging categorizes cervical cancer as an AIDS defining illness.[53] AIDS has been described to occur with a CD4 cell count less than 200 cells/μL; a deficient immune system making the individual to be susceptible to opportunistic infections and malignancies due to impaired immune mechanisms.[54] A study by Oga et al. found that HIV-infected women with low CD4 counts were at increased risk of recurrent lesions and possibly related to the immunosuppression.[49]

The initiation of antiretroviral treatment (ART) for the management of HIV infection has generally proven beneficial to boost the immune system and lowering the susceptibility to opportunistic infections. It is also associated with the reduced risk of CIN occurrence, as well as progression to advanced disease.[49, 55, 56]

The initial treatment modality plays a role in recurrence. A study by Singh et al. showed that LEEP was associated with higher cure rates than cryotherapy although the difference was not statistically significant; and that LEEP seemed to have superior results compared to cryotherapy when used for severe lesions.[26] However, incomplete excision of the lesion also had an effect on recurrence.[31] Another study showed that excisional procedures, including LEEP, for high-grade CIN indicated a very low risk for recurrent disease and potentially negligible risk for invasive cancer.[57] However, this was incumbent on a strict and vigorous follow-up which was offered after treatment.[57] This further strengthens the need for regular (annual) reviews, particularly for those previously treated.

To date, there is no documentation on the risk factors and burden of the recurrence of cervical intraepithelial neoplasia following cryotherapy or LEEP in Swaziland.

CHAPTER 3: METHODOLOGY

3.1. STUDY DESIGN

A retrospective cohort study design was employed.

3.2 STUDY SETTING

The study setting was the Mbabane government hospital (MGH), the national referral hospital, situated in the capital city, Mbabane in Hhohho region. On average, MGH performs 1000 VIA screenings and the positivity ranges between 100 and 300 annually.

3.3 STUDY PARTICIPANT SELECTION

The study population comprised all women who were screened for cervical pre-cancerous lesions, from January 2014 to December 2016 and at initial screening, were VIA positive and underwent cryotherapy or LEEP at MGH, and who subsequently returned for the 6-month follow-up review.

Inclusion Criteria:

Women with the following characteristics were included:

- Infected VIA screening with eligibility for cryotherapy or LEEP
- Pre-menopausal
- Returned for the 6-month follow-up review

Exclusion criteria:

Women who were excluded from the study were:

- Those with overt cervical cancer at initial screening
- Post-menopausal
- Those who did not return for the 6-month review were excluded.

3.4 FACILITY RECORD KEEPING

There is a standard tool for data capturing at the screening clinic and the policy for record keeping in government facilities is 25 years but may be longer depending on space capacity.

3.5. MEASUREMENTS

The medical records of those who underwent either cryotherapy or LEEP procedure were obtained from the facility records administration room after being identified from the screening clinic register (Annex 1). Information which was extracted for the demographics included age, marital status, parity, and use of contraception and medical information using a data collection form (Annex 2). Each woman was given a unique identifier and no patient name was recorded on the data collection form.

Table 1: Scale of measurement for the different variables collected

Variable	Scale of measurement
Age	Numerical data
Sexual debut	Numerical data
Lifetime sexual partners	Numerical – number of sexual partners
Marital status	Categorical (married, single, other)
Parity	Numerical – number of children
District	Nominal (Hhohho, Manzini, Shiselweni, Lubombo)
Educational level	Ordinal (primary, secondary/high, tertiary, none)
Smoking history	Categorical data (current, ex-smoker, never)
STI history	Categorical data (yes, no)
Family history of cancer	Categorical data (yes, no)
Sexual debut	Categorical data (16 years or less, 17-19years, 20 years or more*
Use of contraception	Categorical data (pill, injectable, long term, nil, other)
HIV status	Categorical data (infected, uninfected, unknown)
CD4 count	Categorical data (0 – 200, 350 – 499, 500 or more, unknown, NA!

* categorized in consideration of the adolescent age of up to 19years

! Not applicable. Implied for the HIV uninfected study participants

The outcome measure was recurrence at six months (yes or no). Explanatory variables included variables in the above Table as well as the type of intervention used at the initial visit coded as a categorical variable (LEEP = 0 and cryotherapy = 1). The time interval post-treatment was six months for all the patients.

3.6. DATA MANAGEMENT

3.6.1 Sample size justification

Based on the literature, approximately 10% of women exposed to HPV will develop cervical precancerous lesions, although this is slightly higher among HIV infected women.[20, 58, 59] However, the average recurrence of CIN following treatment is at least 15 % even with the most effective excisional and ablative treatment modalities within 12 months follow-up after treatment.[49]

The sampling frame for this study included the registers of all patients who underwent cryotherapy and LEEP at the screening clinic during the study period (January 2014 to December 2016). On average, the clinic performs 1000 VIA screenings and the VIA positivity ranges from 100 to 300 per year. The derivation of the sample size using G-Power software (version 3.1.9.2) with the following assumptions: odds ratio of 2 or greater, 15% recurrence rate and power of above 80%, yielded an estimated size of 450 for the 3-year study period.

Eventually, the complete sampling method was employed since all the 602 cases met the inclusion criteria. This slightly larger sample, ensured adequate power for the study, and rendered it more robust.[60]

3.6.2. Data collection

The data were collected using a data collection form to capture the socio-demographic and other clinical information of patients who were treated from January 2014 to December 2016. (Annex 2). The hospital records were the source of information where these records were reviewed, and the outcome of the follow-up visit documented.

The data were reviewed for accuracy, consistency, completeness, and the errors were corrected. This was paper-based, and the collected data were transferred manually to a computer and cleaned using Microsoft Excel (2013) software.

3.7 STATISTICAL ANALYSIS

For all analyses, STATA version 13 (Stata Corp., College Station, TX, USA) was used. For descriptive statistics, continuous variables were summarised using the mean and standard deviation if normally distributed and, the median and inter-quartile range for skewed data. For categorical variables, the frequency distribution tables were used to summarise data using numbers and percentages. The Pearson Chi-square test was used to test differences between categorical variables and the student t-test for continuous variables.

Univariate and multivariate logistic regression models were computed to examine the recurrence of CIN for participants who returned for follow-up after cryotherapy or LEEP as first line treatment and socio-demographic/clinical characteristics. All variables which are known *a priori* or with a p-value <0.25 in univariate analysis were considered for inclusion in the final regression models. The backward stepwise elimination method was used to arrive at the final models. Effect estimates are presented as crude or adjusted odds ratios (OR) with 95% CIs and p-values. All p-values <0.05 were considered to be statically significant.

3.8. ETHICAL CONSIDERATIONS

This research undertaking comprised secondary data analysis and the following were considered:

1. Anonymity – no patient names were used. Each patient had a unique identifier and information was only accessed by the principal investigator.
2. Confidentiality was maintained always. The records were safely stored in a lock cupboard.

   Permission to gain access to the facility data was obtained from the facility management (Annex 3). Ethical approval to conduct the study was obtained from the Swaziland Health Ministry Ethics Committee as well as the Faculty of Health Sciences Research Ethics Committee at the University of Pretoria (Ethics reference number: 389/2016 - Annex 4)

3. Upon conclusion of the study, the data sheets will be kept in a sealed box in the data storage room of the School of Health Systems and Public Health at the Faculty of Health Sciences, University of Pretoria for 15 years.

CHAPTER 4: RESULTS

4.1. INTRODUCTION

The total number of patients treated for CIN between 2014 and 2016, was 1205. From these, 602 were eligible study participants who returned for the 6-month follow-up post treatment. The complete sampling method was employed. Figure 4 below outlines the distribution of study participants who returned for follow-up by region. The Mbabane government hospital is in the Hhohho region, which comprised most of the participants (378; 63%).

