The impact of positive margins and crypt involvement in excisional procedures of the cervix on recurrence rates of premalignant diseases of the cervix.

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Plagiarism declaration

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List of abbreviations

AGC	atypical glandular cells
ASCCP	American Society of Colposcopy and Cervical Pathology
ASCUS	atypical squamous cells of undetermined significance
ASC-H	Atypical squamous cells , cannot rule out high-grade squamous intraepithelial lesion
CIN	Cervical intraepithelial neoplasia
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSIL	High grade squamous intraepithelial lesion
LEEP	Loop electrical excision procedure
LLETZ	Large loop excision of the transformation zone
LSIL	Low grade intraepithelial lesion
PPV	Positive predictive value
TOC	Test of cure
WHO	World Health Organisation
NHLS	National Health Laboratory Services

Contents:

1 Introduction

1.1 Cervical cancer

According to the world cancer report of 2014, amongst women cervical cancer has the third highest incidence rate, following breast and colon cancer (1).There were 528 000 new cases reported in 2012 with a mortality of 266 000 of which almost 90% occurred in low-to middle-income countries Worldwide the incidence of cervical cancer was 14 per 100 000. In Sub Saharan Africa the incidence of cervical cancer was the highest at 34.8 per 100 000 compared to breast cancer at 22.5 per 100 000.

According to the 2018 Globocan report not much has changed. Cervical cancer is the fourth most common cancer amongst women with breast cancer still leading (2). It is reported that there were 569 847 new cervical cancer diagnoses made in 2018 (2). There was a slight decrease in incidence now at 13.1 per 100 000 (2). The prevalence is higher in vulnerable populations such as those that are immunocompromised. This is a major contributing factor to rates in sub-Saharan Africa as there is a high prevalence of HIV (1). Incidence of cervical cancer in southern Africa remains the highest worldwide at 43.1 per 100 000 which is an increase from 2014. It is the second most commonly diagnosed cancer in South Africa at an incidence of 44.4 per 100 000 with 12 983 patients diagnosed in 2018 (3).

The Globocan report also found 311 365 deaths reported with a mortality rate of 6.1 per 100 000. Mortality rates are the fourth highest in Southern Africa at 20 per 100 000 (2). Cervical cancer is the leading cause of death from cancer in South Africa with 5 595 deaths reported in 2018 (3). Late presentation with advanced disease is thought to contribute to the higher mortality rates (1). In addition there may be a lack of surgical skill and treatment facilities such as radiation available.

1.2 Aetiology

Human papillomavirus (HPV) is a double stranded DNA virus found on the skin. There about 100 different strains of HPV, with some being oncogenic and others not. Of the oncogenic strains, subtypes 16&18 are responsible for up to 70% of cervical cancer cases. Other oncogenic subtypes are 31, 33, 39, 45, 51, 52, 56, 58, 59, and 68 (4). It is acquired through skin-to-skin contact. Its clinical expression may be in the form of genital warts for which HPV 6 and 11 are mainly responsible, however many may be asymptomatic. Most people will clear the infection within 2 years, but the persistence of HPV infection predisposes women to premalignant and malignant disease (5). Those at risk of progressive

disease are those with compromised immune systems such as HIV positive patients and transplant patient and women on immunosuppressant treatment. Other at risk groups are those infected with high risk strains of HPV, those with persistent HPV infection, and those with a high viral load. Significant risk factors for HPV infection include, young age at sexual debut (<15 years), increased sexual exposure high gravidity, tobacco smokers and those using oral contraceptives (6; 7).

1.3 Prevention

There are primary and secondary methods in preventing cervical cancer. Primary prevention refers to preventing the onset of disease by some form of intervention, implemented before there is any evidence of disease or injury aimed at reducing the incidence of the disease (8). HPV vaccines are available and it has been shown, initially in 2014, to offer protection for up to 9 years depending on the vaccine being used (9). More recently in 2020, data has been published which showed an efficacy of up to 14 years for the quadrivalent vaccine (10). Secondary prevention refers to cervical cytology to screen for pre-malignant disease. The aim is to detect the presence of the disease in an asymptomatic woman. Cytology is done by either conventional pap smears or liquid based cytology. In both types a smear of the cervix is taken and, in the former, placed on a slide or in a liquid based medium as in the latter.

Abnormalities in the cytology are reported according to the 2014 Bethesda classification as low grade intraepithelial (LSIL), high grade intraepithelial lesion (HSIL), atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells of undetermined significance where HSIL cannot be excluded (ASC-H), or atypical glandular cells (AGC)(11). Women with abnormal cytological smears such as HSIL, ASC-H, AGC, and recurrent LSIL require referral for colposcopy for review (12). Low grade lesions have a low potential of progression and mostly regress therefore requiring close follow up and not treatment (13). It has been well documented that the persistence of high grade lesions, which are CIN II and III lesions, untreated can progress to cervical cancer. The process itself can take 10 to 20 years. Women living with HIV are at higher risk of faster progression to malignancy (13). If the colposcopy is in keeping with a high-grade lesion an excisional procedure is preferred, although ablation therapy is also acceptable. Excisional therapy is either, loop electrosurgical excision procedure, which may either be LLETZ or LEEP. Other excisional procedures include cold knife cone biopsy or laser cone biopsy. Ablation therapy is not recommended in patients where the entire transformation zone is not visualised, the lesion covers more than 75% of the surface or the lesion extends into the canal (14). A LLETZ is a therapeutic procedure done on the cervix for treatment of

premalignant disease. A cone biopsy is a diagnostic procedure where a cone shaped resection of the transformation zone and the endocervical canal is done in order to make a diagnosis. Cone biopsy is done for various indications, namely, if the colposcopy is unsatisfactory, when the colposcopy directed biopsy show a lower grade abnormality than seen on cytology and at colposcopy, where the biopsy at colposcopy shows microinvasive carcinoma, cases where endocervical adenocarcinoma is suspected, and finally if colposcopy is not available (15). Excision procedures are categorized according to the American Society of Colposcopy and Cervical Pathology (ASCCP) with the type of excision being done dependant on the type of transformation zone as well as the findings at colposcopy. Loop electrosurgical excision procedures (LEEP) or LLETZ are generally used for type I and 2 procedures. Cone biopsies are of done in type 3 excisional procedures (14). Other treatment modalities include local destructive techniques, namely cryotherapy and laser vaporization. Cryotherapy destroys the tissue by freezing while laser vaporization uses CO2. These modalities are effective in destroying the lesion but due to the fact that there is no histological specimen, the nature of the lesion is unknown and one could potentially be inappropriately treating an early cervical cancer (16).

