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Low grade squamous intraepithelia lesions in HIV-infected women at Chris Hani Baragwanath academic hospital

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LOW GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN HIV-INFECTED WOMEN AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

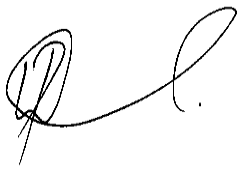
Dr Portia Kenalemang Manamela

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology.

Johannesburg, 2016

Declaration

I, Portia Kenalemang Manamela, declare that this dissertation is my own work. It is being submitted to the Faculty of Health Sciences for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at this or any other University.



this 15th day of November, 2016

Abstract

Background: HIV positive women have a greater risk of cancer of the cervix and they were also found to have high rates of prevalent and incident LSIL and HSIL. Both HIV and Cervical cancer are major public health problems in South Africa. This study will therefore assess whether HIV infected women with a LSIL on Pap smear have clinically significant cervical disease as determined by histology.

Methods: HIV positive women with cytological abnormality on Pap smear are referred to colposcopy according to the SA guidelines. When the colposcopy is more than CIN2 a LLETZ is performed. Data was extracted from a colposcopy database.

Results: There were 652 patients and the mean age was 36.55 years and the median parity was 3.00. There were 266 women (40.80%) who had a histology result of HPV/CIN 1 and 386 women (59.20%) who had a histology result of CIN 2 or more severe lesion.

Conclusion: Our study showed that most of the patients that were referred to our colposcopy clinic had CIN2 or more severe on histology and there were 5 women with invasive disease. The time from performing the Pap smear to colposcopy is extremely long. It is important that women with LSIL be referred to colposcopy as soon as possible.

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TABLE OF CONTENTS

Declaration ii
Abstract iii
Acknowledgements iv
LIST OF ABBREVIATIONS vii
LIST OF FIGURES viii
LIST OF TABLES ix
LIST OF APPENDICES x
CHAPTER 1 1
1.1 Introduction 1
1.2 HIV and Cervical Cancer in South Africa 2
1.3 Natural History of cervical cancer 3
1.4 Risk Factors for HPV Infection 5
1.5 Screening for cervical cancer 5
1.6 Colposcopy 6
1.7 Management of cervical pre-cancer Lesions 7
1.7.1 Management Options for LSIL 8
1.8 Problem statement and justification 9
1.9 Study objectives 11
CHAPTER 2 12
2.1 Methodology 12
2.1.1 Study Design 12
2.1.2 Variables that are recorded in the database 12
2.1.3 Study Setting/Population 12
2.2 Data management 14
2.3 Data analysis 15
2.3.1 Descriptive and Analytical Statistics 15
2.4 Ethics 15
2.5 Funding 15
CHAPTER 3 16
3.1 Results 16
3.1.1 Study population 16
3.1.2 HIV factors 19
3.1.3 Pap smear Findings 20
3.1.4 Colposcopic and Histology Findings 21
3.1.5 Comparison between Colposcopic Diagnosis and Histology Results 24
3.1.6 Margins 26
3.1.7 Comparison of women with CIN 1 with those who have CIN2 or more on histology 26

CHAPTER 4 29
4.1 Discussion 29
4.2 Strengths 34
4.3 Limitations 34
4.4 Recommendations 34
4.4 Conclusion 35
REFERENCES 37
APPENDICES 43

LIST OF ABBREVIATIONS

AGUS	Atypical Glandular Cells of Undetermined Significance
AGC	Atypical Glandular Cells
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ASCUS	Atypical Cells of Undetermined Significance
BV	Bacterial Vaginosis
CDC	Center for Disease Control
CD4	Cluster of Differentiation 4
CEO	Chief Executive Officer
CHBAH	Chris Hani Baragwanath Academic Hospital
CIN	Cervical Intraepithelial Neoplasia
COC	Combined Oral Contraceptive
DMPA	Depot Medroxyprogesterone Acetate
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSIL	High Grade Squamous Intraepithelial Lesion
IQR	Interquartile Range
IUCD	Intrauterine Contraceptive Device
LLETZ	Large Loop Excision of the Transformation Zone
LSIL	Low Grade Squamous Intraepithelial Lesion
NHLS	National Health Laboratory Services
OCP	Oral Contraceptive Pill
Pap smear	Papanicolaou smear
PMTCT	Prevention of Mother to Child Transmission
RR	Relative Risk
SA	South Africa
SD	Standard Deviation
SIL	Squamous Intraepithelial Lesion
TZ	Transformation Zone
VIA	Visual Inspection with Acetic Acid

LIST OF FIGURES

Figure 2.1 Flow diagram showing the management of patients at CHBAH colposcopy clinic

Figure 3.1 Flow diagram showing inclusion and exclusion criteria of study population

Figure 3.2 Method of contraception at the time of presentation

Figure 3.3 Proportion of women smoking cigarette

Figure 3.4 Proportion of women using snuff

Figure 3.5 ART usage

Figure 3.6 Other findings on the Pap smear report

Figure 3.7 Colposcopic diagnoses in women with LSIL on Pap smear

Figure 3.8 Histology results

LIST OF TABLES

Table 3.1 Cervical biopsy methods

Table 3.2 Characteristics of women with invasive disease

Table 3.3 Comparison of colposcopy diagnosis with histology results

Table 3.4 Margins involved

Table 3.5 Demographic details in relation to severity of disease

Table 3.6 Unadjusted risk ratio of CIN2 or more severe lesion

LIST OF APPENDICES

Appendix A – Colposcopy clinic Ethics certificate

Appendix B – Dr Manamela Ethics certificate

Appendix C – CHBAH letter of permission to do research

Appendix D – NHLS letter of permission to use NHLS database

Appendix E – Letter of permission to use CHBAH colposcopy database

Appendix F – Modified Reid Score

Appendix G – Datasheet

Appendix H – Plagiarism form

CHAPTER 1

1.1 Introduction

Cervical cancer is the third leading cause of cancer in women in the world [1] and it is the leading cause of death by cancer in developing countries.[2-6] In South Africa (SA), the overall crude incidence rate of cervical cancer is estimated to be 19.64/100 000.[7] Women with Human Immunodeficiency Virus (HIV) have been shown to have a high risk of Human Papillomavirus (HPV) infection, which is known to be associated with almost all of cancers of the cervix.[2,4]

Both HIV and cervical cancer are major public health problems in South Africa.[3] HIV positive women have a greater risk of cancer of the cervix.[3,5] They were also found to have high rates of prevalent and incident Low Grade Squamous Intraepithelial Lesions (LSIL) and High Grade Squamous Intraepithelial Lesions (HSIL), with relatively low risk of regression to normal from LSIL.[5,8] The progression from low grade to high grade has been different in different populations. Two observational studies and one randomized controlled trial have shown that a progression from LSIL to HSIL in HIV positive women is 14% – 22%.[3,5] HIV positive women are also more prone to having detectable levels of HPV in cervical specimens than HIV negative women.[5] The prevalence of Squamous Intraepithelial Lesions (SIL) among HIV positive women has been estimated to be between 12% to 50%. Moodley J R, Hoffman M, Carrara H et al found a 50% prevalence of cytological abnormalities among HIV positive women in a study done in Cape Town.[3,5]

Prevention of cervical cancer has been possible in developing countries because of effective screening and treatment of pre-cancer lesions.[3] The cervical cancer screening recommendation in developed countries is that HIV positive women should be screened more frequently than HIV negative women. It is recommended that HIV positive women have two Pap smears within the first year after HIV diagnosis and annually thereafter. Women would then be referred for colposcopy for any smear showing Atypical Cells of Undetermined Significance (ASCUS) or a more severe lesion.[3] The new guidelines in SA recommend that all HIV infected women be screened for cervical cancer precursors at diagnosis and then 3 yearly in those with a negative screening test.[9] HIV infected women with ASCUS and more severe are

referred to colposcopy.[10] The problem facing South Africa is that both HIV and cervical cancer are common and resources are limited.[3] Most HIV negative women with LSIL will have a normal Pap smear in 12 months without treatment.[11] HIV positive women are less likely to clear the HPV.[12] Colposcopy may be difficult in women who have a high HPV load and who have warts covering the cervix and vagina. There are, however, no studies assessing the performance of colposcopy in HIV positive women. Referral of women with LSIL to colposcopy increases the workload at colposcopy clinic, and may lead to unnecessary treatment. An aggressive management protocol for HIV positive women with LSIL might do more harm than good. This study is therefore aimed at understanding LSIL in HIV infected women.

1.2 HIV and Cervical Cancer in South Africa

In South Africa (SA), current data on cervical cancer is lacking. This is due to failure of maintaining the pathology-based cancer registry. The last National Cancer Registry report was in 2004 in which 4636 new cases of cervical cancer were recorded, with a crude incidence rate of 19.64 per 100 000/year. Cancer of the cervix is the second commonest cancer among women in South Africa.[7] According to the Globocan 2012 report, the extrapolated incidence of cervical cancer in South Africa was 7735 cases.[13] Cervical cancer was included as one of the Acquired Immune Deficiency Syndrome (AIDS) defining illnesses in 1993.[3,5]

The burden of HIV in Sub-Saharan Africa is high.[14] South Africa has a high burden of HIV and women are more affected than men. Young women have a two-fold HIV seroprevalance rate than men.[10] The estimated prevalence of HIV infection among Gauteng antenatal attendees was 29.9% in 2012 [14]

Immune deficiency in HIV is associated with a rapid progression of premalignant lesions and a slower regression. A study done by Six C, Heard I, Bergeron C et al showed that LSIL progressed to HSIL in 38.1% of HIV positive women with a CD4 count less than 500 cells/mm^3 in a period of one year and there was no progression if the CD4 was more than 500 cells/mm^3.[15] High rates of prevalent SIL in HIV positive women at a relatively young age have been reported. A cross sectional study done in Johannesburg by Finhaber showed an increasing risk of cervical lesions with a

decreasing CD4 count.[16] A prevalence of cervical cancer among HIV positive patients has been shown to be nearly double that of HIV negative patients.[3,17] HIV positive patients present with cervical cancer nearly 10 years earlier than HIV negative patients. Additionally, if their CD4 count is less than 200 cells/mm^3 at presentation they are significantly more likely to have advanced stage of cervical cancer.[17]

1.3 Natural History of cervical cancer

Persistence of high-risk HPV infection has been shown to be an important association with cervical cancer.[18,19] HPV is one of the most common sexually transmitted infections in the world. It has been suggested that most sexually active individuals have been infected with HPV at least once in their lifetime.[20,18] The infection with HPV is usually transient and most individuals clear the virus without any clinical manifestation.[20]

HPV infects the developing immature metaplastic cells of the transformation zone of the cervix.[19] The virus targets the basal cells and these act as reservoirs of the virus once infected.[21] Premalignant and malignant cells arise as a result of HPV DNA integration into the host cellular genome with a resultant overexpression of the viral E6 and E7 oncogenes. Cells acquire a proliferative advantage by escaping growth control exerted by p53 and p105Rb which are inactivated by E6 and E7 proteins respectively.[19] Persistence of infection can lead to cervical cancer precursors which can be low-grade and high-grade squamous intra-epithelial lesions (SIL), subsequently leading to cervical cancer.[21]