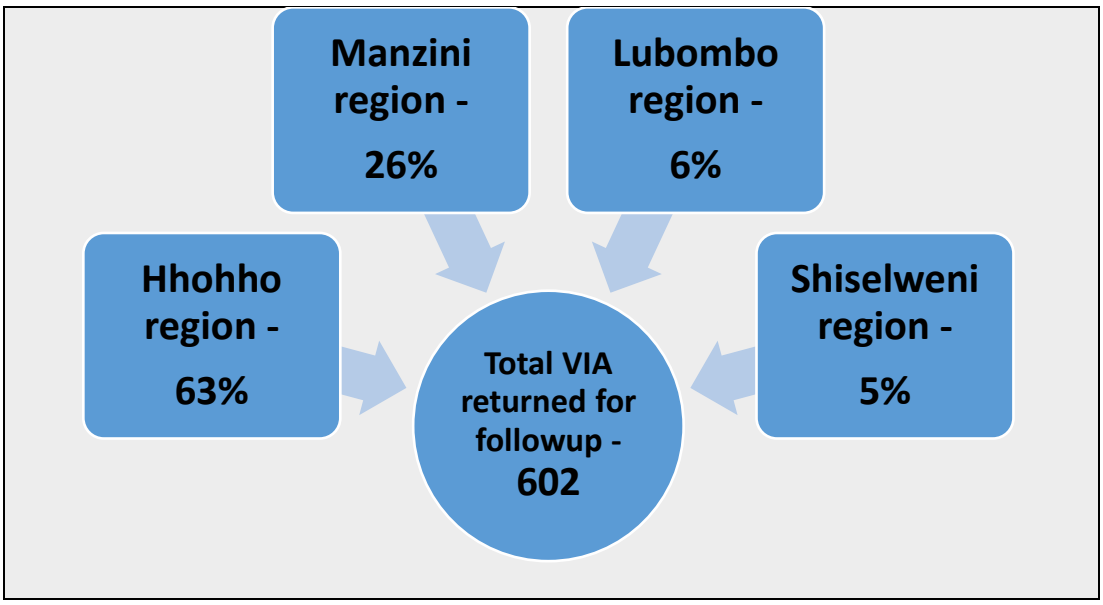


Figure 4: Total participants seen at 6-months follow-up post treatment.

4.2 DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

Table 2 below depicts the demographic characteristics of the study participants with detected CIN through the screening programme of Mbabane government hospital during the period January

2014 to December 2016. The Hhohho region had the highest representation (378; 63%), followed by Manzini region (156; 26%), Lubombo (37; 6%) and Shiselweni (31; 5%). More than half (344; 57%) of the participants had attained secondary/high school education, 110 (18%) and 89 (15%) had tertiary and primary education, respectively. Less than 1% (3) were without education.

The mean age was 30.9 years (SD: 5.7), the oldest at 46 years and the youngest 15 years. Age was normally distributed (Annex 6; Shapiro Wilk p-value=0.105). Most (319; 53%) were single, less than half (271; 45%) were married; and few (12; 2%) were divorced or widowed.

The median sexual debut age was 18 years (youngest at 12 years and the oldest aged 28 years). Sexual debut was not normally distributed (Annex 6: Shapiro Wilk p-value<0.001). Most study participants (386; 64%) had more than two lifetime sexual partners (LSP), the majority of whom had LEEP as the primary mode of treatment.

More than half (378; 63%) of the participants had one to two children, followed by a third who had three to five children (155; 26%). Only 8 (1%) had more than five children and the remaining 61 (10%) were nulliparous. Although this observation was similar for either treatment modality, more nulliparous participants and those with one to two children were treated with LEEP (14% and 66%, respectively) than with cryotherapy (9% and 62%, respectively).

Table 2: Demographic characteristics of study participants treated with either modality

Variable	Total participants treated: N (%)	Participants treated with Cryotherapy: n (%)	Participants treated with LEEP: n (%)	Chi 2 or t-test	p-value
Region				4.25	0.236
Hhohho	378 (63)	276 (64)	102 (59)		
Manzini	156 (26)	104 (24)	52 (30)		
Shiselweni	31 (5)	25 (6)	6 (3)		
Lubombo	37 (6)	24 (6)	15 (8)		
Education level				6.15	0.188
Primary	89 (15)	63 (15)	26 (15)		
Secondary/High	344 (57)	246 (57)	98 (57)		
Tertiary	110 (18)	85 (20)	25 (14)		
No Education	3 (1)	2 (0)	1 (1)		
Unknown	56 (9)	33 (8)	23 (13)		
Marital status				1.58	0.454
Married	271 (45)	198 (46)	73 (42)		
Single	319 (53)	224 (52)	95 (55)		
Other	12 (2)	7 (2)	5 (3)		
Parity				7.98	***0.047**
Nulliparous	61 (10)	37 (9)	24 (14)		
1 – 2 children	378 (63)	264(62)	114 (66)		
3 – 5 children	155 (26)	122 (28)	33 (19)		
>5 children	8 (1)	6 (1)	2 (1)		
Life-sexual-partners				3.27	0.195
1 – 2 LSP	209 (35)	157 (37)	52 (30)		
>2 LSP	386 (64)	266 (62)	120 (69)		
Unknown	7 (1)	6 (1)	1 (1)		
Age (years)	Mean 30.9	30.9	30.9	0.09	0.928
Sexual debut (years)	Median	18			

Note: * statistically significant

#we used the complete case analysis hence for some variables, the total number of study participants is less than 602

LEEP: Loop electrosurgical excision procedure

LSP: Lifetime sexual partners

4.3 CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS

Table 3 below depicts the clinical characteristics of the study participants by treatment. A third of the participants were not on any contraceptives and this remained consistent when stratifying by treatment modality. The long-term contraception commodity was the least used at 3%.

The notable significant clinical characteristics among those treated with either modality, were the HIV status (p=0.024) and ART (p=0.039). More than half (118; 68%) of the HIV infected participants underwent LEEP treatment than cryotherapy. A sizable proportion (101; 28%) of the HIV infected did not have a known CD4 count result. The uninfected (222; 37%) were treated with cryotherapy than LEEP. Few (21; 3%) had an unknown HIV status.

Most participants did not have a history of smoking (94%) or family history of cancer (90%). A third (33%) had a history of sexually transmitted infection treatment.

Table 3: Clinical characteristics of study participants treated with either modality

Variable	Total participants treated: N (%)	Participants treated with Cryotherapy: n (%)	Participants treated with LEEP: n (%)	Chi 2 or t-test	p-value
Contraception				5.27	0.384
Oral	49 (8)	37 (9)	12 (7)		
Injectable	104 (17)	70 (16)	34 (20)		
Long term method	19 (3)	14 (3)	5 (3)		
Other	148 (25)	111 (26)	37 (21)		
None	195 (32)	142 (33)	53 (31)		
Unknown	87 (15)	55 (13)	32 (18)		
HIV status				7.43	**0.024***
HIV infected	359 (60)	241 (56)	118 (68)		
HIV uninfected	222 (37)	172 (40)	50 (29)		
Unknown	21 (3)	16 (4)	5 (3)		
CD4 count (!)				7.74	0.102
0 – 349	70 (11)	48 (11)	22 (13)		
350 – 499	73 (12)	48 (11)	25 (14)		
500 +	137 (23)	91 (21)	46 (27)		
Unknown	101 (17)	70 (17)	31 (18)		
Not applicable	221 (37)	172 (40)	221 (28)		
ART treatment				8.39	**0.039***
Not on ART	82 (14)	52 (12)	30 (17)		
On ART	246 (41)	170 (40)	76 (44)		
Unknown	53 (9)	35 (8)	18 (10)		
Not Applicable	221 (37)	172 (40)	49 (28)		
Cancer family history				1.28	0.528
Yes	62 (10)	41 (10)	21 (12)		
No	539 (90)	387 (90)	152 (88)		
Unknown	1 (0)	1 (0)	0 (0)		
STI treatment				0.18	0.675
Yes	199 (33)	144 (34)	55 (32)		
No	403 (67)	285 (66)	118 (68)		
Unknown					
Smoking History				0.12	0.733
Yes	38 (6)	28 (7)	10 (6)		
No	564 (94)	401 (93)	163 (94)		

Key: * statistically significant
(!) CD4 measured in cells/μL
#we used the complete case analysis hence for some variables, the total number of study participants is less than 602
ART – anti retroviral therapy
LEEP – loop electrosurgical excision procedure
STI – sexually transmitted infection
Total number of participants N= 602: Cryotherapy n=429; LEEP n=173

4.4. RECURRENCE OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

Recurrence occurred in 39 of the 602 who returned at the 6-month review after treatment. Figure 5 below shows that the proportion of cervical intraepithelial neoplasia (CIN) recurrence among all study participants screened with VIA and followed up at 6 months post treatment with either modality at Mbabane government hospital was 6.5%.