1.4 The pathology report

In the pathology report of an excisional procedure of the cervix (LLETZ or Cone biopsy), the pathologist will comment on the grade of cervical intraepithelial neoplasm (CIN) present and if there is microinvasion. The report will also comment on the presence of crypt involvement and whether the ecto- and endo-cervical margins are involved and with what grade of CIN. Current protocols are based on the findings of the report but at present the involvement of the margins and crypts are of unsure significance and follow-up is standard regardless of the above-mentioned findings. This is the focus of this study, to review the impact of these findings with regards to recurrence. Guidelines from ASCCP do state that for patients with positive margins who have no concerns of the effect of treatment on future pregnancy, a repeat excision is acceptable (14).

1.5 Follow-up and impact on services

At the Groote Schuur Hospital colposcopy clinic, we have a high population of HIV positive women. Between 2010 and 2015 of those that were referred with HSIL pap smears, the HIV positive rate was 59.7% (17) .This shows the burden of disease with regards to precancerous lesions and HIV associated disease. Our patients are followed up in the same manner regardless of whether there are risk factors

for recurrence clinically (HIV status, smokers etc) or risk factors histologically, as per the pathology report, as there is no clear evidence to support risk stratification currently. The standard follow-up of the patients at our institution is at six months for a repeat pap smear and colposcopy, then either six months or a year later depending on the pap smear results and findings at colposcopy. Thereafter annually for 3-5 years before returning to normal screening. If we can identify risk factors for recurrence, we could alter the follow-up protocols by keeping those identified as high risk for recurrence in the colposcopy clinic and downgrading the remaining patients to be followed up in the community. This would help decrease the burden of these clinics for follow-up, especially in resource limited areas.

We did a thorough literature review of these specific histological parameters and on the impact of recurrence and hence follow up.

2 Literature review:

There has been a lot of literature written on margin and crypt involvement of excisional procedures of the cervix, and how they affect the rate of recurrence. There currently is no consensus on the impact of the results of the status of the margins or crypts and what this means in the follow up of patients. At present follow up post excisional procedure isn't any different with or without positive margins or crypt involvement.

In 1997 Flannelly et al studied the factors that contributed to the treatment failures following a LLETZ/excisional procedure (18). The study included 1000 women who had LLETZ procedures. They had a 97.7% follow up rate for the first visit which dropped to 31.7% at the 4-year follow-up mark. Although there were only 46.6% cases that had information about the state of the margins available, those that had incomplete excision of the lesion had increased rate of recurrence of CIN, 14% versus 6% in those with complete excision. The period of maximal risk of recurrence was 18 months.

In 2000 Dobbs et al also found a significantly increased risk of recurrence in those with positive margins (RR 8.23) (19). They followed up 394 women who had LLETZ procedures for CIN lesions. The recurrence rate for those with positive margins was 13.3% versus 1.6% in those with complete excisions. The time to recurrence was also noted to be longer in those with complete excision of the lesion, 49 months versus 22 months.

Flannely et al in 2001 looked at whether follow up schedules could be modified according to risk of recurrence (20). They looked at the pattern of recurrence on LLETZ specimens with the objective of reducing surveillance intervals for those identified as low risk and possibly increasing surveillance for those at higher risk. The results showed that disease at the margins as well as the patient being aged more than 50 years at the time of the LLETZ were associated with risk of recurrence of intraepithelial lesions. The risk of recurrence with age over 50 was 25% versus 6.7%. Women who were younger than 50 years had 13% rate of recurrence if margins were positive, whereas women with negative margins had a 5% recurrence rate.

Manchanda el al in 2008 (21) looked at the effects of margin status on recurrence in women over the age of 50. They found that there was a strong association between margin involvement and recurrent CIN, thus recommend that women over the age of 50 with any margin involvement should be considered for repeat LLETZ and not just those with positive endocervical margins. British national guidelines at the time recommended repeat excision in women over 50 years with CIN 3 in the endocervix. (21)

As HPV DNA testing became available, it was also used as another aid in predicting persistent disease. In 2011 Lubrano et al looked at the detection of high risk HPV as well as age and margins in predicting recurrence. In this study 682 women with CIN II and III on LLETZ histology were followed up (22). The Hybrid Capture II was used to detect the high-risk HPV subtypes. There were positive margins in 20% of those who were followed up. Of those with positive margins, most (78%) of them were HSIL at the margins. The number of recurrent or persistent cases in the cohort was 13.9% of which most (77%) were low grade lesions. As with the other studies quoted above, they also found that those with positive margins had a slightly higher rate of recurrence than those without margin involvement (24.8 vs 11.1). The age of the participants and endo or ectocervical margin involvement were not found to be statistically significant factors contributing to rates of recurrence. Twenty-five percent of the cohort were positive for high-risk HPV at the 6 months follow up and of those about 30% had recurrence of CIN of any grade. This study concluded that only positive margins and post conization high risk HPV positive specimens were predictive factors for recurrence (odd ratios were 4.1 and 2.7, respectively). This paper correlates with a similar study published in 2008 by Prato et al (23). In their small cohort of 119 women there was recurrence rate of 23.5% in those with positive margins and 18.5% in those with post conization positive HPV positive testing and most recurred between 3 and 9 months. Participants with negative margins but HPV positive recurred between 16-35 months.