There are more than 40 HPV types that have been associated with mucosal infection of the anogenital tract and these have been classified according to their oncogenic potential as either low- or high-risk HPV types. The low-risk HPV types cause benign hyperproliferative lesions/genital warts, and these are usually HPV 6 and 11 and less commonly 40, 42, 43, 44, 54, 61, 72, 73, and 81. The high-risk oncogenic HPV types are strongly associated with premalignant and malignant cervical lesions and these are usually HPV 16, 18, 45 and 31 and less commonly 33, 35, 39, 51, 52, 56, 58, 59, 68, 69, 73 and 82.[20]

Cervical intraepithelial neoplasia (CIN) is assumed to progress through a long preinvasive state before developing into invasive cancer.[23] Squamous cervical cancer is initiated by infection with the HPV and generally progresses in stages from LSIL to HSIL and HSIL may result in invasive cancer of the cervix.[19] Screening programmes have allowed for the detection of the preinvasive lesions which can be effectively treated. Previous studies have shown that a number of LSIL will spontaneously regress.[23] A meta-analysis representing 27 929 patients found that the rate of regression to normal was 68% for women with ASCUS and 48% for LSIL and 35% for HSIL. [23] The rate of progression to HSIL at 24 months was 7% for ASCUS and 21% for LSIL.[23] The rate of progression to cervical cancer at 24 months was 0.25% for ASCUS, 0.15% for LSIL and 1.44% for HSIL. [23] A comprehensive review and a cohort study of 17 000 women have suggested that the majority of LSIL will regress within 2 years. [23] Bansal N, Wright J D and Cohen C J et al also confirmed these findings which showed that 52% of CIN 1 patients regressed within 1 year while 10% progressed to high grade disease.[23] On the other hand, McCredie, Sharples KJ, Paul C et al showed that the incidence of progression of CIN 3 to invasive cancer of the cervix at 30 years was 31.3% in women that were not treated for CIN 3.[19]

The prevalence of LSIL has been reported to be between 1.6 – 2.4% in population-based surveys.[10] A number of studies have found the prevalence of squamous intraepithelial lesions among HIV-positive women to be 31% to 63%.[24, 25-27] Progression of LSIL was associated with lower CD4 counts and presence of HPV types 16, 18, and 33.[5] Furthermore, the prevalence and degree of dysplasia increases with advancing levels of immunosuppression.[24]

Gaym A, Mashego M and Kharsany A B F *et al*. showed that LSIL was the most common abnormality identified on Pap smears of HIV positive women. The prevalence of LSIL was 9.2% and the prevalence of HSIL was 1.3% in this group of women. [24] They also showed that there was a statistically significant association between HIV infection and abnormal Pap findings. They found that abnormal Pap smears occurred in 36% of HIV-positive women as compared to 10.3% of HIV-negative women.[24] African studies have shown a higher prevalence of 38% of HIV positive women having premalignant cervical lesions at first Pap smear. Omar T,

Schwartz S, Hanrahan C et al study showed that 21.5% of women with a baseline of LSIL progressed to HSIL at 5.5 months follow-up. [10] Progression was associated with a low CD4 count. Women having CD4 counts less than 200 cells/mm^3 had a two-fold increased risk of progression from LSIL to HSIL.[10]

1.4 Risk Factors for HPV Infection

Risk factors for HPV infection include early age of coitarche and number of sexual partners.[4] Other risk factors include history of other HPV-mediated neoplasia, immunosuppression, prior sexually transmitted infection, low socioeconomic status and history of smoking. Use of oral contraceptive pills (OCP) increases the risk of cervical cancer in women infected with HPV, but there is no evidence that OCPs increase the risk of HPV infection.[4]

1.5 Screening for cervical cancer

Cervical cancer screening programmes are aimed at reducing mortality and morbidity resulting from cervical cancer. The existence of premalignant lesions of the cervix can be detected through screening and these lesions can subsequently be managed accordingly.[8] Early treatment options of premalignant lesions are effective in preventing cervical cancer.[4]

Methods used for cervical cancer screening include Pap smear, visual inspection with 5% acetic acid and Human Papillomavirus detection.[28] Pap smear is recognized as an effective, successful and well accepted test for cervical cancer screening.[2] Furthermore, certain high risk HPV types associated with cervical cancer have been identified. There are different tests available for detecting high risk HPV DNA and these tests can be used for both screening and diagnosis.[8] These HPV tests, however, are not available in public hospitals due to high costs.[21]

Sankaranarayanan R, Nene BM, Dinshaw KA et al conducted a randomized cervical cancer screening trial in 52 villages in India, with a total of 142 701 healthy women between the ages of 30 and 59 years. [22] The groups were randomized to undertake screening by HPV testing, cytological testing, visual inspection with acetic acid (VIA), or standard of care (no screening). Women with positive screening tests were referred for colposcopy and biopsy and for treatment if lesions were detected. The

hazard ratios for the incidence of advanced cancer and death in the HPV testing groups were 0.47 (95% CI: 0.32 – 0.69) and 0.52 (95% CI: 0.32 – 0.83) respectively, as compared with the control group. There were no significant reductions in the numbers of advanced cancers or deaths observed in the cytological testing and the VIA group compared with the control group. This study showed that alternative strategies to cytology are effective in identifying premalignant lesions and therefore reducing the incidence of cervical cancer. The problem with HPV DNA testing is that it is extremely expensive.[22]

1.6 Colposcopy

Colposcopy may be used as a screening tool in some areas and it is also used as a diagnostic aid.[29] In most clinics in SA and at the colposcopy clinic at CHBAH, colposcopy is used for diagnosis and to plan a punch biopsy or to decide on treatment with Large Loop Excision of the Transformation Zone (LLETZ). Colposcopy has a sensitivity of 91.3% and a specificity of 24.6% in a meta-analysis conducted by Underwood M, Arbyn M, Parry-Smith W et al.[30] Using scoring systems like the Reid score and the Swede score may assist in making colposcopy more accurate.[31]

The Reid score is a systematic and objective colposcopic method of grading the severity of premalignant cervical lesions. The index considers four attributes of premalignant cervical lesions. The first colposcopic sign considers the colour (intensity) of aceto-whitening, the second sign considers the nature of the lesion margin and surface contour of acetowhite areas, the third sign considers vascular changes or features and the fourth considers the colour changes after iodine application. The first three signs are evaluated following application of 3-5% acetic acid to the cervix. The last sign is dependent on a preliminary score of the first three signs and is determined after Lugol's iodine application to the cervix.[32] Common cervical findings on colposcopy include acetowhite changes, punctations, mosaicism and abnormal vessels in women with CIN.[4]

The Swede score is a scoring system developed by Strander B, Ellstrom-Andersson A Franzen S et al for colposcopic examination that uses five variables. These variables include acetowhiteness, margins and surface, vessels, lesion size and iodine staining. Each variable gets assigned a score of 0, 1 or 2 to predict a high

grade lesion on histology after a cone or biopsy. The possible total score is 0-10 and a score of ≥5 points showed HSIL and a score of ≥8 had a specificity of 90%.[33] This system is also used to identify patients with low grade lesions or normal findings and in their study, Strander B, Ellstrom-Andersson A, Franzen S et al showed that 17% of patients only needed to be followed up with colposcopy or cytology instead of biopsy.[33]

1.7 Management of cervical pre-cancer Lesions

Treatment of CIN is indicated in women with CIN2 and CIN3. The treatment of cervical squamous intraepithelial neoplasia is the removal or ablation of the transformation zone (TZ). Destructive methods of the TZ include cryotherapy, coagulation and laser ablation, while excisional methods include cold knife cone biopsy, laser excision, Large Loop Excision of the Transformation Zone (LLETZ) and hysterectomy.[29,34]

Colposcopy is not a reliable method for recognising glandular disease or microinvasive cancer. Histology obtained by a LLETZ procedure has revealed between 0.6% and 1% of colposcopically unsuspected microinvasive disease. [29] According to Cantor SB, Cardenas-Turanzas M, Cox DD et al, colposcopy had a sensitivity of 0.983 and a specificity of 0.451 in the detection of LSIL when using a disease threshold of HSIL. They also found a sensitivity of 0.714 and a specificity of 0.813 for the detection of HSIL.[35]

The choice of method for the treatment of CIN is based on the size of the lesion, the age of the patient, HIV status, the patient's desire for future pregnancy and other gynaecological problems.[29,34] Hysterectomy is indicated in women with other gynaecological problems and when future pregnancy is not desired. Ablative methods are useful for women who wish to retain their fertility, but these methods have limitations in that they require histological diagnosis and they cannot be performed in women with a very atrophic cervix and where the lesion involves more than 2 quadrants of the cervix. [36] Other excisional methods like LLETZ and cold knife cone may be used in women who wish to retain their fertility.[36]

Excision using a wire loop was first described by Cartier, in 1981, and he used this as a means of biopsy. [39] The process has evolved to loops which are larger and with different shapes and sizes thus allowing tailoring of treatment based on colposcopic diagnosis. This procedure is performed under local anaesthesia, is easy to perform and has proven to be acceptable to patients and is available in many hospitals.[37]

Two large meta-analytical reviews were done to look at the relative obstetric risks associated with excisional methods of cervical biopsy.[29,38] The review by Kyrgiou M, Koliopoulos G, Martin-Hirsch P et al studied preterm pregnancy-related outcomes in women who subsequently conceived after being treated for CIN by cold knife cone biopsy, laser ablation, laser cone and LLETZ. The review showed an increased risk for preterm labour (Relative Risk, RR 1.7) in women who had been treated by LLETZ compared to women who had not had treatment. Similarly there was an increased risk for low birth weight and premature rupture of membranes.[29,38] There was a marginally non-significant association between laser conisation and preterm delivery (RR 1.71).[38] Laser ablation was not associated with any obstetric complications mentioned above. Cold knife cone, however was associated with a significant increase in low birth weight (RR 2.53), preterm delivery (RR 2.59) and caesarean section (RR 3.17).[38]

It has also been shown in the literature that the risk of preterm birth increased with a greater depth of excision of the cervix. In a case control study done by Castanon A, Landy R, Brocklehurst P et al, there was a 15.3% risk of preterm birth in women who had large excisions of ≥15 mm and there was a 7.2% risk in patients with a punch biopsy or excisions ≤10 mm compared with a 6.7% risk in the general population.[37] Short term complications of excisional biopsy included incomplete excision, which can lead to the increased risk of the residual disease being present in the subsequent follow up of the patient.[29]