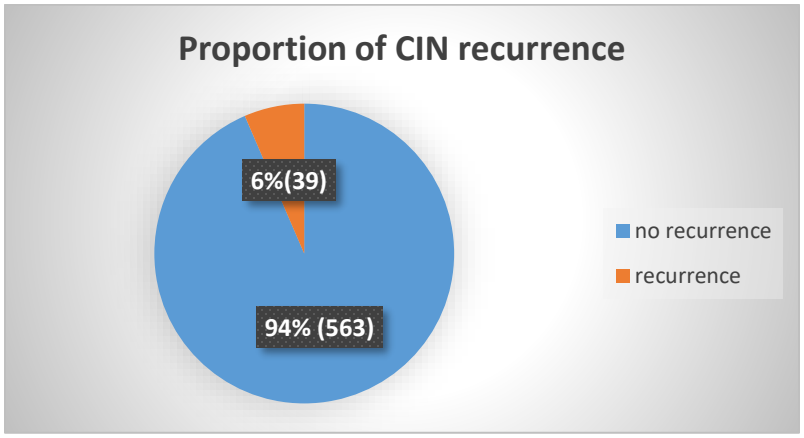


Figure 5: CIN recurrence at 6 months follow up among all study participants screened with a significant VIA result during the study period

Total recurrences = 39
Total VIA positive treated during the period = 602
Proportion of CIN recurrence = 39/602 *100 = 6.5%

4.5. RECURRENCE OF CIN BY VARIABLE

The proportion of recurrence by variables is outlined in Table 4 below. Most (25; 64%) recurrences were in the Hhohho region, with the least (3; 8%) in both the Lubombo and Shiselweni regions. The mean age of study participants who had recurrence was 30.1 years (SD 4.92; 95%CI 28.5-31.7); and for those with no recurrence was at 30.9 years with a higher standard deviation and narrower confidence interval. The median sexual debut age for study participants with recurrence and for those without recurrence was 17 and 18 years, respectively.

More than half (23/39; 59%) of those with recurrence were married. Among those with recurrence, two thirds (27; 69%) were HIV infected, and a third (11; 28%) were uninfected. Recurrence was noted in participants who reported to have had more than 2 lifetime sexual partners (24; 65%) and those with a sexual debut of less than 20 years (34; 87%).

Table 4: Proportion of recurrence by explanatory variable

Variable	Recurrence (n; %) N = 39	No recurrence (n; %) N = 563
Region		
Hhohho	25 (64)	353 (63)
Manzini	8 (21)	148 (26)
Shiselweni	3 (8)	28 (5)
Lubombo	3 (8)	34 (6)
Age (mean years)	30.1 (95%CI: 28.5-31.7)	30.9 (95%CI 30.5-31.4)
Nil/Primary	9 (24)	83 (14)
Secondary/High	19 (49)	325 (58)
Tertiary	6 (15)	104 (19)
Marital status		
Married	23 (59)	248 (44)
Single	16 (41)	303 (54)
Other	0 (0)	12 (2)
HIV status		
HIV infected	27 (69)	332 (59)
HIV uninfected	11 (28)	211 (37)
Unknown	1 (3)	20 (4)
Contraception		
Oral	3 (8)	46 (8)
Injectable	7 (18)	97 (17)
Long term method	1 (3)	18 (3)
Other	10 (26)	138 (25)
None/Unknown	24 (45)	264 (47)
Parity		
Nulliparous	4 (10)	57 (10)
1 – 2 children	22 (57)	356 (63)
> 3 children	13 (33)	150 (27)
Lifetime Sexual Partners		
0 – 2	13 (33)	196 (35)
>2	24 (62)	362 (64)
Unknown	2 (5)	5 (1)
Sexual debut		
$\leq$ 16 years	12 (31)	159 (28)
< 20 years	22 (56)	274 (49)
$\geq$ 20 years	5 (13)	130 (23)
STI treatment		
Yes	10 (26)	189 (34)
No	29 (74)	374 (66)
Cancer family history		
Yes	3 (8)	59 (11)
No	36 (92)	503 (89)
Smoking History		
Yes	3 (8)	35 (6)
No	36 (92)	528 (94)
Sexual debut (mean years)	17.3 (95%CI: 16.6-17.9)	17.9 (95%CI 17.7-18-1)

4.6. RECURRENCE OF CIN BY REPRODUCTIVE HEALTH VARIABLES

Table 5 below outlines recurrence of CIN by reproductive health variables. The odds of recurrence for those with an unknown lifetime sexual partners (LSP) were six times in comparison to those who had 1-2 LSP (OR 6.03; 95%CI 1.07-34.13; p=0.042).

Table 5: Recurrence of CIN and reproductive health variables

Variable	N (#)	Recurrence OR (95% CI)	p-value
Lifetime SP	602		
1 – 2		1 (reference)	
>2		1.00 (0.50 – 2.01)	0.999
Unknown		**6.03 (1.07 – 34.13)**	***0.042**
Sexual debut			
16 years or less		1 (reference)	
17 – 19 years		1.06 (0.51 – 2.21)	0.868
20 years or more		0.51 (0.18 – 1.48)	0.216
Contraception	602		
Oral		1 (reference)	
Injectable		1.11 (0.27 – 4.47)	0.887
Long term method		0.85 (0.08 – 8.74)	0.893
Other		1.11 (0.29 – 4.21)	0.877
None		1.01 (0.27 – 3.71)	0.993
Unknown		1.14 (0.27 – 4.76)	0.862
Parity	594		
0		1 (reference)	
1 – 2		0.88 (0.29 – 2.64)	0.821
3 – 5		1.30 (0.41 – 4.17)	0.654
>5		1 (omitted)	
STI treatment	602		
Yes		1 (reference)	
No		1.47 (0.70 – 3.07)	0.311

Key:
(#) complete case analysis was used hence for some variables, the total number of study participants is less than 602
*statistically significant
SP - sexual partners
STI - sexually transmitted infection

4.7. RECURRENCE OF CIN BY HIV STATUS, CD4 CELL COUNT AND ART UPTAKE

Table 6 below outlines recurrence by HIV status, CD4 cell count, and ART uptake. The odds of recurrence were 67% less for study participants on ART than those who were not on ART (OR 0.33; 95%CI 0.14-0.75; p=0.008). The HIV uninfected (who did not need ART) had 69% less odds of CIN recurrence post treatment than the HIV infected (OR 0.31; 95%CI 0.13-0.72, p=0.007). Being on ART was significantly protective against recurrence of CIN post treatment.