Not all papers however found positive margins alone to be a significant contributing factor to recurrence. In 2010 Treacy et al asked whether a more detailed evaluation of excision margins could

refine cytologic follow-up of women post LLETZ for high grade dysplasia (24). They followed up 2321 women with LLETZ specimens that had high grade lesions over a 12-year period looking for any grade of dysplasia post procedure. The detailed evaluation they looked at were positive margins, grade of dysplasia at the margins, negative margins, as well as the site of the margins (endo versus ectocervix). They had cytological and histological follow-up information available for 66% of the women. Just over half of the specimens had positive margins on histology. Of those who had positive margins 85% had high grade dysplasia. There was however very little difference in the rate of recurrent lesions in the two groups. Those who had high grade lesions at the margins had a 20.7% recurrence rate versus 18.9% in those who had low grade lesions at the margins, showing that there was no statistical significance in the grade of dysplasia at the margins. Participants with complete excision of lesions also had a 14.7% recurrence rate. Participants with low grade lesions at the margins and those that had negative margins were compared for recurrence and it was found not to be statistically significant. They however didn't compare the recurrence rates of those that had high grade lesions at the margins with those that had negative margins for statistical significance. What this study highlights are that those with positive margins regardless of the grade of dysplasia at the margins are at risk of recurrence. It also illustrates that those with negative margins are still at risk of recurrence, hence the state of the margins alone cannot be used to stratify those at risk of recurrence.

A 2015 study done by Palmer et al disputed whether the LLETZ excision margins predict residual disease in women who have undergone post-treatment cervical cytology and high-risk papillomavirus testing (25). This was a retrospective cohort study that looked at women who had been treated for CIN and had subsequent high-risk HPV test of cure (TOC) six months post LLETZ. In summary they found that although women with positive margins were likely to have high grade follow-up cytology, this finding was not statistically different from those with negative margins. There was also no correlation of positive margins and high-risk HPV status at TOC. The paper challenges whether margin status should in fact be reported on at all if there is no impact on recurrence rates.

Zhu et al also looked at factors that influenced the persistence or recurrence of HSIL. The age of the patient was a strong independent predictor of persistence or recurrence (26). Their study population included women who had loop electrosurgical excision procedures (LEEP) done and HSIL at the margins. The rate of persistence or recurrence was 11%. In this select group of patients they looked at the factors that influenced recurrence like depth and thickness of LEEP specimens, number of involved margins, and age amongst others. On analysis an age of 35 and above was the only independent risk factor that influenced recurrence or persistent rate with the relative risk being 14% compared to 3% in those aged less than 35 years old. The suggestion from this paper was that patients

over 35 should have more definitive management at follow up with repeat LEEP or hysterectomy as opposed to the younger age group who would be followed up with colposcopy and biopsy.

In 2016 Liss et al published a paper where they looked at various factors that were predictors of positive margins at the time of LEEP (27). They found that a history of smoking and high grade squamous intraepithelial lesion on the preceding Pap smear to be significantly associated with positive margins. With the aim of preventing positive margins which is high risk for recurrence the suggestion was that patients with these risk factors have their LEEP procedures done under anaesthesia in theatre allowing better access and the ease with which to do a deeper and a larger LEEP as an attempt to achieve clear margins. This recommendation may not always be feasible in all settings.

A South African study was conducted by Adam et al in 2008 (28). They looked at the predictors of persistent disease after treatment. The study was conducted at the 'see and treat' colposcopy clinic at Chris Hani Baragwanath Academic hospital. It included 1186 women who were sent to the colposcopy clinic of which 1016 had LLETZ's done. Other than the usual criteria for performing LLETZ, they also performed them on all HIV positive women who had LSIL or more on cytology or CIN 1 or more on colposcopy. Although these where not standard indications for LLETZ the authors noted that this was done because these patients were high risk for progression or recurrence due to their HIV status. The women were followed up at six monthly intervals but however there was a 41.3% loss to follow up rate. Of those that were followed up, 49% of them had persistently abnormal cells as defined by any abnormality seen on pap smear. At an odds ratio of 10.7, they found dysplasia at both ecto and endocervical margins increased the risk of persistent disease. On further analysis they also reported that disease at either margin doubled the risk of recurrence compared to those with no disease at the margin. HIV co-infection also increased the likelihood of recurrence especially in those patients with a CD4 count less than 500. Interestingly however, unlike other studies, they happened to find the increased age of the patient to be protective in the risk of recurrence. This result is particularly interesting as it conflicts with the study by Flannely et al that put women aged above 50 at higher risk. It also is different from the statistics of South Africa where the lifetime risk of invasive carcinoma of the cervix is 28.7 per 100 000 and increases to 136.4 per 100 000 in those women aged 65-69 years old (20)(28).

The columnar epithelium of the cervix forms invaginations that go into the canal forming crypts. Part of the histology report of an excisional procedure will report on the involvement of the crypts, which can also be involved with CIN. It is postulated that the involvement of the crypts themselves is indicative of an aggressive lesion, a higher chance of recurrence or it represents a lesion with a bigger volume (29).There are a few studies that look at the involvement of the crypts as an independent risk

factor for recurrence. In 2013 a study conducted by Kodampur et al confirmed this. They had a cohort of 1013 women in a 2-year period who had a LLETZ done, 30% of these women had negative margins and of those 37% had positive crypt involvement. They found in this group of women there was an increased risk of recurrence and a need for retreatment of 2.7-fold. This suggested that the involvement of the crypts was in independent risk factor for recurrence of disease (29). A more recent study published in 2015 by Papoutsis et al came to the same conclusion. In this study, 526 women who had LLETZ procedures done were followed up for two years. Those that had CIN 2-3 on pre-treatment punch biopsies and endocervical crypt involvement were at high risk for cytological recurrence. (30).