1.7.1 Management Options for LSIL

Prevention of cervical cancer relies on the detection of cervical cancer precursors and this is performed using the Pap smear in SA. There are 3 management options for patients with LSIL on a Pap smear: refer to colposcopy; do an HPV test or repeat the Pap smear. Women with abnormal smears are referred for colposcopy and in some clinics the diagnosis is confirmed with a punch biopsy after which treatment

occurs.[21] In SA and in many other developing countries diagnosis is combined with treatment where immediate treatment with excision of the TZ is performed at the first colposcopic visit. This protocol is used to minimize loss to follow-up and to avoid long waiting periods due to poor infrastructure and scarce resources.[39] The specimen is then sent for histology after excision and this gives the final diagnosis. Treatment with ablative technologies needs to be based on the histological specimen.[7]

In view of the increased risk of cervical disease among HIV positive women, the Center for Disease Control (CDC) recommended that all HIV positive women should have a Pap smear performed as part of their initial evaluation when diagnosed with HIV. If the Pap smear should be found to be negative, the smear should be repeated in six months. Women who have had at least two negative Pap smears and never had an abnormal smear can have a Pap smear performed every 12 months.[5] The guidelines in SA recommend that all HIV infected women be screened for cervical cancer precursors at diagnosis and then 3 yearly in those with a negative screening test.[9] HIV infected women with an ASCUS and more severe are referred to colposcopy.[10]

Results from a few observational studies and one clinical trial suggested that observation without excisional therapy was appropriate management for HIV positive women with LGSIL or CIN 1. However, LLETZ has been recommended as the preferred method of treatment should treatment be required.[5] LLETZ may be used for excising the TZ as a simple outpatient procedure under local anesthetic infiltration, enabling comprehensive histological evaluation of the entire specimen. This therefore enables a true diagnosis, establishment of the excision margins and possibility of microinvasive or glandular disease.[32]

1.8 Problem statement and justification

Most cervical cancer guidelines, including the South African guidelines, recommend that HIV positive women with any cytological abnormality of ASCUS and more severe get referred to colposcopy.[7,9] Studies have confirmed the high HPV prevalence in HIV infected women.[7,9] The prevalence of LSIL is therefore also high. It is however not clear how many of these women with LSIL are just HPV infection or dysplasia. Such information may assist in the management of HIV infected women with a LSIL

on their Pap smear and this information will add to our existing knowledge on the subject.

1.9 Study objectives

1. To describe characteristics of HIV infected women with LSIL on Pap smear.
2. To compare women with CIN 1 on histology with those who have CIN 2/CIN3 on histology.
3. To compare the colposcopy findings to the histology results
4. To describe the proportion of women who have been overcalled at colposcopy and the proportion of women who were undercalled at colposcopy.

CHAPTER 2

2.1 Methodology

2.1.1 Study Design

This study is a cross sectional analysis of data from the colposcopy clinic database at Chris Hani Baragwanath Academic Hospital (CHBAH) from April 2003 to Dec 2013. The data was prospectively entered into the colposcopy database as patients were seen at the colposcopy clinic.

2.1.2 Variables that are recorded in the database

Information on the demographic details including age and parity, HIV status, CD4 count, ART use, current contraceptive use, smoking, snuff usage, cervical cytology results, colposcopy findings and histology was recorded in the database. Cervical cytology was performed using the conventional Papanicolaou smear and reported using the Bethesda Classification. Colposcopy at CHBAH was done by consultants or registrars under consultant supervision. Finesse I® and Finesse II® colposcopic machines were used in the colposcopy clinic and relevant sizes of the UtahLoop® or C-LETZ® were used to perform the LLETZ. Colposcopic diagnoses were made using the Modified Reid Score (Appendix F).

2.1.3 Study Setting/Population

Chris Hani Baragwanath Academic Hospital is a tertiary hospital situated in a large urban township called Soweto. The population of Soweto is comprised of economic migrants from rural South Africa and neighbouring African countries as well as political refugees from neighbouring African countries. The township mainly comprises black South Africans from different economic and educational backgrounds.

The CHBAH colposcopy clinic is a referral centre for Soweto and the Southern parts of Gauteng Province. According to the SA guidelines, HIV positive women with any cytological abnormality on Pap smear should be referred to colposcopy.[9] HIV positive women with LSIL on Pap smear were referred to Chris Hani Baragwanath Academic Hospital Gynaecology Outpatients according to referral guidelines (Fig 1).

HIV positive women with cervical cytology showing LSIL or more and colposcopy of CIN1 were treated immediately prior to 2008 because of the concern that CIN1 would progress more rapidly in HIV infected women. After 2008, a LLETZ was performed immediately in women where the colposcopy was equal to or more than CIN2 or if the colposcopy was inadequate or if the cervix was covered with warts.

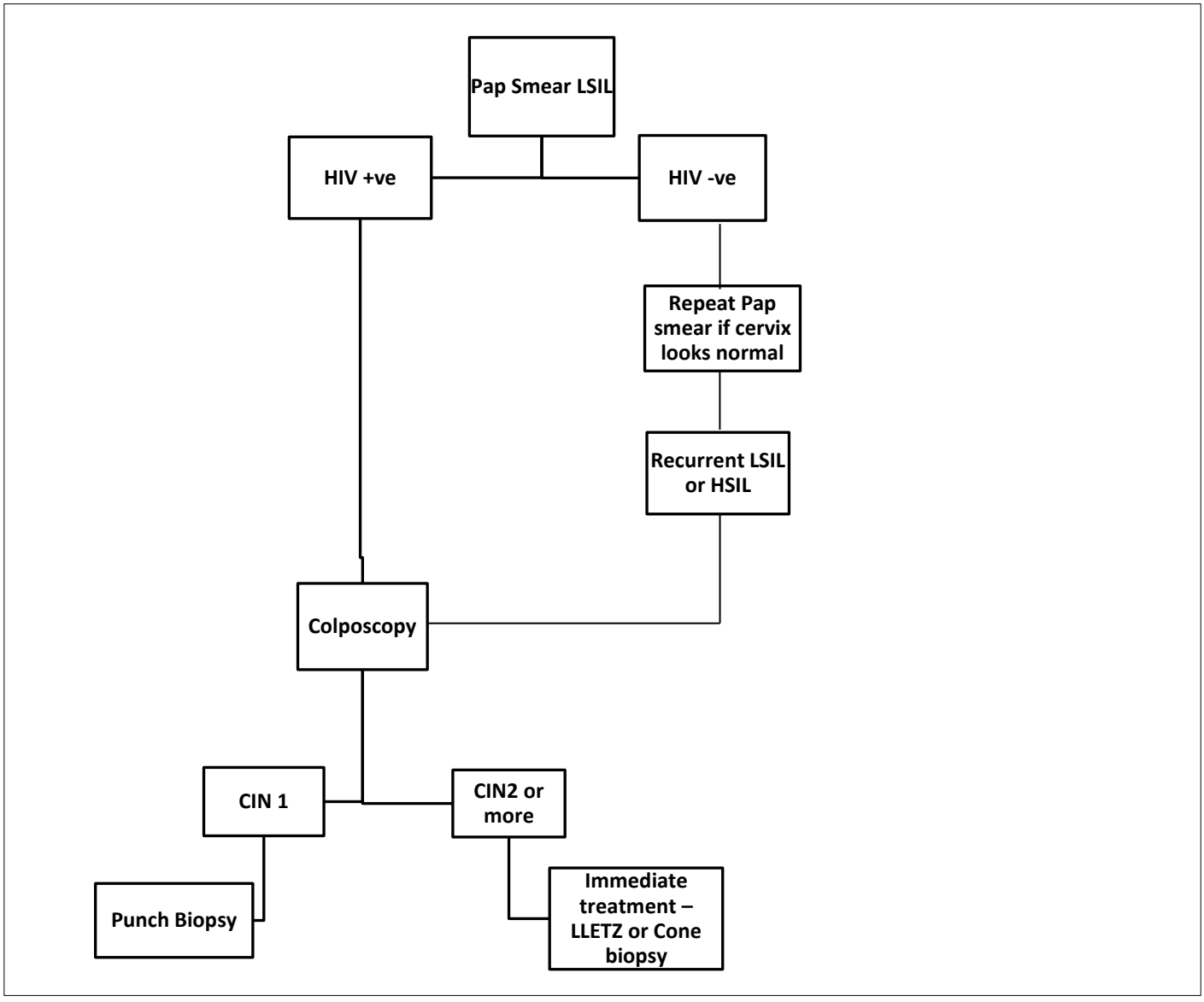


Figure 2.1 Flow diagram showing the management of patients at CHBAH colposcopy clinic

2.2 Data management

On the 8th April 2014, the data was extracted from the database. All relevant variables were entered into an Excel spreadsheet and subsequently coded for analysis. Duplicate records, HIV negative patients and patients with Pap smears that were not LSIL were thereafter excluded from the analysis. The next steps involved rechecking the missing data on the NHLS system and on the CHBAH record database for accuracy. The data was exported to a statistical programme and data analysis was performed using STATA statistical software (version 11).

After 2006, all women who did not know their HIV status, or who have tested negative more than 6 months prior to their first colposcopy at visit CHBAH were offered an HIV test (Personal communication). Patients that came to the clinic with an unknown HIV status at first visit were considered HIV positive at that time if they tested HIV positive within 6 months of the first colposcopy visit.

The following variables were extracted from the database: Age, HIV status, CD4 count, ART use, parity, contraceptive use, smoking, use of snuff, Pap smear results, colposcopic diagnosis and histology, dates of Pap smear and dates of colposcopy. Two new variables were created using the histology result: HPV or CIN1 (as a proxy for overtreatment) and CIN2 and more severe (as a proxy for appropriate treatment).

At Chris Hani Baragwanath Academic Hospital (CHBAH), colposcopy with or without LLETZ is performed by specialist gynaecologists or by supervised registrars. Colposcopic diagnosis is performed using a method based on the Modified Reid Colposcopic Index. All pap smears were performed using the conventional method. Most Pap smears were performed by the National Health Laboratory Services (NHLS) and some of the women had their Pap smears done by private laboratories. All histological examinations were performed and processed by the NHLS which has a strict internal and external quality control.

2.3 Data analysis

2.3.1 Descriptive and Analytical Statistics

All categorical variables are described using frequencies and percentages. Continuous variables are described using means and standard deviation (SD) and/or medians with IQRs. A new outcome variable was created: HPV/CIN1 and CIN2 and more severe. CIN2 was used as a cut-off because this is the threshold for treatment at this clinic.

Comparisons were made using the Chi square test for categorical variables and the Student T-test or Wilcoxon rank for comparing continuous variables. A logistic regression analysis was used to find the strength of associations. A p-value of <0.05 is considered significant.

2.4 Ethics

All women at the colposcopy clinic were counseled and requested to sign an informed consent form that allowed their data to be used for audit, follow-up and research (Ethics clearance certificate for the Database – M080603/M040609). The ethics application was updated and the certificate is attached (Appendix A). Women that declined to give consent were treated the same way as all other patients.

Ethics approval to do this study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Clearance certificate number: M140220 (Appendix B). Approval to conduct the study was also obtained from the CEO at Chris Hani Baragwanath Hospital (Appendix C) and database gate keepers for NHLS for the use of Pap smear and Histology results (Appendix D). Permission was obtained from Dr Y Adam, Prof CJ van Gelderen, Dr F Kabir, Dr K Kgomo and Dr A Lekha for use of information from the colposcopy database (Appendix E).