Table 6: Recurrence by HIV status, CD4 count, and ART uptake

Variable	N	Recurrence Adjusted OR (95% CI)	p-value
ART treatment	602		
Not on ART		1 (reference)	
On ART		0.33 (0.14 – 0.75)	**0.008**
Unknown		0.35 (0.09 – 1.30)	0.118
Not applicable		0.31 (0.13 – 0.72)	0.007
HIV status	602		
HIV infected		1 (reference)	
HIV uninfected		0.64 (0.31 – 1.32)	0.227
Unknown		0.61 (0.08 – 4.76)	0.079
CD4 count (cells/μL)	602		
0 – 349		1 (reference)	
350 – 499		1.6 (0.50 – 5.15)	0.431
500 or more		0.70 (0.21 – 2.29)	0.555
Unknown		1.12 (0.35 – 3.57)	0.850
Not applicable		0.68 (0.23 – 2.03)	0.491

4.8. RECURRENCE OF CIN COMPARING TREATMENT MODALITIES

Table 7 below depicts recurrence for study participants who underwent cryotherapy. The HIV uninfected study participants had 41% reduced odds for recurrence than HIV infected study participants (adjusted OR 0.59; 95%CI 0.29-1.23; p=0.048). Participants who were HIV infected and on ART had an almost 70% reduced odds for recurrence post cryotherapy than participants who were not on ART (adjusted OR 0.3; 95%CI 0.13-0.71; p=0.006) whereas participants not needing ART because they were HIV uninfected had significantly reduced odds for recurrence post cryotherapy (adjusted OR 0.27, 95%CI 0.11-0.64; p=0.003).

Participants with an unknown number of lifetime sexual partners (LSP) had more than five times increased odds for recurrence than participants who had one to two LSP (p=0.050).

Table 7: Recurrence after cryotherapy as first line treatment adjusting for different variables

Variable	(N)[#]	Recurrence OR (95% CI)	p-value
Treatment			
Cryotherapy	602	2.32 (0.95 – 5.64)	**0.063**

Variable		Recurrence Adjusted OR (95% CI)	p-value
ART treatment	602	**2.61 (1.06 – 6.42)**	**0.047**
Not on ART		1	
On ART		**0.31 (0.13 – 0.71)**	**0.006**
Unknown		0.34 (0.09 – 1.27)	0.108
Not applicable		**0.27 (0.11 – 0.64)**	**0.003**
HIV status	602	**2.47 (1.01 – 6.02)**	**0.022**
HIV infected		1	
HIV uninfected		0.59 (0.29 – 1.23)	0.048
Unknown		0.57 (0.07 – 4.46)	0.600
CD4 count (cells/μL)	602	**2.46 (1.01 – 6.03)**	**0.048**
0 – 200		1	
350 – 499		1.64 (0.51 – 5.32)	0.407
500 or more		0.71 (0.22 – 2.33)	0.572
Unknown		1.11 (0.35 – 3.57)	0.857
Not applicable		0.64 (0.21 – 1.90)	0.418
Contraception	602	2.35 (0.96 – 5.73)	0.060
Oral		1	
Injectable		1.17 (0.29 – 4.77)	0.823
Long term method		0.86 (0.08 – 8.89)	0.901
Other		1.12 (0.30 – 4.24)	0.872
None		1.02 (0.28 – 3.79)	0.971
Unknown		1.24 (0.29 – 5.23)	0.768
Parity	594	2.30 (0.95 – 5.60)	0.066
No children		1	
1 – 2 children		0.82 (0.27 – 2.49)	0.732
3 – 5 children		1.15 (0.36 – 3.72))	0.813
>5 children		1 (omitted)	
Lifetime SP	602	2.27 (0.93 – 5.54)	0.072
1 – 2		1.04 (0.52 – 2.10)	0.906
>2		1 (omitted)	
Unknown		**5.70 (1.00 – 32.59)**	**0.050**
Sexual debut	602	2.28 (0.93 – 5.57)	0.070
16 years or less		1	
17 -19 years		0.99 (0.48 – 2.08)	
20 years or more		0.50 (0.17 – 1.46)	0.991

STI treatment	602	2.34 (0.96 – 5.69)	0.205
Yes		1	
No		1.49 (0.71 – 3.13)	0.290
Family Hx of cancer	601	2.31 (0.95 – 5.62)	0.065
Yes		1	
No		1.35 (0.40 – 4.55)	0.623
Smoking History	602	2.32 (0.95 – 5.63)	0.064
Yes		1	
No		0.81 (0.24 – 2.77)	0.735
Education level	602	2.46 (1.00 – 6.03)	0.050
Primary		1	
Secondary/High		0.59 (1.39)	0.
Tertiary		0.56 (0.18 – 1.67)	0.295
None		5.41 (0.42 – 69.37)	0.194
No information		1.09 (0.33 – 3.54)	0.887

#we used the complete case analysis hence for some variables, the total number of study participants is less than 602

Table 8 below shows that the study participants who underwent LEEP, on ART (AOR 0.31; 95%CI 0.13-0.71; p=0.006) and HIV uninfected (AOR 0.27; 95%CI 0.11-0.64; p=0.003) had about 70% reduced odds for CIN recurrence (69% and 73%, respectively).

Table 8: Recurrence after LEEP as first line treatment adjusting for different variables

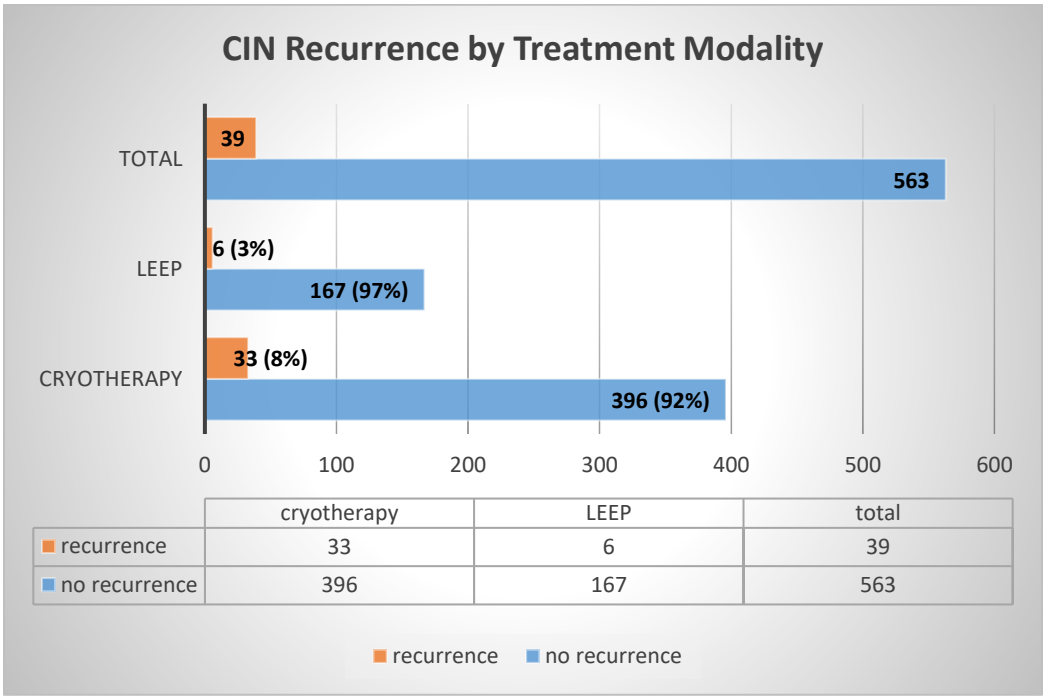
Variable	N #	Recurrence OR (95% CI)	p-value
Treatment	602		
LEEP		0.43 (0.18 – 1.04)	**0.063**
Variable		**Recurrence Adjusted OR (95% CI)**	**p-value**
HIV status	602	**0.41 (0.17 – 0.99)**	**0.048**
HIV infected		1 (reference)	
HIV uninfected		0.59 (0.29 – 1.23)	0.158
Unknown		0.57 (0.74 – 4.46)	0.600
ART treatment	602	**0.38 (0.16 – 0.94)**	**0.037**
Not on ART		1 (reference)	
On ART		**0.31 (0.13 – 0.71)**	**0.006**
Unknown		0.34 (0.09 – 1.27)	0.108
Not applicable		**0.27 (0.11 – 0.64)**	**0.003**
CD4 count	602	0.41 (0.17 – 0.99)	0.048
0 – 200		1 (reference)	
350 – 499		1.64 (0.51 – 5.32)	0.407
500 or more		0.71 (0.22 – 2.33)	0.572
Unknown		1.11 (0.35 – 3.57)	0.857
Not applicable		0.64 (0.21 – 1.90)	0.418
Contraception	602	0.43 (0.17 – 1.04)	0.060
Oral		1	
Injectable		1.17 (0.29 – 4.77)	0.823
Long term method		0.86 (0.84 – 8.89)	0.901
Other		1.12 (0.29 – 4.24)	0.872
None		1.02 (0.28 – 3.79)	0.971
Unknown		1.24 (0.29 – 5.23)	0.768
Parity	594	0.43 (0.18 – 1.06)	0.066
No children		1	
1 – 2 children		0.82 (0.27 – 2.49)	0.732
3 – 5 children		1.15 (0.36 – 3.72)	0.813
>5 children		1 (omitted)	
Lifetime SP	602	0.44 (0.18 – 1.07)	0.072
1 – 2		1.04 (0.52 – 2.10)	0.906
>2		1 (omitted)	