After a thorough review of the available literature it is evident that there is no clear consensus of the impact of margin and crypt involvement on the recurrence of pre-malignant disease. There is no robust data favouring one or the other. It is also evident that there are too few studies that were conducted in Southern Africa and other limited resource settings with our burden of disease and burden of HIV. In a clinical setting where disease burden overwhelms the system, a more tailored follow up limited to those at high risk for recurrence may be more appropriate. This study aims to review the impact of margins and crypt involvement in cervical excision specimens at Groote Schuur Hospital to assess if there is evidence to adjust our follow-up protocols.

3 Rationale for the study

Although most of the literature suggests that the state of the margins and crypts in excisional specimens of the cervix has an impact on the rate of recurrence of pre-malignant lesions, in clinical practice follow-up of these patients is unchanged. In a low resource setting such as our own, we are interested in finding out if we can tailor our follow-up protocols according to significant risk factors.

4 Aim

The aim of the study was to assess the impact of margin and crypt involvement on recurrence rates in patients who had excisional procedures of the cervix over a 5-year period in a limited resource setting.

5 Specific Objectives

5.1 Primary objectives:

1. Describe the demographic data of women who have attended and had either a LLETZ or cone biopsy at the colposcopy clinic at Groote Schuur hospital in 2010
2. To compare recurrence rates between women with no margin involvement versus women with either endo, ecto, or dual margin involvement over a 5-year period.
3. To compare recurrence rates between women with no crypt involvement with women with crypt involvement over a 5-year period.
4. To document the follow up defaulting rate at each year.

5.2 Secondary objectives

1. To document how many excisional specimens had cancer with/without CIN
2. To document how many excisional specimens had CIN II and III only
3. To document how many of the specimens with CIN II and III only had margin involvement
4. To document how many of the specimens with CIN II and III only had crypt involvement

6 Research design and methodology

6.1 Study design

This is a retrospective review of women attending the colposcopy clinic at Groote Schuur Hospital in 2010 and follow-up over a 5-year period

6.2 Study population

All women who had been identified with an abnormal cervical screening test and who had been referred to and attended the colposcopy clinic at Groote Schuur Hospital in 2010 where a cervical excisional procedure was offered and performed. These folder numbers were extracted from the colposcopy database.

Inclusion criteria:

- All women who had a LLETZ or a Cone biopsy in 2010
- All women who had histology and cytology results available on the national health lab system(NHLS) regardless of whether they followed up at GSH or their local clinics.

Exclusion criteria:

- All women with macroscopic cancer who had wedge biopsies of the cervix
- All initial excisional procedures that had microinvasion on the histology were included in the demographic data but excluded from the final analysis.
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6.3 Data Collection

The period of evaluation was between 2010 and 2015. Patients seen at the Groote Schuur Hospital colposcopy clinic that had cervical excisional procedures done in 2010 were followed up for the five years following their procedure. Folder numbers and demographic data was retrieved from the colposcopy database. A folder review was done on the available folders. The NHLS was used to review the histology reports as well as cytology results. The DISA lab system was accessed for the results.

Data was collected and entered in a data collection sheet that was created for the study. Different variables were categorized numerically. Demographics were documented. Pathology results were coded according to the highest level of abnormality seen at the margins. Recurrence was based on high grade cytology or histology. Histology took precedence over cytology when both were available. High grade cytology defined as HSIL or ASC-H and high-grade histology defined as CIN II or CIN III. There was no differentiation made between recurrent and persistent disease in the database, they are all coded as recurrence. Some patients had missed their follow up and re-presented when referred again with an abnormal pap smear making it difficult to differentiate between recurrent and persistent disease. The year that patients were lost-to-follow-up was defined as the year they missed their follow-up appointment.

The data collection sheets used, are attached as Appendix A & B.

6.4 Sample size

As this is a descriptive study the sample size was not powered.

6.5 Statistical Analysis

Data was analysed using Microsoft Excel and SPSS (Version 26). Descriptive data was presented as means and standard deviations for continuous variables, and as proportions for categorical data. Chi-square tests were used to compare recurrence rates and lost to follow-up rates between women with (a) endo-margin involvement and those without, (b) ecto-margin involvement and those without, (c) dual-margin involvement and those without, and (d) crypt- involvement and those without. Statistical significance was regarded as $p< 0.05$ as confidence intervals set at 95%.

7 Ethical considerations

The study was a retrospective descriptive study in keeping with the declaration of Helsinki (31). Information used was extracted from a colposcopy research database that has ongoing ethical approval with the Faculty of Health Sciences's Human Ethics Research Council (Ref no 344/2011).