2.5 Funding

Stationery was funded by the researcher.

CHAPTER 3

3.1 Results

The initial section of this chapter describes the study population including demographic information and HIV factors. The subsequent sections present other Pap smear, colposcopy and histology findings. This is followed by conducting a comparison between women with a histology result of CIN 1/HPV to women with CIN2 and more severe lesions. The chapter concludes by conducting an analysis of overtreatment and under treatment of women with LSIL.

3.1.1 Study population

There were a total of 6159 women on the colposcopy clinic database. Of the 6159 women, 3895 (63.24%) were HIV positive women referred to CHBAH colposcopy clinic between 1st April 2003 and 31st December 2013. Of these, 660 women were referred with LSIL. Eight (8) women were excluded from the study as they did not have histology results. The resultant 652 women formed part of the analysis. Of the 652 women, 75 had a recurrent LSIL. The flow diagram below illustrates the above-mentioned sample criteria.

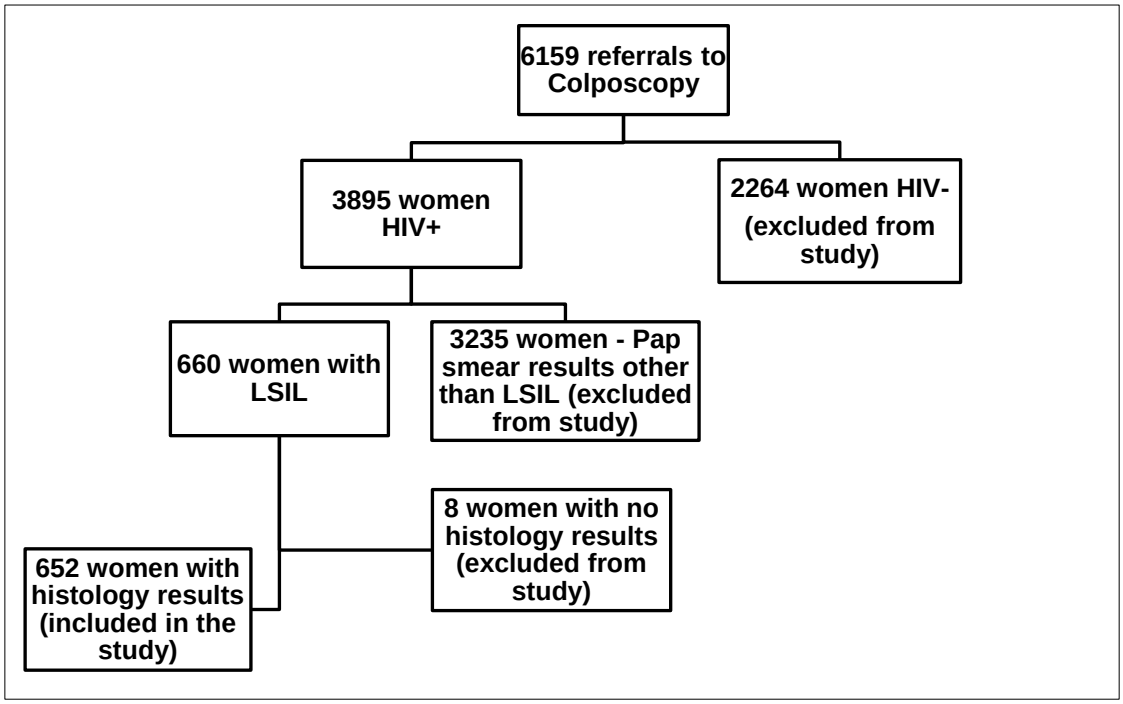


Figure 3.1 Flow diagram showing inclusion and exclusion criteria of study population

Age and parity

The median age was 36.00 years (IQR= 31.00-42.00) with a range of 18.00 to 66.00 years and a mean of 36.55 (SD± 7.68). The median parity was 3 (IQR= 2-5) and the mean was 3.22 (SD± 1.75) with a range of 0 to 10.

Contraception

As illustrated in figure 3.2 below, the largest proportion of women (n=285; 43.71%) only used condoms as a method of contraception. A significant number of women (n=152; 23%) were not using any form of contraceptive method, however, this may be explained by the fact that some of the women were either not in their reproductive years or not sexually active.

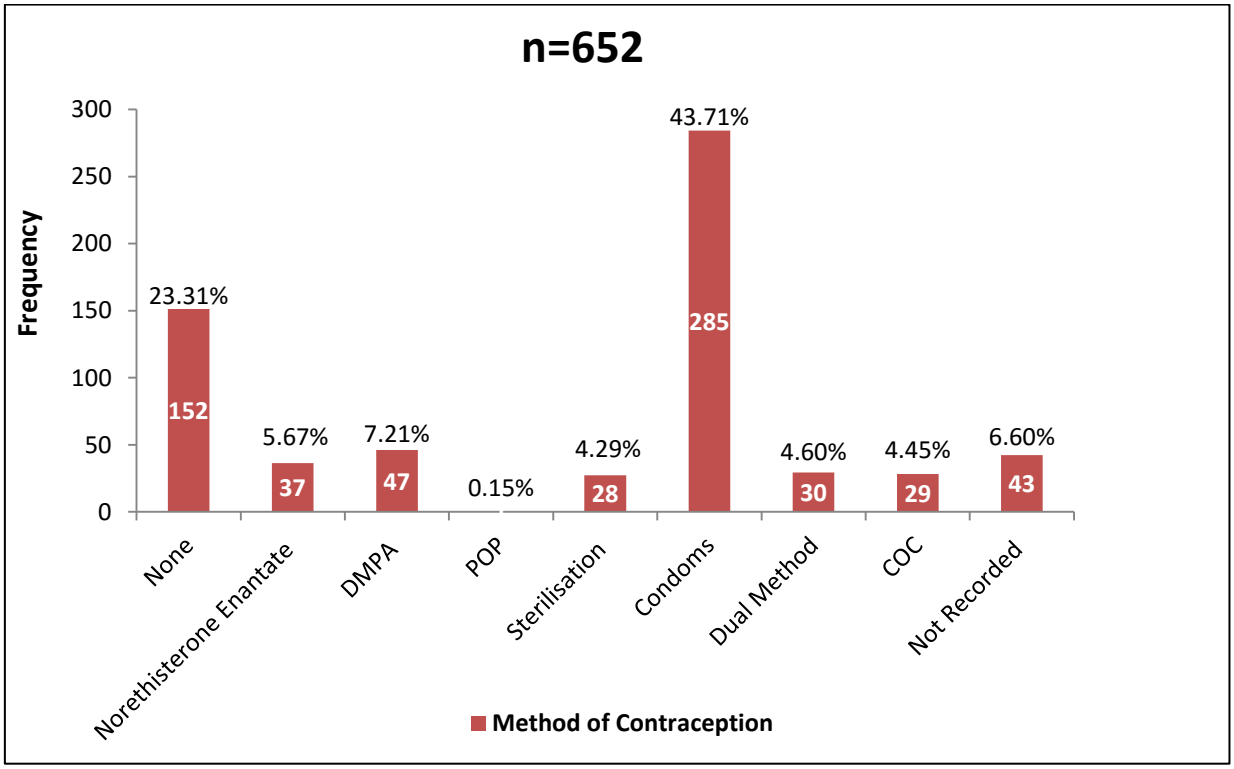


Figure 3.2 Method of contraception at the time of presentation

*Dual method indicates the use of condoms together with any hormonal contraceptive method
** POP users (n=1)

Cigarette Smoking

Of the entire database, only 87 women's smoking records were recorded. This was because smoking was only captured from 2010 in the database. From the sample of 87 women, 5 women (5.75 %) were recorded as smokers. Figure 3.3 below is a clear illustration of the proportion of smokers to non-smokers.

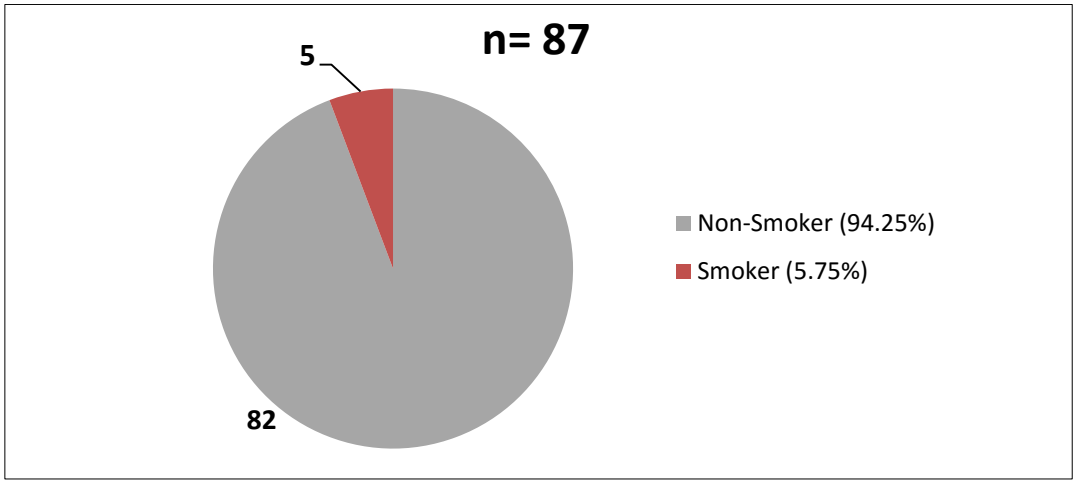


Figure 3.3 Proportion of women smoking cigarette

Snuff Users

Only 87 snuff usage information was recorded in the database. This information was only being recorded from the year 2010. From the sample of 87 women, 14 women (16.09%) were recorded as snuff users. Figure 3.4 below indicates the proportion of snuff users to non-users.

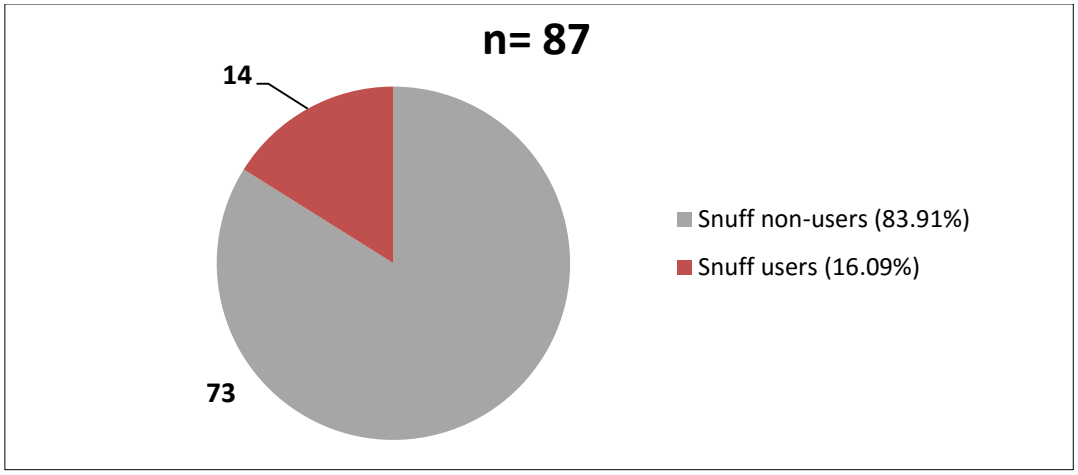


Figure 3.4 Proportion of women using snuff

3.1.2 HIV factors

HIV Duration

The duration of HIV infection was only recorded after 2010 and was only known in 58 women. The median interval between HIV diagnosis and the first colposcopy visit was 50.32 months (IQR= 24.6 – 104.2) and the range was between 12 days to a maximum of 212 months. The mean interval recorded was 63.05 months (SD± 48.2).