Unknown		5.70 (1.00 – 32.59)	0.050
Sexual debut	602	0.44 (0.18 – 1.07)	0.070
16 years or less		1	
17 – 19 years		0.99 (0.48 – 2.08)	0.991
20 years or more		0.50 (0.17 – 1.46)	0.205
STI treatment	602	0.42 (0.18 – 1.04)	0.061
Yes		1	
No		1.49 (0.71 – 3.12)	0.295
Family Hx of cancer	601	0.43 (0.18 – 1.05)	0.065
Yes		1	
No		1.35 (0.40 – 4.55)	0.623
Smoking History	602	0.43 (0.18 – 1.05)	0.064
Yes		1	
No		0.81 (0.24 – 2.77)	0.735
Marital status	590	0.44 (0.18 – 1.08)	0.074
Married		1	
Single		0.58 (0.30 – 1.12)	0.105

*statistically significant p-value

#we used the complete case analysis hence for some variables, the total number of study participants is less than 601

Figure 6 below shows CIN recurrence by treatment modality. A significant finding was that, the recurrence was mainly among those treated with cryotherapy (33/39; 85%), than those who underwent LEEP (6/39; 15%) during the study period (p=0.037).



Pearson chi2 = 3.63; p=0.057

Figure 6: Recurrence of CIN at 6 months follow up post treatment

4.9. RISK FACTORS FOR CIN RECURRENCE: UNIVARIABLE AND MULTIVARIABLE LOGISTIC REGRESSION

Post adjustment for other covariates the odds of recurrence among the study participants treated with LEEP was reduced by almost 60% than those who underwent cryotherapy (AOR 0.38; 95% CI 0.16-0.94; p=0.037) (Table 9). The model demonstrated that only ART had significance to the development of CIN recurrence post treatment with either modality. ART reduced the odds of recurrence by about 70% (AOR 0.31; 95%CI 0.32-0.71; p=0.006) and HIV uninfected study participants had 73% reduced odds to have recurrence (AOR 0.27; 95%CI 0.11-0.64; p=0.003).

Table 9: Univariable and multivariable analysis to determine the risk factors for recurrence

Variable	Univariable analysis		#Multivariable analysis	
	OR (95% CI)	p-value	AOR (95% CI)	p-value
ART treatment*				0.024
Not on ART	1 (reference)		1 (reference)	
On ART	0.33 (0.14 – 0.75)	**0.008**	0.31 (0.32 – 0.71)	**0.006**
Unknown	0.35 (0.09 – 1.31)	0.118	0.34 (0.09 – 1.27)	0.108
Not applicable	0.31 (0.13 – 0.72)	**0.007**	0.27 (0.11 – 0.64)	**0.003**
Treatment*				
Cryotherapy	1 (reference)		1 (reference)	
LEEP	0.43 (0.18 – 1.05)	0.063	0.38 (0.16 – 0.94)	**0.037**
Sexual debut*	0.88 (0.76 – 1.02)	0.089		
Marital status*				
Married	1 (reference)			
Single	0.57 (0.29 – 1.10)	0.094		
Lifetime SP*				
1 – 2	1 (reference)			
>2	1.00 (0.50 – 2.01)	0.999		
Unknown	6.03 (1.07 – 34.12)	0.042		
Education level*				
Primary	1 (reference)			
Secondary/High	0.59 (0.25 – 1.40)	0.233		
Tertiary	0.58 (0.19 – 1.75)	0.337		
None	5.06 (0.41 – 62.17)	0.205		
Unknown	0.99 (0.31 – 3.20)	0.990		
HIV status*				
HIV infected	1 (reference)			
HIV uninfected	0.64 (0.31 – 1.32)	0.227		
Unknown	0.61 (0.08 – 4.76)	0.641		
Age	0.97 (0.92 – 1.03)	0.384		
CD4 count				
0 – 200	1 (reference)			
350 – 499	1.60 (0.50 – 5.15)	0.431		
500 or more	0.70 (0.22 – 2.33)	0.555		
Unknown	1.12 (0.35 – 3.57)	0.850		
Not applicable	0.68 (0.23 – 2.03)	0.491		
STI treatment				
Yes	1 (reference)			
No	1.47 (0.70 – 3.07)	0.311		

Family Hx of cancer		
Yes	1 (reference)	
No	1.41 (0.42 – 4.71)	0.579
Smoking History		
Yes	1 (reference)	
No	0.80 (0.23 – 2.71)	0.715
Parity		
No children		
0 – 2 children	1 (reference)	
3 – 5 children	1.10 (0.53 – 2.27)	0.795
>5 children	1 (omitted)	
Contraception		
Oral	1 (reference)	
Injectable	1.11 (0.27 – 4.47)	0.887
Long-term method	0.85 (0.08 – 8.74)	0.893
Other	1.11 (0.29 – 4.21)	0.877
None	1.01 (0.27 – 3.71)	0.993
Unknown	1.14 (0.27 – 4.76)	0.862

#The initial multivariate logistic regression analysis run included ART, treatment given, sexual debut, marital status, lifetime sexual partners, educational level, and HIV status removing variables one at a time. ART and treatment were included in the final model (p>0.05). Final model Pearson goodness-to-fit p = 0.05

* Variables used in full multiple regression model

Age showed a linear relationship to CIN recurrence as confirmed by the Box-Tidwell test (p=0.206) for age and p=0.199 for age). Sexual debut also showed a linear relationship to CIN recurrence (p=0.177 for sexual debut and p=0.590 for sexual debut).

CHAPTER 5: DISCUSSION

5.1. INTRODUCTION

The main objective of the research was to determine the burden of cervical intraepithelial neoplasia recurrence at six months follow-up post treatment with either cryotherapy or LEEP between January 2014 and December 2016 at Mbabane government hospital (MGH) located in the Hhohho region of Swaziland. This section entails the discussion of the findings of the current study and interpreted in conjunction with other published studies from the literature.

5.2. FACTORS INFLUENCING CIN RECURRENCE

5.2.1. Demographic characteristics

Most of the study participants were from the Hhohho region, followed by the Manzini region. Although MGH is the national referral hospital, the expectation would be a higher attendance by participants from the local region. Geographically, Manzini is proximal to Hhohho region. The road infrastructure allows for easier access of from Manzini to MGH than it does for other regions which could explain the seeming low follow up from the other regions.

It is probable that some of the study participants either returned to their regional hospitals or were completely lost to follow up. Chirenje Z.M. et al. found that access to cervical cancer screening remains a challenge in Sub-Saharan Africa.[61] The return for review rate of our study participants from other regions may also highlight challenges to access.