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Appendix A

DATA COLLECTING SHEET

participant number	age	gravity	parity	cntraception 1=menopause 2=COC 3=injection 4=IUCD 5=pregnant 6=T/L 7=condom 8=implanon 9=none	HIV serology 1=negative 2= not on treatment 3=1st line 4=second line 5=defaulted 6=unknown	CD4 1=N/A 2=unknown 3=>500 4=<500	viral load 1= N/A 2=unknown	medical history 1=diabetes 2=TB 3=transplant 4= autoimmune disease 5= none 6=other	smoker 1=yes 2=no 3=unknown	procedure performed 1=LLETZ 2=Cone biopsy	HPV 1=yes 2=no 3=unknown	CIN 1=cin 1 2=cin 2 3=cin 3 4=no cin	other findings 1=cervicitis/ infection 2=microinvation 3=nil 4=other	endo-margin involvement 1=no involvement 2=CIN1 3=CIN2 4=CIN3 5=HPV 6=other	ecto-margin involvement 1=no involvement 2=CIN1 3=CIN2 4=CIN3 5=HPV 6=other	crypt involvement 1=yes 2=no 3=unknown
1	28		2	3	1	1	1	5	1	1	1	3	1	1	1	1
2	21		1	3	3	4	2	5	2	1	1	3	3	1	1	1
3	52		2	1	3	185	2	5	1	2	1	3	2	1	2	1
4	42		2	3	3	4	LDL	5	2	1	2	1	3	2	1	2
5	31		1	9	6	1	1	5	2	1	1	3	3	1	1	1
6	44		3	1	1	1	1	6	1	1	1	2	3	1	5	1
7	49		4	1	4	4	2	5	2	1	1	1	3	5	1	2
8	38		4	9	1	1	1	5	1	1	1	2	3	1	1	1
9	33		3	3	1	1	1	5	1	1	1	3	3	1	1	1
10	50		2	1	1	1	1	5	2	1	1	2	1	1	1	1
11	51		5	1	2	294	9400	1	2	2	1	2	3	1	2	1
12	38	3	3	3	2	428	2	6	2	1	1	1	1	2	2	1
13	41	2	2	9	2	2	2	5	1	2	1	3	3	4	4	1
14	33	2	2	3	6	1	1	5	1	1	1	3	1	4	1	1
15	45	2	2	3	1	1	1	5	1	1	1	3	3	1	1	1
16	34	3	3	3	1	1	1	5	2	1	1	2	3	1	1	1
17	45	4	4	3	6	1	1	5	2	1	1	2	3	1	1	1
18	37	5	5	3	1	1	1	5	2	1	1	3	3	1	1	1
19	36	3	3	9	1	1	1	5	2	1	1	1	1	1	1	2
20	48	3	2	9	1	1	1	5	1	1	1	3	3	4	1	1
21	38	3	3	3	2	4	2	5	2	1	1	3	3	3	2	1
22	41	5	2	3	2	2	2	6	2	1	1	3	3	1	1	1
23	32		3	3	2	2	2	5	2	1	1	3	3	4	1	1
24	35	3	3	3	3	3	2	6	2	2	1	3	2	6	6	1
25	37	2	2	6	2	164	2	6	2	1	1	2	3	1	1	1

APPENDIX B

DATA COLLECTION SHEET 2- Follow up

pap smear 2010 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9=no pap	pap smear 2011 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9=no pap	pap smear 2012 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9=no pap	pap smear 2013 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9=no-pap	pap smear 2014 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9= no pap	pap smear 2015 1=NILM 2=LSIL 3=HSIL 4=ASCUS 5=ASC-H 6=AGC 7=other 8=N/A 9= no pap	year of recurrence 1=2011 2=2012 3=2013 4=2014 5=2015 6=N/A 7= persistance 8=no recurrence	loss to follow-up 0=2010 1=2011 2=2012 3=2013 4=2014 5=2015 6=no loss to follow up	repeat procedure 1=LLETZ 2=cone 3=N/A 4=hyst
9	1	1	1	1	9	8	6	3
9	4	3	1	1	1	8	6	3
9	9	9	9	9	9	6	1	3
9	1	9	9	9	9	8	6	3
9	9	9	9	9	9	6	0	3
1	9	9	2	9	9	6	0	3
9	1	1	1	1	9	8	6	3
9	1	9	9	9	9	6	2	3
9	1	9	3	3	1	3	5	2
9	1	1	1	1	9	8	6	3
9	1	1	1	1	9	8	6	3
9	1	9	9	9	1	8	6	3
2	2	2	2	3	3	4	6	2
9	1	9	1	9	9	6	4	3
1	1	1	1	1	4	8	6	3
1	1	1	1	1	9	8	5	3
1	1	1	1	1	9	8	5	3
1	1	1	1	1	9	8	5	3
9	1	9	9	9	9	6	2	3
5	9	9	3	1	9	8	2	2
1	9	9	9	9	9	6	1	3
9	1	1	1	9	9	8	4	3
9	9	9	9	9	9	6	0	3
9	9	9	9	9	9	6	0	3
1	1	1	9	9	9	6	3	3

Publication ready article for submission to the South African Journal of Gynaecological Oncology (SAJGO)

THE IMPACT OF POSITIVE MARGINS AND CRYPT INVOLVEMENT IN EXCISIONAL PROCEDURES OF THE CERVIX ON RECURRENCE RATES OF PREMALIGNANT DISEASE OF THE CERVIX

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ABSTRACT

BACKGROUND

Recurrent disease after cervical excisional procedures has been linked to many factors. We aim to determine if positive margins and crypt involvement increased the rate of recurrence of premalignant disease in patients who had excisional procedures.

METHODS

In this retrospective review of the colposcopy database, patient records and pathology database, women who had cervical excisional procedures at the Groote Schuur Hospital colposcopy clinic in

2010 were followed up until 2015. Recurrence was based on high grade cytology or histology at follow up. Chi-square tests were used to compare recurrence rates.

RESULTS

Two hundred and seventy women were included in the final analysis. 130 women had CIN 3 and 94 had CIN 2 at the excisional procedure. Eighty five (31.5%) had endo-margin involvement, 46 (17%) had ecto-margin involvement, and 24 (8.9%) had dual margin involvement. Two hundred and thirteen (79.2%) had crypt involvement. Recurrence occurred in 30 (19.4%) of the 155 patients we had follow up data on. Of those that recurred, 19 ($P<0.001$) had positive endo-margin involvement, 10 ($P=0.007$) had ecto-margin involvement, 9 ($P<0.001$) had dual margin involvement, and 28 ($P=0.058$) had crypt involvement. 155 women (43%) were lost to follow-up

CONCLUSION

Positive margins at excisional procedure of the cervix have a statistically significant increased risk of recurrence of pre-malignant disease. There was a trend towards recurrence of disease in those who have crypt involvement. In limited resource setting follow up protocols can be adjusted so that women without margin involvement can be seen at longer intervals.