CD4 count

The CD4 count was known in 584 out of 652 women. At the point of first visit, the median CD4 count recorded was 275.00 cells/mm^3 (IQR = 173.50 – 434.00) and the mean was 321.00 cells/mm^3 (SD± 217.25). The number of women with a CD4 count less than or equal to 200 cells/mm^3 was 190 (32.53%); women with a CD4 count above 200 cells/µL and below 350 cells/mm^3 was 180 (30.82%) and 214 women (36.64%) had a CD4 count above 350 cells/mm^3.

ART usage

There were 312 women (47.85 %) using ART and 74 (11.35%) women had been exposed to ART as a prevention of mother to child transmission (PMTCT) before their colposcopy clinic visit, however, they were not using ART for their own health. A further 200 women (30.67%) had never used ART and 66 women (10.12%) had no ART information recorded in the database. Figure 3.5 below demonstrates the various ART treatment findings.

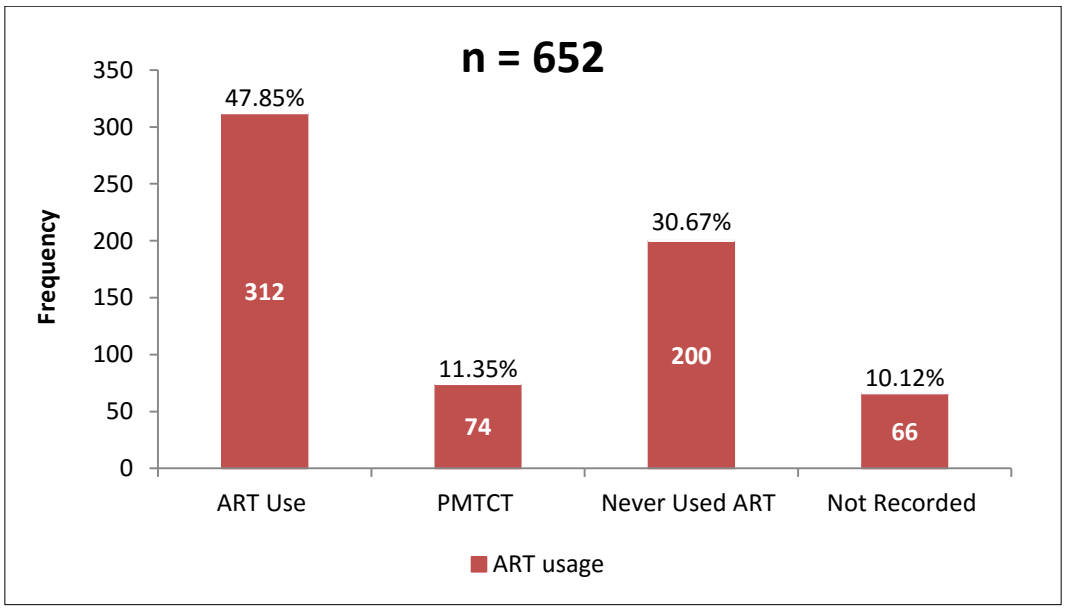


Figure 3.5 ART usage

3.1.3 Pap smear Findings

Time Interval between the Pap smear and Colposcopy Assessments

The median time between the Pap smear being performed and the initial colposcopy visit was 210.00 days (IQR= 132.00 - 313.00). The minimum time interval reported was 8.00 days and the maximum reported time interval was 2134.00 day (approximately 5 years and 10 months). The mean time interval was 239.19 with a standard deviation of ±183.62.

Pap smear Report Findings

There were 99 women (15.18%) that had other findings on their Pap smear results. Bacterial Vaginosis was found in 78 women (11.96%), 15 women (2.30%) had Candidiasis and 6 women (0.92%) had Trichomonas on their Pap smear results. Figure 3.6 below illustrates the proportion of women who had these findings.

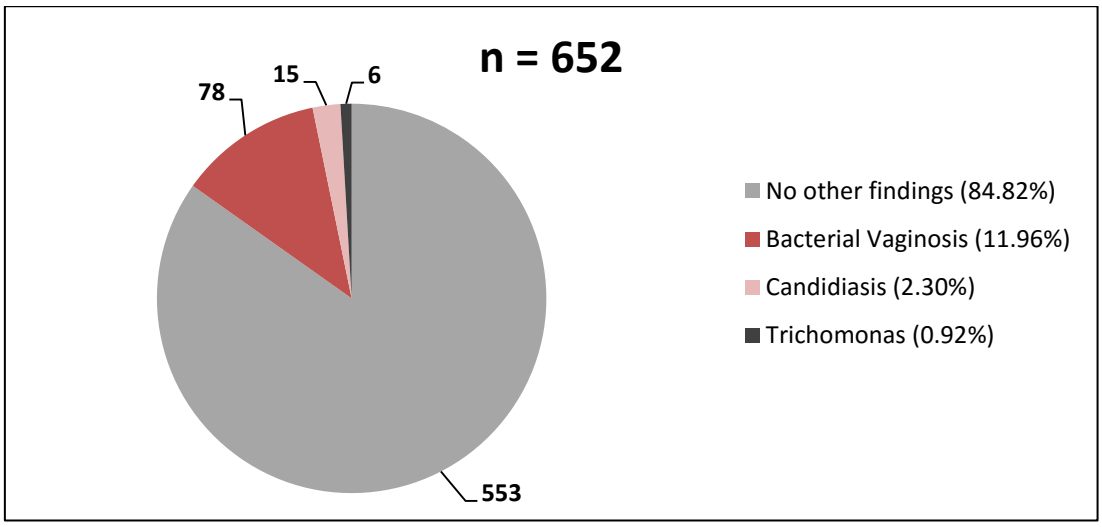


Figure 3.6 Other findings on the Pap smear report

3.1.4 Colposcopic and Histology Findings

Colposcopic Report Findings

The colposcopic diagnosis was not recorded in 28 women (4.29%) and a diagnosis could not be made in 31 women (4.75%). The most common colposcopic diagnosis was CIN2 as reflected in figure 3.7.

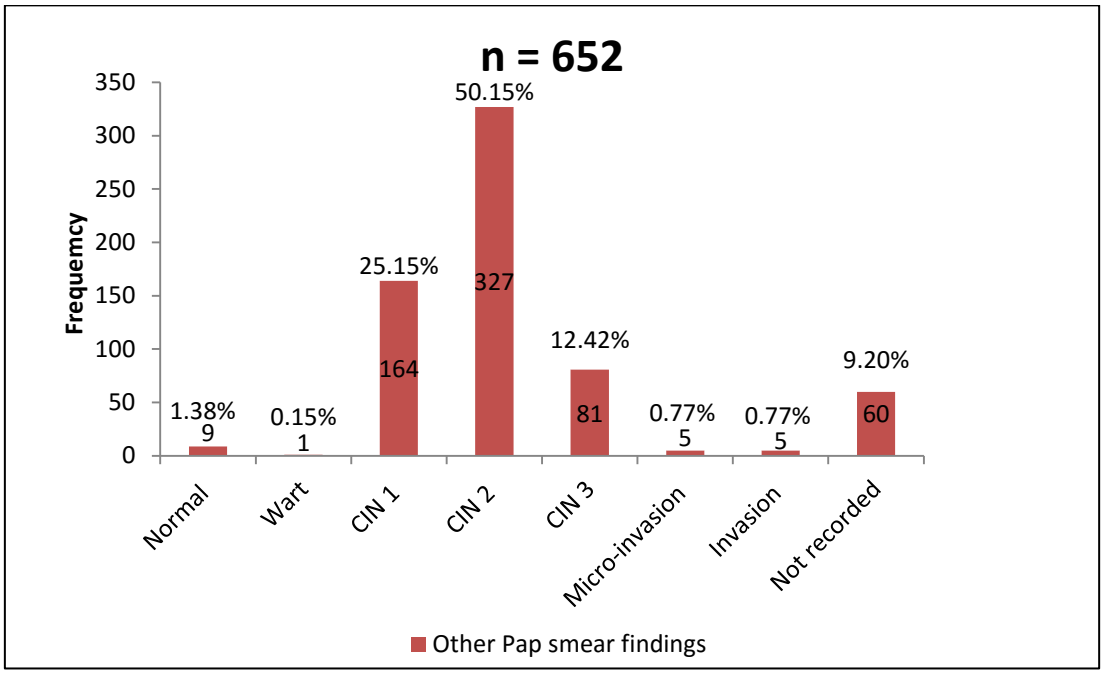


Figure 3.7 Colposcopic diagnoses in women with LSIL on Pap smear

Methods used for obtaining Histology specimens

Histology in this study was obtained by Punch biopsy, Loop excision (of the transformation zone), cold knife cone and hysterectomy as illustrated in table 3.1 below.

Hysterectomy, which is generally not a common method used for the management of premalignant lesions, was performed on 6 women (0.92%). The factors contributing to the hysterectomy being performed were as a result of a large lesion, the inability to see the cervix and inability to perform a loop and other concomitant gynaecological conditions requiring hysterectomy.

LLETZ was performed either under local anaesthesia or under general anaesthesia. In the study population, 7 women had LLETZ performed under general anaesthesia, whereas 569 women had LLETZ performed under local anaesthesia. The reasons for LLETZ being performed under general anaesthesia are attributable to either inability to insert the speculum due to pain and/or severe discomfort; difficulty visualizing the cervix; a large lesion or the patient requesting general anaesthesia.

Table 3.1 Cervical biopsy methods

Method	Frequency	Percentage (%)
LLETZ (local anaesthesia)	569	87.27
LLETZ (general anaesthesia)	7	1.07
Loop Biopsy (local anaesthesia)	11	1.69
Cone Biopsy	2	0.31
Punch Biopsy	9	1.39
Hysterectomy	6	0.92
Not recorded	48	7.36
Total	**652**	**100.00**

Histology

There were 266 women (40.80%) who had an histology result of HPV/CIN 1 and 386 women (59.20%) had an histology result of CIN 2 or more severe lesion. Figure 3.8 displays the breakdown of different histological categories. There were no histology reports that were normal.