Age and sexual debut

The study participants were aged between 15 and 46 years; and adolescents accounted for 2% (9/601). This is not unique to Swaziland since a study by Vetrano et al. described the risk factors of CIN in adolescents and the age range for their study was 12 to 21 years. [62] Mbulawa et al. investigated human papillomavirus (HPV) prevalence among South African adolescents and young women, with an age range of 16 to 22 years.[63]

According to the 2014 Swaziland multiple indicator cluster survey (MICS), prevalence of sexual debut before 15 years was 3%.[64] In our study, the median sexual debut age was 18 years. Early sexual debut increases the risk for cervical precancer and invasive cancer. Individuals who are sexually exposed before the age of 20 years have an increased risk for cervical cancer.[65]

Marital status

After treatment for cervical intraepithelial neoplasia, the requirement is abstinence for six weeks. The majority of the treated participants reported to be single (53%). This may not necessarily mean that fewer married participants needed treatment but rather that they needed consent for the procedure from their partners.[66] Although not quantified, but some did not return for treatment

Educational background

A plethora of research confirm that an individual's level of education affects their health-seeking behaviour. According to the Journal of Educational Policy and Entrepreneurial Research (JEPER), participants with high educational level reported higher score on health seeking behavior scale. Cervical cancer screening relates largely to health seeking behaviour since in the pre-cancer stage is non-symptomatic.[67]

An understanding of the disease is what can prompt an individual to access screening services. The findings of this study are congruent with the JEPER conclusion since the 1% without education were shown to have a five times greater chance of recurrence, although this was not statistically significant. The majority who attained secondary education (57%) had 42% reduced odds for recurrence. However, this was not statistically significant and we cannot conclude on this because the sample size was not powered to allow for a robust conclusion.

Smoking history

Smoking has been shown as a risk factor for cervical cancer.[25, 68] There was a larger representation of smokers in this sample of women treated for pre-cancerous lesion compared to the 2014 survey (MICS) results; 6% and 1.3%, respectively. However the survey was unable to demonstrate that those who smoked were at higher risk of precancerous lesions compared to non-smokers, nonetheless, the sample size was not sufficient to allow the drawing of any conclusion.[64]

5.2.2. Clinical Characteristics

Most (360/602; 60%) of the study participants were HIV infected and this is congruent with Heard et al. who found that the risk cervical intraepithelial neoplasia was higher among HIV infected women.[20]

The majority of HIV infected study participants had LEEP (69%; 116/169) as the first line of treatment than cryotherapy (56%; 239/427). This could indicate the advanced state of the cervical lesion at initial presentation beyond the eligibility criteria for cryotherapy, and the documented faster progression of disease in those infected with HIV thus requiring more extensive treatment (LEEP), as well as the fact that the disease burden is disproportionately higher in those infected with HIV compared to the uninfected. However, the majority were on ART and had a CD4 count of 500 cells/μL or more.

CIN Recurrence, HIV Status and ART Uptake

The immune status invariably affects the risk for CIN recurrence.[51, 69] This study showed that the HIV infection status, CD4 count, and whether a participant was on ART treatment or not were significant predictors of recurrence. The findings from this study are consistent for both treatment modalities; the positive HIV status and lack of ART were statistically significant predictors of recurrence ($p=0.017$ and $p=0.031$, respectively). These were the only significant predictors of recurrence for both modalities of treatment in this study. Other factors did not significantly predict recurrence.

The recurrence of CIN at six months review post treatment among the HIV infected study participants on anti-retroviral therapy and their HIV uninfected counterparts is somewhat similar, attesting to the documented immune boosting characteristics of ART.[55, 56] Although, this study did not consider the grade of CIN before treatment and at review, nor the excision margins post LEEP. Kelly et al. found that the early initiation of ART and sustained adherence is likely to reduce incidence and progression of SIL and CIN, and eventually the incidence of invasive cervical cancer.[55]

From this study, only the HIV uninfected and those on ART significantly had reduced odds for CIN recurrence 6 months post treatment. Clifford et al. affirm that the impact of HAART on standardized incidence ratios for cervical cancer or non-acquired immunodeficiency syndrome-defining cancers was unclear.[70] This was similar to the findings by Clifford et al. who noted that with HIV, the risk is increased for cervical pre-cancer than for invasive cervical cancer.[61]

Study participants with a lower CD4 cell count had an increased risk of recurrence than those with a CD4 cell count of 500 or more. But, the CD4 cell count was not a significant predictor for CIN recurrence. Oga et al. found that immunosuppression plays a crucial role in the recurrence of CIN lesions; not only does the HIV infected status increase CIN recurrence risk, but a low CD4 cell count in those on ART.[49] Conversely, our study found that anti-retroviral therapy decreased the odds for recurrence. But, Huckho et al. found that only the CD4 cell count was associated with CIN recurrence and not HAART.[46]

Swaziland has a high HIV prevalence and the majority of patients needing cervical precancer treatment are HIV infected highlighting the burden of cervical pre-cancer disease in this population.[6] In addition, more recurrence of the pre-cancer occurred with the HIV infected (69%) than with the HIV uninfected (28%). Introduction of the HPV vaccine would be beneficial to Swazi women especially the HIV infected women because it would decrease the burden of CIN and consequently cervical cancer in this population and overall.

The country is burdened with high risk subtypes including 16, 18, 31, 33, 45.[35] A better vaccine to introduce would be the nine-valent vaccine that contains subtypes 6, 11, 16, 18, 31, 33, 45, 52, and 58.[28] This vaccine also includes protection against the low risk subtypes 6 and 11 responsible for causing genital warts. Other HPV-related cancers (including vagina, vulva, oropharyngeal, anal, penile, and cutaneous carcinomas), can be curbed through the introduction of HPV vaccination.[71] The vaccine has also been shown to be effective in reducing CIN recurrence after treatment.[36]

5.2.3. CIN Recurrence and Treatment Modality

For all the study participants, there is no masking of the recurrence by the vaccine since it is not yet rolled out in Swaziland. CIN screening and treatment is undertaken with the aim of curbing progression of the dysplasia (CIN) to invasive cervical cancer. This study found that the recurrence of CIN at 6 months' follow-up after treatment with either cryotherapy or LEEP at Mbabane government hospital was 6.5%. However, this could be an under estimation since all patients who were treated did not return for follow up at the stipulated time.

A study by Huchko MJ et al. found that 7.1% of their study population presented with recurrence of high grade CIN (CIN 2 or more) at 6 months after undergoing LEEP. [46] The analysis by treatment modality indicate that CIN recurrence among study participants treated with cryotherapy

(8%) than with LEEP (3%). For this study, participants who underwent LEEP had 57% reduced odds of CIN recurrence. Although, this was not statistically significant. Other studies reported similar findings noting reduced CIN recurrence with LEEP compared to cryotherapy.[41, 72]

CIN recurrence depends on a variety of factors which include initial CIN grade, treatment type, and age among others contributors.[38] From our study, the majority of participants who had recurrence were between 20 and 29 years. This is contrary to the findings by Zhu et al. who found increased CIN recurrence in the older age group (>35years).[73]

5.2.4. CIN Recurrence and Reproductive Health Status

Lifetime Sexual Partners

The reproductive health characteristics influencing CIN recurrence as noted in the literature were comparable with our study findings. The number of lifetime sexual partners affects the risk of CIN recurrence. Our study found that the high number of lifetime sexual partners increased the odds of cervical pre-cancer recurrence post treatment. Women who did not disclose the number of their sexual partners (or for whom the information was not recorded), had six times increased odds of CIN recurrence (p=0.042).