INTRODUCTION

According to the 2018 Globocan report cervical cancer is the fourth most common cancer amongst women with breast cancer still leading (2). It is reported that there were 569 847 new cervical cancer diagnoses made in 2018 (2). There was a slight decrease in incidence at 13.1 per 100 000, compared to 14 per 100 000 in 2014 (1) (2). The prevalence is higher in vulnerable populations such as those that are immunocompromised. This is a major contributing factor to rates in sub-Saharan Africa as there is a high prevalence of HIV (1). Incidence of cervical cancer in southern Africa remains the highest worldwide at 43.1 per 100 000 which is an increase from 2014 (1). It is the second most commonly diagnosed cancer in South Africa at an incidence of 44.4 per 100 000 with 12 983 patients diagnosed in 2018 (3).

The Globocan report also found 311 365 deaths reported with a mortality rate of 6.1 per 100 000. Mortality rates are the fourth highest in Southern Africa at 20 per 100 000 (2). Cervical cancer is the leading cause of death from cancer in South Africa with 5 595 deaths reported in 2018 (3). Late presentation with advanced disease is thought to contribute to the higher mortality rates (1). In addition there may be a lack of surgical skill and treatment facilities such as radiation available.

Human papilloma virus (HPV), the causative agent for cervical cancer, is a double stranded DNA virus acquired through skin-to-skin contact. Most people will clear the infection within 2 years, but the persistence of HPV infection predisposes women to premalignant disease (5). Oncogenic HPV strains, subtypes 16 & 18 are responsible for up to 70% of cervical cancer cases (4). Those with compromised immune systems such as transplant patients and HIV infection are at increased risk of persistent disease and smoking is also a contributory factor (5).

Cervical cytology is done to screen for premalignant lesions. Abnormalities in the cytology are reported, according to the 2014 Bethesda classification (11). Women with abnormal cytological smears require referral to colposcopy for review (12). Persistence of high grade lesions, which are cervical

intraepithelial neoplasm (CIN) II and III lesions, can progress to cervical cancer which can take 10 to 20 years (13). If colposcopy is in keeping with a high-grade lesion an excision procedure will be done (32). Excisional procedures are termed type 1, 2, and 3 depending on the type of transformation zone and the findings at colposcopy (14). Other treatment modalities include local destructive techniques, namely cryotherapy and laser vaporization. These modalities are effective in destroying the lesion but leave no specimen for histology therefore one could potentially be inappropriately treating an early cervical cancer (16).

The pathology report will comment on the grade of CIN, the presence of microinvasion, crypt involvement and whether the ecto- and/or endo-cervical margins are involved and the relevant grade involved.

Current literature is ambivalent about positive margins alone as a predictor for recurrent or residual disease(19-22). Our study aims to see the effects of positive margins and crypt involvement on recurrence of pre-malignant disease in patients attending the colposcopy clinic at Groote Schuur Hospital (GSH).

MATERIALS AND METHODS

In this retrospective review we followed up women who had an excisional procedure of the cervix at the colposcopy clinic at GSH in Cape Town, South Africa in 2010. Women who attended the clinic in 2010 were followed up over a five-year period. The clinic adopts the see and treat method. Patients with clinically high-grade lesions visualized on colposcopy were offered immediate treatment. All specimens were sent for histology. The standard follow-up is at four to six months for a repeat pap smear and colposcopy, then either six months or a year later depending on the pap smear results and findings at colposcopy. Thereafter annually for 3-5 years before returning to normal screening.