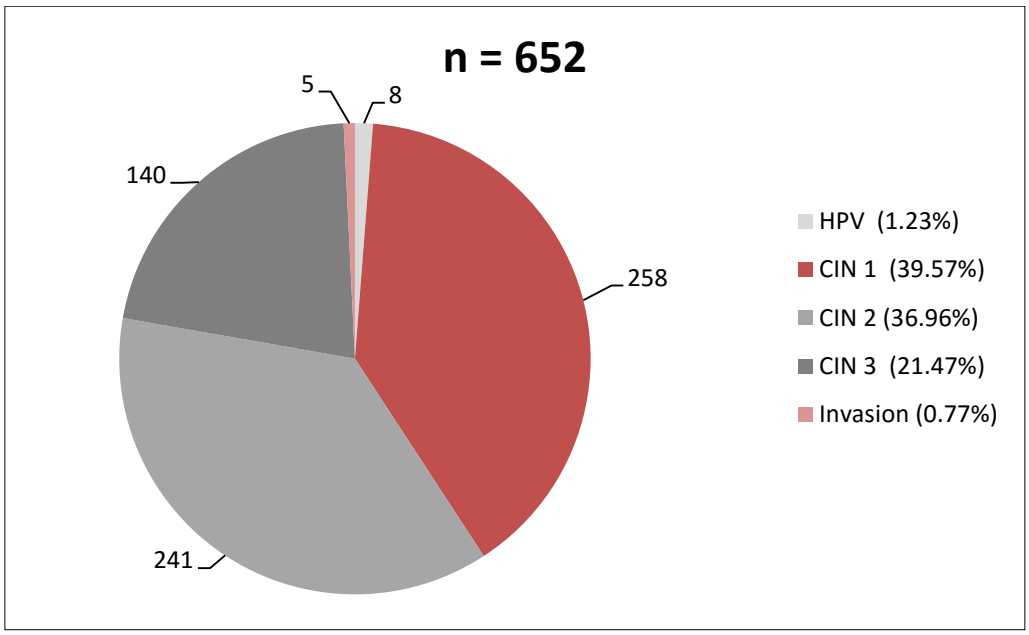


Figure 3.8 Histology results

Histology of invasive disease

There were 5 women with histology of invasive disease. The staging of these cancers are not recorded in the database as these women are referred for oncological assessment and treatment. Table 3.2 below describes these women. The mean age of women with invasive cervical cancer was 27.2 years (SD± 8.84), mean parity was 3.4 (SD± 1.52), mean CD4 count was 333.00 cells/mm^3 (SD± 165.70) and the mean time to colposcopy was 229.4 days (SD±131.27).

Table 3.2 Characteristics of women with invasive disease

	Parity	Time from Pap to 1st visit	CD4 (cells/mm^3)	Age	Use of ART
Patient 1	1	436	175	45	No ART
Patient 2	3	155	403	44	ART
Patient 3	1	208	unknown	54	No ART
Patient 4	2	90	534	30	No ART
Patient 5	3	258	223	49	No ART

3.1.5 Comparison between Colposcopic Diagnosis and Histology Results

A total of 591 women had histology results as well as a colposcopic diagnosis. The histology results were compared to the colposcopic findings. The analysis in table 3.2 below is a summary of the relationship between findings from each procedure. There were no histology reports that were normal. Each diagnosis is explained in the text that follows the table.

Table 3.3 Comparison of colposcopy diagnosis with histology results

Colposcopy / **Histology**	**CIN1**	**CIN2**	**CIN3**	**Micro-invasion**	**Invasion**	**Normal**	**Total**
HPV	3.00 (1.83%)	3.00 (0.92%)	1.00 (1.23%)	0.00 (0.00%)	0.00 (0.00%)	0.00 (0.00%)	**7.00**
CIN1	**99.00 (60.37%)**	113.00 (34.56%)	11.00 (13.58%)	1.00 (20.00%)	0.00 (0.00%)	5.00 (55.56%)	**229.00**
CIN 2	41.00 (25.00%)	**134.00 (40.98%)**	43.00 (53.09%)	0.00 (0.00%)	0.00 (0.00%)	1.00 (11.11%)	**219.00**
CIN 3	21.00 (12.80%)	76.00 (23.24%)	**26.00 (32.10%)**	3.00 (60.00%)	2.00 (40.00%)	3.00 (33.33%)	**131.00**
Microinvasion	0.00 (0.00%)	0.00 (0.00%)	0.00 (0.00%)	**0.00 (0.00%)**	0.00 (0.00%)	0.00 (0.00%)	**0.00**
Invasion	0.00 (0.00%)	1.00 (0.31%)	0.00 (0.00%)	1.00 (20.00%)	**3.00 (60.00%)**	0.00 (0.00%)	**5.00**
Normal	0.00 (0.00%)	0.00 (0.00%)	0.00 (0.00%)	0.00 (0.00%)	0.00 (0.00%)	**0.00 (0.00%)**	**0.00**
Total	**164.00**	**327.00**	**81.00**	**5.00**	**5.00**	**9.00**	**591.00**

Colposcopy diagnosis of CIN1- compared to histology findings

Of the 164 women that were diagnosed with CIN1 on colposcopy, 99 women (60.37%) were diagnosed with CIN1 on histology. On the other hand, 41 women (25.00%) were diagnosed with CIN2 and 21 women (12.80%) with CIN3 on histology. This represents a total of 62 women (37.00%) who were underdiagnosed at colposcopy. A further 3 women (1.83%) who had CIN1 on colposcopy only had HPV diagnosis on histology.

Colposcopy diagnosis of CIN2 – compared to histology findings

Out of the 327 women who were diagnosed as CIN2 at colposcopy, 113 (34.56%) had CIN 1 and 3 women (0.92%) had HPV on histology. This represents a total of 116 (35.48%) women who were over-diagnosed based on their colposcopy findings in comparison to their histology findings. Conversely, 76 women (23.24%) had CIN 3 and 1 woman (0.31%) had invasion on histology, indicating that a total of 77 women (23.55%) were underdiagnosed at colposcopy. There was absolute correlation in 134 women (40.98%), who were therefore correctly diagnosed.

Colposcopy diagnosis of CIN3 – compared to histology findings

There were 81women that had CIN 3 at colposcopy. Out of the 81 women, 1 woman (1.23%) had HPV, 11 women (13.58%) had CIN 1 and 43 women (53.09%) had CIN 2 on histology, hence 55 women (67.90%) were over-diagnosed at colposcopy. Twenty-six women (32.10%) had CIN 3 diagnosis on both procedures, which demonstrates a low proportion of accuracy of colposcopy for a diagnosis of CIN3 in women with a LSIL on Pap smear. In this category, no patients were underdiagnosed at colposcopy.

Colposcopic diagnosis of ≥CIN2 combined

CIN 2 and more severe is the threshold above which a LLETZ is performed at this clinic. There were 418 women in this group who were diagnosed with CIN 2 and more severe. Of these 289 (69.14%) would have been appropriately treated.

Colposcopic Diagnosis of Microinvasion – compared to histology findings

There were 5 women that were diagnosed as microinvasion at colposcopy. Out of these 5 women, 1 woman (20.00%) had CIN 1 and 3 women (60.00%) had CIN3 on histology, respectively – indicating 80% patient over-diagnosis at colposcopy. The remaining women (20.00%) had a histology diagnosis of invasion, reflecting underdiagnosis at colposcopy. There was no accurate diagnosis of microinvasion using colposcopy.

Colposcopic Diagnosis of Invasion – compared with histology findings

There were 5 women that had invasion as a diagnosis at colposcopy. Out of the 5 women, 2 women (40%) had CIN 3 on histology, reflecting the number of women that

were over-diagnosed at colposcopy. However, 3 of the 5 women (60%) were accurately diagnosed at colposcopy as invasion as this was confirmed histologically.

'Normal' Colposcopy finding – compared with histology findings

There were 9 women that had a normal colposcopy. Out of the 9 women, 5 women (55.56%) had CIN 1 on histology, 1 woman (11.11%) had CIN 2 on histology and 3 women (33.33%) had CIN 3 on histology. All women (100%) in this category were under diagnosed as none of the histology results were normal.

3.1.6 Margins

Over 50% of women had clear margins, 4.98% of women had involvement of both margins and 5.31% had involvement of the endocervical margins. Involvement of margins is shown in table 3.4 below.

Table 3.4 Margins involved

Margins	Frequency	Percentage (%)
Both	31	5.15
Ectocervical	203	33.72
Endocervical	33	5.48
Margins clear	321	53.32
Unknown	14	2.36
Total	**602**	**100.00**

3.1.7 Comparison of women with CIN 1 with those who have CIN2 or more on histology

Table 3.5 below is a comparison of factors according to demographic details in relation to severity of disease. The table displays the statistical difference between the women who had CIN1/HPV and the women who had CIN2 or more severe on histology. There were too few women where smoking and snuff were recorded. Due to the minimal sample size of smoking and use of snuff, there was no statistical difference of snuff use or smoking in the 2 histology categories hence it has not been represented in the table.

Table 3.5 Demographic details in relation to severity of disease

	HPV/CIN1 n=266 (40.80%)	≥CIN2 n= 386 (59.20%)	p-value
Mean Age 36.55 (SD±7.68)	37.14 (SD± 7.97)	36.15 (SD± 7.45)	0.00 (t-test)
Mean Time from Pap to Colposcopy (days)	266.73 (SD± 232.89)	220.69 (SD± 138.48)	0.00 (t-test)
Use of ART N=312 (47.93%)	n=149/266 (56.02%)	n=163/386 (42.34%)	0.00 (chi2)
Mean HIV duration in days (years) – only known in 58 patients	2105.70 (1527.95) (5.77 years)	1586.38 (1322.77) (4.35 years)	0.00 (t-test)
Use of condoms (comparator) N=285 (71.43%)	n=131/266 (SD± 79.39)	n=154/386 (SD± 65.81)	0.00
Use of *any hormonal contraception N=114 (28.57%)	n=34/266 (12.87%)	n=80/386 (20.73%)	
Parity			
Nulliparous	n=41/266 (15.41%)	n=45/386 (11.66%)	0.16 (chi)
Parous women N=564 (86.77%)	n=224/266 (84.21%)	n=340/386 (88.08%)	

*** Depo Provera, COC, POP or Norethisterone**

The women who had HPV/CIN1 were older (37.14 years) than the women who had CIN2/CIN3 (36.15 years). This age difference is not clinically significant even though it is statistically significant. The time from Pap smear to colposcopy was longer (266.73 days) among women who had HPV/CIN1 compared with women who had CIN2/CIN3. Women who had HPV/CIN 1 had a longer duration of HIV infection (5.77 years) and had a higher proportion of ART use (56.02% vs. 42.34%). The different contraceptive methods were analysed to determine the relationship between the different methods and the probability of having HPV/CIN1 or CIN 2/CIN3. The women who had HPV/CIN1 had a higher proportion of condom use compared to hormonal contraception as opposed to the women with CIN2/CIN3, their proportions were 49.29% and 39.90% respectively. On the other hand the women with HPV/CIN1 had a lower proportion of hormonal contraception use compared to women with CIN2/CIN3, their proportions were 12.87% and 20.73% respectively. There was no statistical difference with respect to parity between these 2 groups of women.