It is also possible that those who did not disclose information about lifetime sexual partners probably had more than two sexual partners; hence the increased odds for recurrence. Khan et al. found that women who had more than five lifetime sexual partners had lower rates of CIN regression .[28]

Sexual Debut

Sexual debut is also noted as a significant risk factor for CIN recurrence.[26] Our study found that the odds of CIN recurrence among participants who had a sexual debut at 20 years or older, was half that of those who had an earlier sexual debut. Louis et al. found that women with an earlier sexual debut (16 years or younger) had more than two-fold risk for developing invasive cervical cancer than those with a later debut at 21 years or older.[26]

Contraception

In our study, contraception had no bearing on recurrence of CIN. This is in line with other studies which found that hormonal contraception did not increase the rate of CIN recurrence or its persistence.[74, 75]

Sexually Transmitted Diseases

Sexually transmitted infections also have a bearing on CIN progression according to Mitra A. et al who stated that, "CIN disease severity is associated with increasing vaginal microbiota diversity and may be involved in regulating viral persistence and disease progression. However, for our study participants, there was no demonstration of an association of STI's with recurrence of CIN. In our study, we could not demonstrate the effect of pregnancy on the recurrence of CIN because only two participants returned for review pregnant.

CHAPTER 6: LIMITATIONS

6.1. SELECTION BIAS

Selection bias can occur whenever there are systematic or directional errors when participants are sampled into a study.[76]

a. Patients who never returned for follow up were invariably excluded from the study.

b. Patients who may have returned for follow up outside the stipulated study period.

6.2. INFORMATION BIAS

Information bias can occur whenever there is a systematic or directional error in how measurement or information is derived from participants.[76]

a. Information recorded in the medical registers from where the study data were collected, was not specifically tailored for the study. Hence, there were several missing variables which were important in drawing inferences and conclusion(s). These included:
    i. Smoking history – if a study participant was noted to be a smoker, the duration and pack years were not documented
    ii. Contraception – the duration on a commodity was not documented.

b. There was a significant number of study participants with an unknown HIV status of which one had CIN recurrence. The twenty who did not have recurrence could have either been infected or uninfected; and this would have significantly affected the study findings.

c. This study did not consider the grade of CIN before treatment and at review, nor the excision margins post LEEP. From literature, CIN recurrence is also affected by the initial grade and at treatment and if after LEEP there was a neoplasia free margin. In our study, the recurrence CIN was not document hence we cannot conclude if there was progression of the lesion or whether it was the same grade. For LEEP, the excision margins were not document hence we cannot conclude if the recurrence was actual or whether it was a residue from the initial treatment.

6.3. GENERALISABILITY OF STUDY RESULTS

The study is not generalizable since it was confined to one hospital setting. Although, cervical cancer screening and treatment are offered at 14 facilities, the dynamics of Mbabane government hospital are different from other facilities. Hence, we cannot conclude that our findings are an epitome of the situation in Swaziland.

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

7.1. CONCLUSION

Cervical intraepithelial neoplasia (CIN) is a treatable and curable condition. However, CIN and recurrence thereof remain a challenge for many health systems in developing countries, including Swaziland. Recurring CIN (including, poorly treated CIN) is recognized as the continual impediment in cervical cancer control and its elimination. This study set out to determine the proportion of CIN recurrence six months after treatment with cryotherapy or LEEP at Mbabane government hospital in order to enable evidence-based decision-making by the Health Ministry, to improve the cervical screening programme for better prevention and control of CIN, as well as cervical cancer.

In this study, there were proportionately more HIV infected participants with CIN recurrence than those who were HIV uninfected. The significant findings which emerged from this study is that ART reduced the odds of CIN recurrence. The findings from this study are consistent for both treatment modalities; the positive HIV status and lack of ART were statistically significant predictors of CIN recurrence. The *Test and Start* program for HIV is paramount and the most beneficial for the reduction of CIN recurrence. The program recommends immediate commencement of ART regardless of CD4 cell count. Based on the study findings, a CD4 cell count of 500 cells/μL or more appears to be protective against CIN recurrence. Thus, to minimize the recurrence of CIN, HIV infected women need to be initiated on ART regardless of the treatment modality.

The study results reflect that evidence-based, targeted strategies are mandatory in order to promote healthy behaviour. Delayed sexual debut (post adolescence) emerged to be protective against CIN recurrence post treatment with either cryotherapy or LEEP. Reproductive health promotion should comprise education to encourage delayed sexual debut (after 20 years of age) and allow individuals to make informed decisions to also curb sexually transmitted infections and diseases which pose an increased risk for CIN recurrence, as well as CIN progression to invasive cancer.

Swaziland has the highest burden of HIV globally, and this is an indirect indicator of the cervical pre-cancer disease burden in this population. The challenge of ever increasing cervical cancer and other HPV-related cancers in Swaziland can be overcome through the introduction of the HPV vaccine, both as a means of primary prevention (for the sexually naïve) and secondary prevention (for those already infected with HPV), is paramount. Vaccination of boys should be considered due to its advantage to reduce the risk of HPV transmission to sexual partners, lowering the infectious pool of HPV in the general population and eventually HPV-related diseases for both men and women.[77] The benefits of the vaccine are worthwhile and far outweigh the cost to realize a healthy nation.

7.2. RECOMMENDATIONS

The study provides several important insights into factors which may influence CIN recurrence post treatment for future health service practice. The information could also be used to develop targeted interventions aimed at improving CIN recurrence and, in turn, the cervical cancer screening programme.

7.2.1. Strengthening the HIV *Test and Start* Programme

Every HIV infected individual should be initiated on treatment without delay. This study demonstrated that participants on ART had better CIN treatment outcomes than those not on treatment. Beyond ART commencement, treatment adherence must also be emphasized as the study revealed that those with a CD4 cell count of less than 500 cells/μL fared worse.

7.2.2. Health Education and Promotion

The reproductive health education must cascade to the lowest level to strengthen health seeking behaviour on the importance of cervical cancer screening and prevention of risk factors for cervical cancer. The health promotion package should include education on the risk factors associated with cervical pre-cancer (also including more than two lifetime sexual partners).

7.2.3. Health Service Factors

The benefits of treatment should be clearly communicated to the patient by the healthcare provider. The patient should also be alerted of the importance of uninterrupted treatment and be involved in

setting treatment goals. Barriers or obstacles to treatment, if any, should be discussed and patient be helped to device strategies to overcome them.

Conversely, the health programmes targeted at the adolescent age group should clearly communicate the risk factors of CIN and its recurrence in order to encourage delayed sexual debut beyond the adolescent age.

7.2.4. Further research

7.2.4.1. The study highlighted the challenge of loss to follow up, as only 50% of those screened for cervical cancer returned at the 6-month review to the treating facility. A study on the outcomes for treated patients is warranted which will also quantify the magnitude of loss to follow up and reasons thereof.

7.2.4.2. The health seeking behaviour patterns of the Swazi population. Most of disease that can be screened for are associated with health seeking behaviours. Knowing the patterns of health seeking behaviours can guide programming.