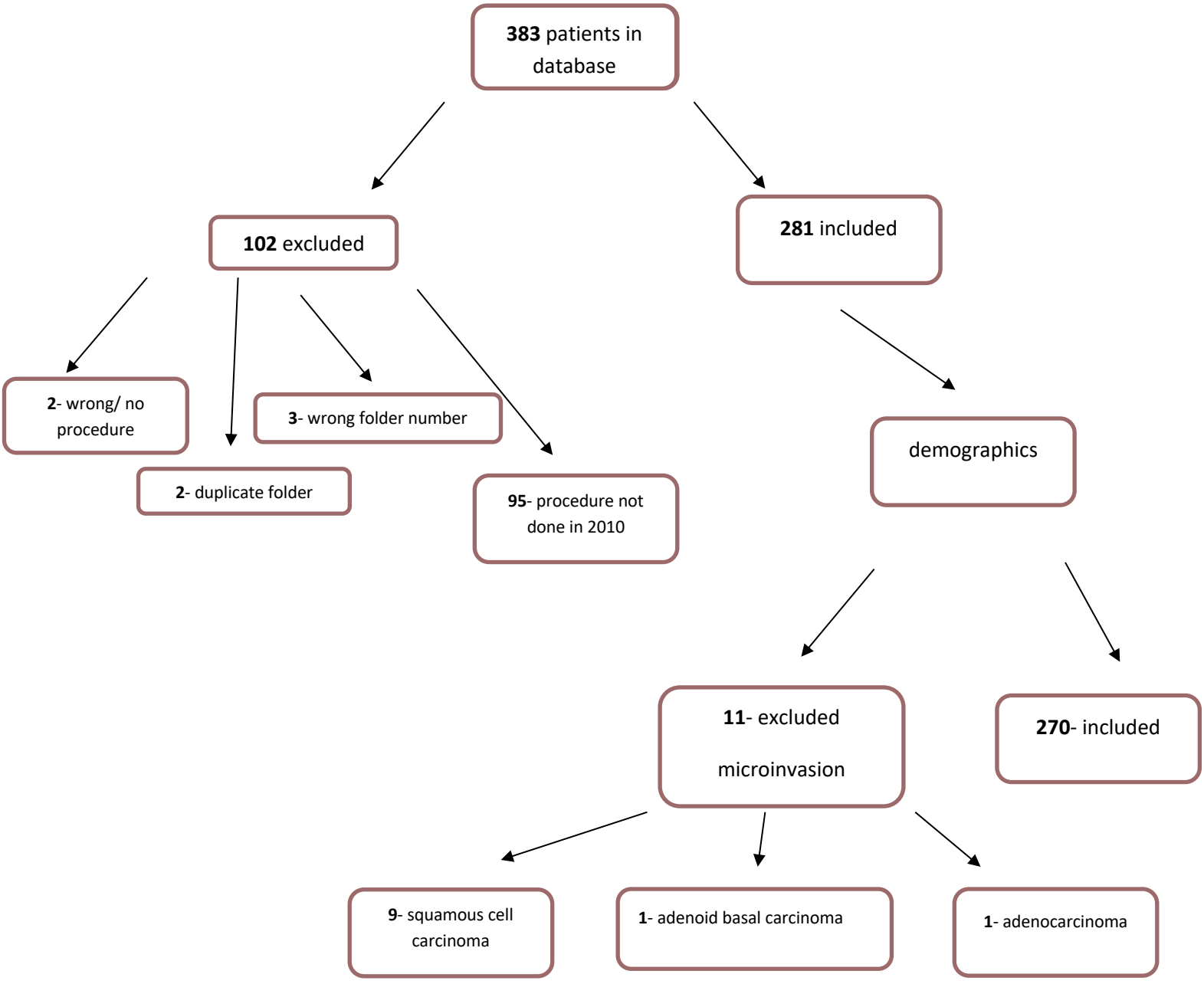
The colposcopy database of our clinic was used to extract the folder numbers of the patients who had excisional procedures in 2010. Additional information was obtained from patient folders and the national health lab system (NHLS). Demographic data was collected. Pathology results were coded according to the highest level of abnormality seen at the margins. Patients were coded as having a recurrence based on high grade cytology (HSIL or ASC-H) or histology (CIN II or CIN III) at follow up. Histology took precedence over cytology when both were available. There was no differentiation made between recurrent and persistent disease. It was all coded as recurrence. Some patients had missed their follow up and re-presented upon referred again with an abnormal pap smear making it difficult to differentiate between recurrence and persistent disease. Loss-to-follow-up was defined as the year they missed their follow-up appointment. The colposcopy database as well as the study was approved by the University of Cape Town's Faculty of Health Science's Human Research Ethics committee and in keeping with the Declaration of Helsinki (31) .

Data was analysed using Microsoft Excel and SPSS (Version 26). Descriptive data was presented as means and standard deviations for continuous variables, and as proportions for categorical data. Chi-square tests were used to compare recurrence rates and loss to follow-up rates between women with (a) endo-margin involvement and those without, (b) ecto-margin involvement and those without, (c) dual-margin involvement and those without, and (d) crypt- involvement and those without. Statistical significance was regarded as $p< 0.05$ as confidence intervals were set at 95%.

RESULTS

There were 383 patients who had excisional procedures of the cervix in 2010 recorded on the colposcopy database. Of these,102 of those were excluded. And the demographics for the remaining 281 patients were collected

Diagram 1: Flow chart



A. Demographic data (N=281)

Table 1: Demographics

	No.	Percentage
Age (mean)	37.6 (20-69)	
Parity	2.3 (0-9)	
Contraception (N =246) 1. Injection 2. COC 3. Implanon 4. IUCD 5. Tubal Ligation 6. Condom 7. None	 ▪ 120 ▪ 15 ▪ 1 ▪ 2 ▪ 16 ▪ 12 ▪ 80	 ▪ 48.8 ▪ 6.1 ▪ 0.4 ▪ 0.8 ▪ 6.5 ▪ 4.9 ▪ 32.5
HIV status (N=281) Unknown Negative Positive (N=176) ▪ No treatment ▪ First line ▪ Second line	 22 83 176 ▪ 65 ▪ 100 ▪ 11	 7.8 29.6 62.6 ▪ 36.9 ▪ 56.8 ▪ 6.3
CD4 count (N=176) Unknown >500 <500	 30 16 130	 17 9 74
Viral load (N=176) Unknown LDL >1000 <1000	 138 27 6 5	 78 15 4 3
Smoking (N=281) Yes No Unknown	 53 211 17	 19 75 6
Relevant medical history (N=281) None Diabetes TB Autoimmune disease Other	 214 9 16 1 41	 76 3 6 0.4 14.6

The mean age was 37.6 years (SD = 8.42, Range = 20 – 69 years), 74% were between the ages of 30-49 years. On average the women had a parity of 2.3 (SD = 1.5, Range = 0 - 9).

35 women (12.5%) were menopausal and thus not included in the contraception group. The majority of the group (120; 48.8%) used injectable contraception followed by a third (n=80) not using any contraceptive method.

Eighty three women (29.6%) were self-reported HIV negative and 22 (7.8%) did not know their HIV status. The remaining 176 (62,6%) were HIV positive, of which 65 women were not on treatment, 100 on first line and 11 on second line treatment. The majority (130; 74%) had a CD4 count below 500. Most (N=138; 78%) of the HIV positive patients had unknown viral loads. Twenty-seven patients (15%) had suppressed viral loads.

Fifty-three (19%) women smoked and 211 (75%) were non-smokers. The remaining 17 (6%) were unknown as it was not documented in the patient files or the database.

The majority (241;86%) of women had no medical history of note if HIV was excluded. Those with significant histories included 9 (3%) diabetics, 16 (6%) who had TB previously or on current treatment and one (0.4%) who had an autoimmune disease.