Risk of CIN2 or more severe lesion in women with LSIL on Pap smear

Table 3.6 below shows the unadjusted risk ratio of CIN2 or more severe lesion. The following variables, namely, duration of HIV infection, snuff usage, smoking, parity, age and time from Pap smear to presentation had no statistical significance of an increased risk of having CIN2 and more severe lesion. However, women who used hormonal contraception compared to women who used condoms were 2 times more likely to have CIN2 and more severe lesion. HIV infection was also significantly associated with having CIN 2 or more on histology. Women who used ART were 43% less likely to have a more severe lesion and there was an approximately 20% less likely chance of having a more severe lesion with a higher CD4 count category.

Table 3.6 Unadjusted risk ratio of CIN2 or more severe lesion

	Unadjusted OR	p-value
Duration of HIV infection in weeks	1.00	0.20
Time form Pap to colposcopy in days	0.998	0.00
Use of snuff compared to no use	1.06	0.92
Smoking compared to no smoking	1.13	0.89
Parity	0.99	0.88
Nulliparous compared to parous women	1.38	0.163
Age (in years)	0.98	0.10
ART use compared to no use	0.57	0.00
CD4 count	0.99	0.00
CD4 category compared to <200 cells/mm^3		
≥200	0.81	0.00
Hormonal contraception compared to condoms	2.00	0.003

CHAPTER 4

4.1 Discussion

The objectives of this study were to describe the characteristics of HIV infected women with LSIL on Pap smear, compare the characteristics of women with CIN 1 on histology with those who have CIN 2 or more on histology, compare the colposcopy findings to the histology results and finally to describe the proportion of women who have been overcalled at colposcopy and the proportion of women who were undercalled at colposcopy. In this chapter, the results, limitations and strengths of the study will be discussed, followed by a conclusion and recommendations.

In our study we found that about 59.20% of patients with LSIL on Pap smear had clinically significant disease on histology. This finding is much higher than in a study done by Soto-Wright V, Samuelson R, McLellan R et al where they found that 9%-16% of patients with LSIL on Pap smear subsequently had CIN2 to CIN3 on biopsy.[40] A South African study by Lindeque also found that about 15–30% of women with LSIL on cytology were expected to have CIN 2 or 3 on biopsy.[41] The IQR from when the Pap smear was performed and the first colposcopy visit was 132.00 – 313.00 days, therefore a repeat Pap smear was not indicated in 75% of patients in our study. It is not our practice to repeat Pap smears even in women who arrive after a year of having had a Pap smear as this would further delay the colposcopic assessment. We can only postulate that in less than 25% of patients a repeat pap smear may have shown persistence, or progression. There were no patients with normal histology in this study.

In a study done by Omar T, Schwartz S, Hanrahan C et al, also in a Soweto population they found that in HIV positive women with LSIL on baseline smear, there was a 0.8% progression rate to ASC-H and 21.5% to HSIL at five months repeat smear.[10] In contrast, though, a study done by Saayman F, van Gelderen C J, Michelow P et al showed that there was no significant increased risk of up or down-grading of dysplasia with an interval of 6 months or more than 6 months between a Pap smear and histology. They also found that the use of ART had no effect on the risk of invasive disease or the upgrading of dysplasia. [11]

We also found that there were 5 women (0.77%) that had invasion on histology. This finding is slightly higher than in other studies that showed that up to 0.5% of patients with LSIL on Pap smear will have invasive cancer.[42] However, in these studies there were no HIV positive patients. The difference in this group of 5 women was not tested against the study population because of the small numbers. Only 1 out of 5 women were on ART as the threshold for ART use was higher at the time of this study. These are important findings as they help us keep in mind that we do not understand the natural history of CIN in HIV positive women. It may be that progression is sooner in HIV positive women. A study done at the Charlotte Maxheke Johannesburg Academic hospital showed that HIV infected women presented with cervical cancer 10 years earlier than HIV negative women.[17] These findings support the ongoing practice that HIV positive women should be referred to colposcopy as soon as they have LSIL on their Pap smears.

The mean age in our study was 36 years and this is similar to the peak age for development of cervical precursor lesions. The age and parity, in our study, were not associated with a more severe CIN lesion and this was also a finding in the study done by Omar T, Schwartz S, Hanrahan C et al.[10]

In our study, 32.53% of women had a CD4 count less than 200 cells/mm^3. This is less than the finding in Firnhaber's study where they found 42% of women (both LSIL and HSIL) in their study had a CD4 count of <200 cells/mm^3.[16] In our study the mean CD4 count was low at 275.00 cells/mm^3 and this is consistent with the known association of a low CD4 count with cervical dysplasia. Furthermore, the prevalence and degree of dysplasia increases with advancing levels of immunosuppression.[24] A low CD 4 count of ≤200 cells/mm^3 proved to be a significant risk factor for severe disease in both our study and Firnhaber's study.[16] This fact was also true for progression to higher grade lesions in Omar's study.[10] Finhaber C, Van Le H, Pettifor A et al also showed that women with CD4 counts <200 cells/mm^3 had a higher prevalence of HPV 16 as well as high grade lesions.[16] In a study done by Clark, progression of LSIL to HSIL was associated with lower CD4 counts and presence of HPV types 16, 18, and 33 [5]. Regrettably in our study we did not have information regarding HPV subtypes as HPV testing was not specifically performed.

Due to our study being retrospective and that viral load testing was not standard of care at the time of the study, we were unable to obtain information on viral load. We were also unable to determine the duration of ART usage and whether the duration had a protective effect against the risk of having CIN 2 or more severe lesions. Of importance, though, is that there was a higher percentage of women using ART in our study than in previous studies of HIV in SA, which is promising.

Condom use was high at 43.17% and this was associated with a lower risk of CIN2 or more severe lesions in our study and this finding was consistent with the results in Firnhaber's study. Condom usage was much higher in Firnhaber's study, with a reported 75.4% condom usage, however, their study was consistent with our study in that they also found condom use was associated with a lower risk of HSIL compared to non-condom users.[16] It is interesting and encouraging that condoms are a common contraceptive method in our study population. This might be because women that are HIV positive are more likely to use condoms when they know their status and/or clinics are promoting condom use to patients.

Hormonal contraceptive use, specifically combined oral contraceptives (COC), has been associated with an increased risk of cervical cancer or upgrade of dysplasia in women infected with HPV.[4] In our study we found that women that used hormonal contraceptives were 2 times more likely than women who used condoms to have CIN2 and more severe lesion.

Intuitively, one would have expected that the progression of CIN would be higher in HIV patients and in patients with a longer duration of HIV infection. However, HIV duration was not associated with a statistically significant increased risk of having a more severe cervical lesion. This could possibly be attributed to patients seeking HIV treatment earlier.

The colposcopy clinic at CHBAH uses a "see-and-treat" policy in order to avoid loss of patient follow-up. The disadvantage of this management policy is that patients may be exposed to overtreatment. As a result, patients with a lower-grade lesion or with a normal cervix may receive inappropriate and excessive treatment, and they may be exposed to bleeding and infection unnecessarily.[39]

Overtreatment can be defined based on either a conservative or a more aggressive approach to the treatment of squamous intraepithelial lesions. In a conservative approach, the appropriate treatment threshold is defined as a diagnosis of high-grade SIL or worse. This approach states the numerator as the number of patients who were treated and found to have a normal or low-grade SIL at histology. As a result, this approach yields more patients who are overtreated at the time of the diagnostic visit. With a more aggressive approach, the appropriate treatment is when histology results show a low-grade SIL or worse. The numerator for overtreatment with this approach is the number of patients treated and found to have normal histological results and no SIL.[39]

In our study we found that 129 women (21.83%) were over diagnosed and therefore overtreated – this is using CIN 2 and more severe lesion as a threshold for treatment. There is therefore a greater risk of overtreatment of HIV positive women with LSIL who are referred to a colposcopy clinic where immediate treatment is practiced. However colposcopy was better in predicting a clinically significant lesion when the colposcopic diagnosis was CIN3 or more.

Punch biopsies may therefore be important in women with CIN 2 and less severe disease (on colposcopy) in order to reduce overtreatment. However punch biopsies have not been shown to be very accurate when compared to cone biopsies and LLETZ.[30]

In a study done by Bigrigg MA, Codling BW, Pearson P et al, the reported overtreatment was 27.9% if the threshold was a pathology report of low-grade SIL and 4.7% if the pathology report was negative.[44] In another study done by Keijser KG, Kenemans P, van der Zanden PH et al, patients diagnosed with any grade of CIN were treated and they reported overtreatment of 13.3% for low-grade SIL and of 7.0% for a negative pathology report.[45] Megevand E, Van Wyk W, Knight B et al had overtreatment of 21.8% in low-grade SIL or normal histology results.[46] However, these women were HIV negative.

Histology was considered the ‘gold standard’ diagnostic tool in our study and the threshold for treatment in our colposcopy clinic was considered to be CIN 2 since 2006. Omar T, Schwartz S, Hanrahan C et al found that colposcopy had a sensitivity

of 54%-85% for the detection of CIN 3.[10] We found that 61 of the 591 women (10.49%) were underdiagnosed. This is concerning as the risk associated with underdiagnosis is that patients that don't return for their results can end up being lost to follow-up and thus risk progressing to cervical cancer.[39]

In comparison to the other levels of cervical dysplasia, CIN1 had the highest rate of underdiagnosis. A possible reason to account for this difference could be that the colposcopists were influenced by prior knowledge that the patients had LSIL on their Pap smear and therefore expected a CIN1 diagnosis on colposcopy. This demonstrates the importance of adhering to the diagnostic protocols of colposcopy and therefore correct treatment for the patients.

Colposcopy is not a reliable method of recognizing glandular disease or early invasive cancer. [29] Our study confirmed these findings as we found that colposcopy was an unreliable tool for diagnosing microinvasion (0.00%).

LLETZ is considered both a diagnostic and therapeutic procedure and it provides a conservative management approach to the treatment of HSIL. In some patients, however, cervical lesions persist or recur and positive margins have been identified as an important predictive factor. Having both margins positive carries the highest risk of recurrence as compared to one margin or no margins being positive.[43,47] Moreover, Zhu M, He Y, Baak JPA et al found in their study that the persistence rate of cervical lesions in patients with HSIL was 11.3%. They also found that women who were over 35 years of age were 4.6 times more likely to have persistence of or recurrence of cervical lesions.[48] In our study, 4.98% of women had involvement of both margins and 5.31% had involvement of the endocervical margins. In a study done by Kabir F, van Gelderen C, McIntyre J et al, using the same database as ours, they found involvement of both margins in 12.66%,[43] which is much higher than in our study. Possible reasons to account for this difference is that our study only looked at HIV positive patients with low grade SIL and the study by Kabir F, van Gelderen C, McIntyre J et al looked at HIV positive patients with both LSIL and HSIL. LSILs on Pap smear may be associated with smaller lesions and we had lower rates of both margin involvements compared to Kabir's study. High-risk HPV testing has been shown to be a useful predictor of recurrence, however, HPV testing was not done in our study.