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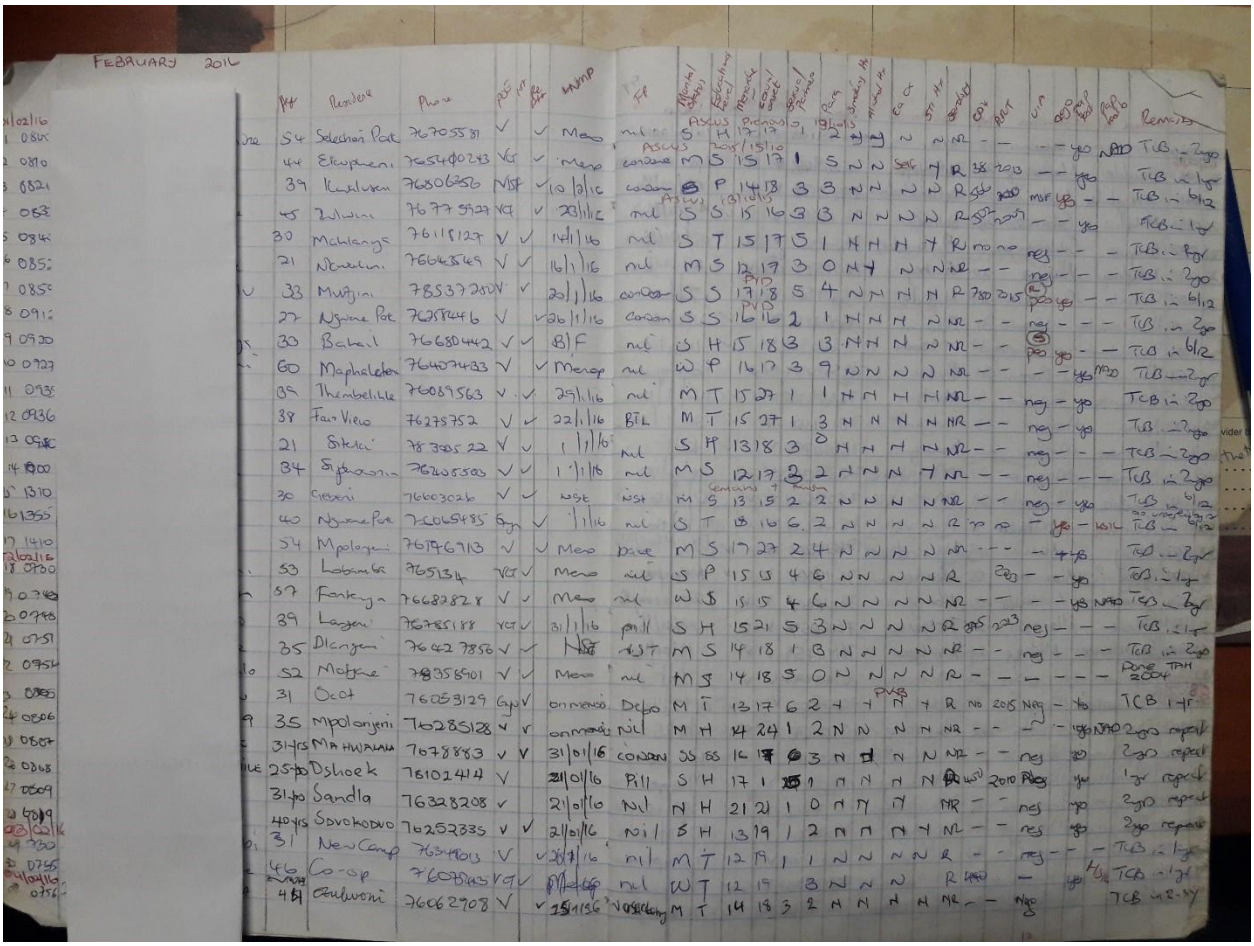
ANNEXES

ANNEX 1: Copy of the clinic register



ANNEX 2: Data collection form

Form #:

Data collection: cervical cancer screening for women in Swaziland

All information collected must be:

1. As seen on the records and interpreted by data collector
2. Be anonymous (no patients name)
3. Confidential

Date completed: ___ ___ / ___ ___ / 2 0 1 ___

Facility Name: _____ **Facility ID:** ☐☐

❒ **(1) Mbabane Government Hospital**

1. SOCIO-DEMOGRAPHICS

Age at initial treatment (in years)			
Marital status	1 – Married 2 – Single 3 – Other(specify)		
Level of education	01 – Primary 02 – Secondary 03 – Tertiary 04 – None		
District	1– Hhohho 2 – Manzini 3 – Shiselweni 4 – Lubombo		
Smoking History	01 – Current 02 – Ex-smoker 03 – Never	Duration in years	__ years

Age at first sexual intercourse	___Years		
Life time sexual partners			
Pregnancy status on review			
Parity	▌1 – between 0 and 2 ▌2 – between 3 and 5 ▌3 – more than 5		
Use of Contraception	▌1 – Pill ▌2 – InjecTable ▌3 – Long Term ▌4 – Other	Duration of contraception use	__ Years

2. MEDICAL INFORMATION

Family History of cancers			▌(1)Yes	▌(2) No	▌(3) Not Recorded
Previously screened for cervical cancer					
Treated for precancerous lesion					
If yes : **Cryotherapy** Date: __/__/__ (yy/mm/dd)	▌(1)Yes	▌(2) No	**LEEP**	▌(1) Yes	▌(2) No
Other treatment:					
Outcome of follow up	▌Treated ▌Repeat Cryotherapy ▌Needs LEEP ▌Advanced Disease				
HIV infected			▌(1)Yes	▌(2) No	▌(3) Not Recorded
If Infected, treated for opportunistic infections			▌(1)Yes	▌(2) No	▌(3) Not Recorded
Treated for STIs			▌(1)Yes	▌(2) No	▌(3) Not Recorded

ANNEX 3: Permission Letter from Mbabane Government Hospital Management

Permission to access Records / Files / Data base at the Mbabane Goverment Hospital

To: Senior Medical Officer/Information Officer
M G Hospital

Dr Mahaliyana

From: The Investigator
Sexual Reproductive Health

Dr Simangele Mthethwa-Hleta

Re: Permission to do research at Mbabane Government Hospital

Dr Simangele Mthethwa-Hleta is a researcher working at the Sexual reproductive health unit/Ministry of Health. I am requesting permission to conduct a study on the Mbabane Government Hospital grounds that involves access to patient records.

The request is lodged with you in terms of the requirements of the Promotion of Access to Information Act. No. 2 of 2000.

The title of the study is: **Recurrence of cervical intraepithelial neoplasia post cryotherapy and Loop Electrosurgical Excision Procedure in HIV positive women versus HIV negative women in two hospitals in Swaziland**

The researcher request access to the following information:

Access to the clinical files, record book and the data base.

We intend to publish the findings of the study in a professional journal and/ or at professional meeting like symposia, congresses, or other meetings of such a nature.

We intend to protect the personal identity of the patients by assigning each patient a random code number.

We undertake not to proceed with the study until we have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and the Swaziland Ethics Committee

Yours sincerely

Signature of the Principle Investigator

Permission to do the research study at this hospital and to access the information as requested, is hereby approved.

Senior Medical Officer

Mbabane Govt Hospital Hospital
Dr P.S. Mahaliyana

Signature of the CEO/SMO

MBABANE GOVERNMENT HOSPITAL
SMO
11 OCT 2016
P.O. BOX
MBABANE
SWAZILAND

Hospital Official Stamp

ANNEX 4: Research Ethics Committee Approval Letter

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 28 August 2018.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

20/10/2016

Approval Certificate
New Application

Ethics Reference No.: 389/2016

Title: Recurrence of cervical intraepithelial neoplasia post cryotherapy and Loop Electrosurgical Excision Procedure in HIV positive women verses HIV negative.

Dear Dr Simangele MthethwaHleta

The **New Application** as supported by documents specified in your cover letter dated 12/10/2016 for your research received on the 12/10/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 19/10/2016.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**389/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

☎ 012 356 3084 deepeka.behari@up.ac.za / fhsethics@up.ac.za http://www.up.ac.za/healthethics
Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

ANNEX 5: Research Ethics Committee Amendment Approval Letter

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

26/04/2018

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No: 389/2016

Title: Recurrence of cervical intraepithelial neoplasia at six months post cryotherapy or Loop Electrosurgical Excision Procedure at Mbabane Government Hospital Swaziland.

Dear Dr Simangele MthethwaHleta

The **Amendment** as described in your documents specified in your cover letter dated 4/04/2018 received on 4/04/2018 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 25/04/2018.

Please note the following about your ethics amendment:
- Please remember to use your protocol number (**389/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committe may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics amendment is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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ANNEX 6: Distribution of the age and sexual debut of the study participants