4.2 Strengths

The strength of our study is that all our patients had histology as a ‘gold standard’ of diagnosis.

4.3 Limitations

This is a retrospective study and therefore missing information was not obtainable. Other variables for risk factors for carcinoma of the cervix have not been collected in the database, e.g. sexual history, coitarche and number of sexual partners. Smoking and use of snuff were only added in the database from 2010.

Another limitation of this study is that it is a cross sectional study with a long interval between LSIL and colposcopy/biopsy. We were also not able to determine HPV subtypes in our study population because it is not standard of care. HIV parameters change over time and we did not know how long these women were on ART, we also did not have information on their viral loads and we did not have serial CD4 counts. This database is a clinical database and missing information could not be obtained.

4.4 Recommendations

Recommendations for further research:

A prospective study which may assist in finding factors that will increase the sensitivity and the specificity of colposcopy, thereby reducing the risks of both over and under treatment.

Markers of HPV infection:

A prospective study to determine the common HPV subtypes in our population would be beneficial and will add to our knowledge and understanding of LSIL in HIV positive patients. The knowledge of HPV subtyping may assist in the screening and prediction of patients who will progress to more severe lesions and those who will be at risk of persistence. Palefsky JM, Minkoff H, Kalish LA et al found the prevalence of HPV in HIV positive women to be 20%-34%, resulting in these patients being about 5 times more likely to have high risk HPV infection than in HIV negative women.[49]

Clinical recommendations:

Addressing the long waiting time between Pap smear and colposcopy will help in reducing the chances of women with LSIL progressing to more severe lesions by the time they present for colposcopy.

Our recommendation is that women who are HIV positive should still be referred to colposcopy. In this study we found 5 women with invasive cervical cancer and previously, from the same population, in a study done by Saayman F, van Gelderen C J, Michelow P et al, this number was 4 women.[11] South Africa has one of the highest HIV prevalence rates in the world. HIV positive women are living relatively long lives because of improved access to ART and consequently at a higher risk of progression from CIN 2/3 to invasive cervical cancer.[28] It is therefore imperative that HIV positive women with LSIL be promptly managed to avoid the progression to cervical cancer. Starting ART earlier and continuing with the ART that was started during pregnancy will assist in improving patient's health and therefore reducing disease progression.

It is important that there is ongoing quality control in colposcopy. Using a uniform scoring system for all colposcopists may reduce the chances of over and under diagnosis, thereby ensuring that patients are properly diagnosed and appropriately treated.

4.4 Conclusion

We found that HIV infected women with LSIL on Pap smear had a high frequency of clinically significant cervical disease (n=386 [59.20%]). At CHBAH colposcopy clinic we use a threshold for treatment of CIN 2 at colposcopy. From this study it is evident that using a threshold for treatment of CIN3 in women with a Pap smear report of LSIL will lead to an overtreatment of 12/91 (13.91%) and using a threshold of CIN 2 for treatment at colposcopy would result in an overtreatment of 30.86%.

We would recommend that for women with LSIL on Pap smear and a colposcopy finding of CIN 1, treatment should be based on histology obtained by a punch biopsy. For women with CIN 2 on colposcopy treatment with immediate LLETZ should be individualized according to adequacy of the colposcopy, age of the patient and desire

for pregnancy. In cases where the colposcopy is a CIN 3, a LLETZ may be performed immediately in this setting.

The long time interval between the Pap smear and the colposcopy visit is a concern that needs to be addressed by the Department of Health. The fact that we found five women with invasive disease in this group is a concern, although we are uncertain whether these women were candidates for a biopsy at the time that they had the pap smear. The South African guidelines state that HIV positive women with any abnormality should be referred to colposcopy. Cytology services in the Public Health sector have also not been able to produce reports with results within four weeks. There is a long waiting time for colposcopy. (personal communication)

We found a significant association of low CD4 (<200 cells/mm^3) with a clinically significant lesion of CIN2 and more severe. The use of ART was associated with a lower frequency of a clinically significant lesion. This study therefore further emphasizes the need for ART at diagnosis of HIV infection.

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APPENDICES

Appendix A

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

22 November 2013

Dr Yasmin Adam
Head
Dept of Obstetrics & Gynaecology
CH Baragwanath Academic Hospital
University

Sent by email to: yasminadam@gmail.com

Dear Dr Adam

RE: Protocol M080603/M040609
Request for Ethics Approval to Continue the Coloscopy Database at Chris Hani Baragwanath Academic Hospital

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (medical) has reviewed and approved the following amendments on the abovementioned protocols as detailed in your letter dated 05 November 2013:

- Using an International Scoring System 'Modified Reid Score"
- Collecting Gynaecological Information
- Adding a section on Sexual History and Condom Use

Thank you for keeping us informed and updated.

Yours sincerely,

Anisa Keshav
Administrator
Human Research Ethics Committee (Medical)

Appendix B

R14/49 Dr Portia Manamela

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140220

NAME: (Principal Investigator)	Dr Portia Manamela
DEPARTMENT:	Obstetrics and Gynaecology Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	An Evaluation of Low Grade Squamous Intraepithelial Lesions in HIV-Infected Women at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	28/02/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Yasmin Adam
APPROVED BY:	Professor P Cleaton-Jones, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/06/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 14th January 2014

TITLE OF PROJECT:
Evaluation Of HIV Positive Women With Low Grade SIL Who Underwent Colposcopy At Chris Hani Baragwanath Academic Hospital

UNIVERSITY: Witwatersrand

Principal Investigator: Dr P.K Manamela

Department: Obstetrics and Gynaecology

Supervisor : Dr Y. Adam

Permission Head Department (where research conducted): **Yes**

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

Recommended
(On behalf of the MAC)
Date: 14/01/2014

Approved/Not Approved
Hospital Management
Date: 17/01/14

Appendix D

NATIONAL HEALTH LABORATORY SERVICE
UNIVERSITY OF THE WITWATERSRAND – JOHANNESBURG

SCHOOL OF PATHOLOGY
Division of Anatomical Pathology

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Division of Anatomical Pathology
Faculty of Health Sciences
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Professor MJ Hale MBChB (Rhodesia) FCPath (SA). LRCP, LRCS, LRCP&S (Edinburgh & Glasgow)
Professor & Head: Division of Anatomical Pathology,

Human Research Ethics Committee (Medical)
University of the Witwatersrand
Johannesburg
20000

May 20, 2014

Re: Consent for access to NHLS database

This letter serves to confirm that the Department of Anatomical Pathology at the University of the Witwatersrand and NHLS is happy to assist Dr P Manamela with her study entitled "An evaluation of low grade squamous intraepithelial lesions in HIV-infected women at Chris Hani Baragwanath Hospital"

Notwithstanding the requirement that research projects should comprise the researchers work only, it is recognized that publication of such work is encouraged. In the event that the information used comprises the diagnosis only then joint authorship from a member of staff in the Department of Anatomical Pathology would not be expected. However should additional information be extracted from the report for purposes of further interpretation such as morphological details and immunohistochemical profiles, it would be expected that this would be done in conjunction with a member of staff in the Department of Anatomical Pathology and that joint authorship would follow in resulting publications. Dr Manamela will be in contact with the Department of Anatomical Pathology in respect of this.

Assuring you of the Department of Anatomical Pathology's co-operation in this and future research projects.

With best wishes.

Yours sincerely,

Professor MJ Hale
Head: Department of Anatomical Pathology

20/5/2014
Date

Appendix E

Colposcopy and Cervical Screening Clinic
Department of Obstetrics & Gynaecology
Chris Hani Baragwanath Hospital
PO Bertsham 2013
Johannesburg

Tel: +2711 933-8153
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e-mail:vangel@pixie.co.za
yasminadam@gmail.com

To Whom It May Concern

06 February 2014

Re: Use of Colposcopy Database

This is to certify that Dr Portia Manamela may use the above database and extract data from it for the purpose of her work on "An evaluation of LSIL in HIV infected women at CHBAH".

CJ VAN GELDEREN MB ChB FRCOG FCOG(SA)
Professor Emeritus, Obstetrics and Gynaecology
Chris Hani Baragwanath Academic Hospital and
The University of the Witwatersrand.

Y ADAM MB BCh FCOG(SA)
Principal Specialist
Department of Obstetrics and Gynaecology,
Chris Hani Baragwanath Academic Hospital and
The University of the Witwatersrand.

Appendix F

Modified Reid colposcopic index

Feature	0 points	1 point	2 points
Colour of acetowhite (AW) area	Low-intensity acetowhitening; snow-white, shiny AW; indistinct AW; transparent AW; AW beyond the transformation zone	Grey-white AW with shiny surface	Dull, oyster-white; Grey
AW lesion margin and surface configuration	Feathered margins; angular, jagged lesions; flat lesions with indistinct margins; microcondylomatous or micropapillary surface	Regular lesions with smooth, straight outlines	Rolled, peeling edges; internal demarcations (a central area of highgrade change and peripheral area of lowgrade change)
Vessels	Fine/uniform vessels; poorly formed patterns of fine punctuations and/or fine mosaic; vessels beyond the margin of transformation zone; fine vessels within microcondylomatous or micropapillary lesions	Absent vessels	Well defined coarse punctation or coarse mosaic
Iodine staining	Positive iodine uptake giving mahogany brown colour; negative uptake of lesions scoring 3 points or less on above three categories	Partial iodine up-take by a lesion scoring 4 or more points on above three categories – variegated, speckled appearance	Negative iodine uptake by a lesion scoring 4 or more points on the above three criteria

Adapted from Coppleson *et al.*, 1993 b[50]

Scoring: A score of 0 to 2 points = Likely to be CIN 1; 3-4 points = Overlapping lesion: likely to be CIN 1 - 2; 5 to 8 points = Likely to be CIN 2 - 3 lesions.

Appendix G

DATA SHEET

The following variables will be extracted from the colposcopy database:

Age ☐

Parity ☐

Current Contraception:

Type of contraception	**(tick the relevant type)**
COC	
Condoms	
DMPA	
Nur-Isterate (Nurethisterone enantate)	
Progestogen only pill	
IUCD	
Sterilisation	
None	
Other	

CD4 count (within 6 months) ☐

ART use ☐ Y ☐ N

Colposcopy diagnosis as recorded in the database ☐

Type of biopsy Punch biopsy ☐

LLETZ ☐

Cone biopsy ☐

Hysterectomy (if performed) ☐

Histology results	Normal	☐
	HPV only	☐
	Cervicitis only	☐
	CIN 1	☐
	CIN 2	☐
	CIN 3	☐
	Microinvasion	☐
	Invasion	☐
	Other	☐