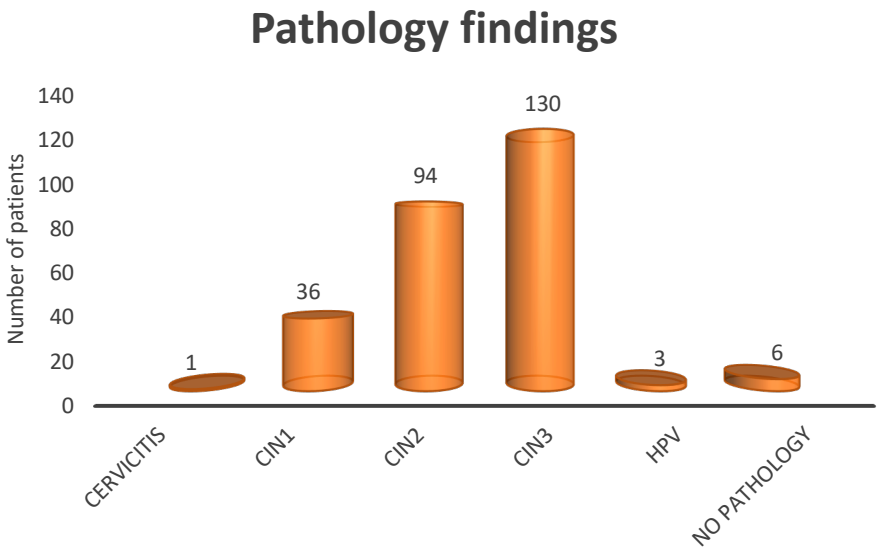
B. Outcomes data

There were 248 LLETZ procedures done and 33 Cone biopsies. Eleven patients had micro invasion diagnosed and were excluded from the rest of the analysis. Majority (N=224; 83%) of the patients had high risk lesions (CIN II & III) with only 6 patients (2%) having no pathology.

Table 2: Pathology findings

Pathology findings	N=270	Percentage
CIN3	130	48.2
CIN2	94	34.8
CIN1/HPV	39 (36+3)	14.4
Cervicitis	1	0.4
No Pathology	6	2.2

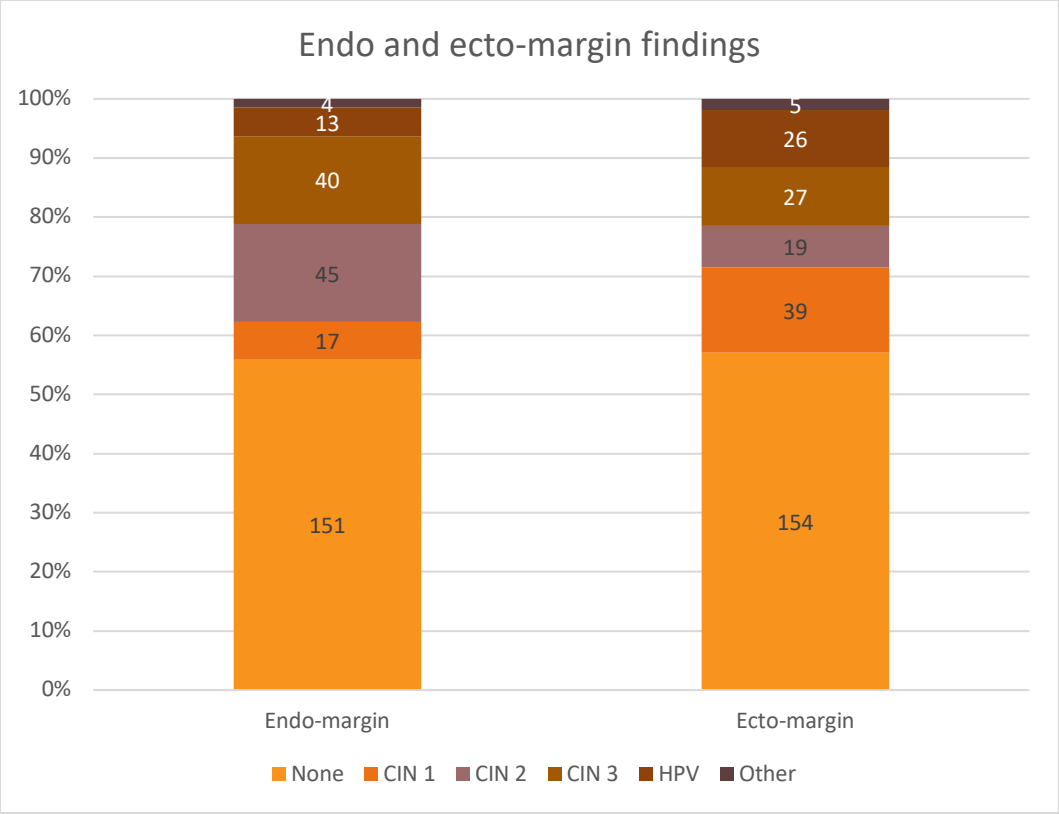
Figure 1: Pathology findings (N=270)



Margin involvement was defined as having CIN II or CIN III present at either the endocervix, ectocervix or both margins. Eighty-five of 270 (31.5%) had endo-margin involvement, 46 (17%) had ecto-margin involvement, and 24 (8.9%) as had dual involvement. There was no marginal involvement in 115 specimens.

Ectocervix mainly had CIN III involvement and in the endocervical margin there was mainly CIN II involvement. Of those that had dual involvement, the majority (54%) had CIN III at both margins.

Figure 2: Margin findings (N=270)



Crypts involvement was present in 213 (78.9%) patients. Fifty-six (20.7%) of them had no crypt involvement. One pathology report had no comment on the crypt involvement.

Recurrence of pre-malignant lesions was found in 30 (19.4%) of the 155 patients who were followed up for the duration of the 5 years. There were 115 women (43%) who were lost to follow-up. Women with endo, ecto, and dual-margin involvement were significantly more likely to have a recurrence compared to those without. There was a trend towards higher recurrence in those with crypt involvement compared to those without crypt-involvement (p= 058).

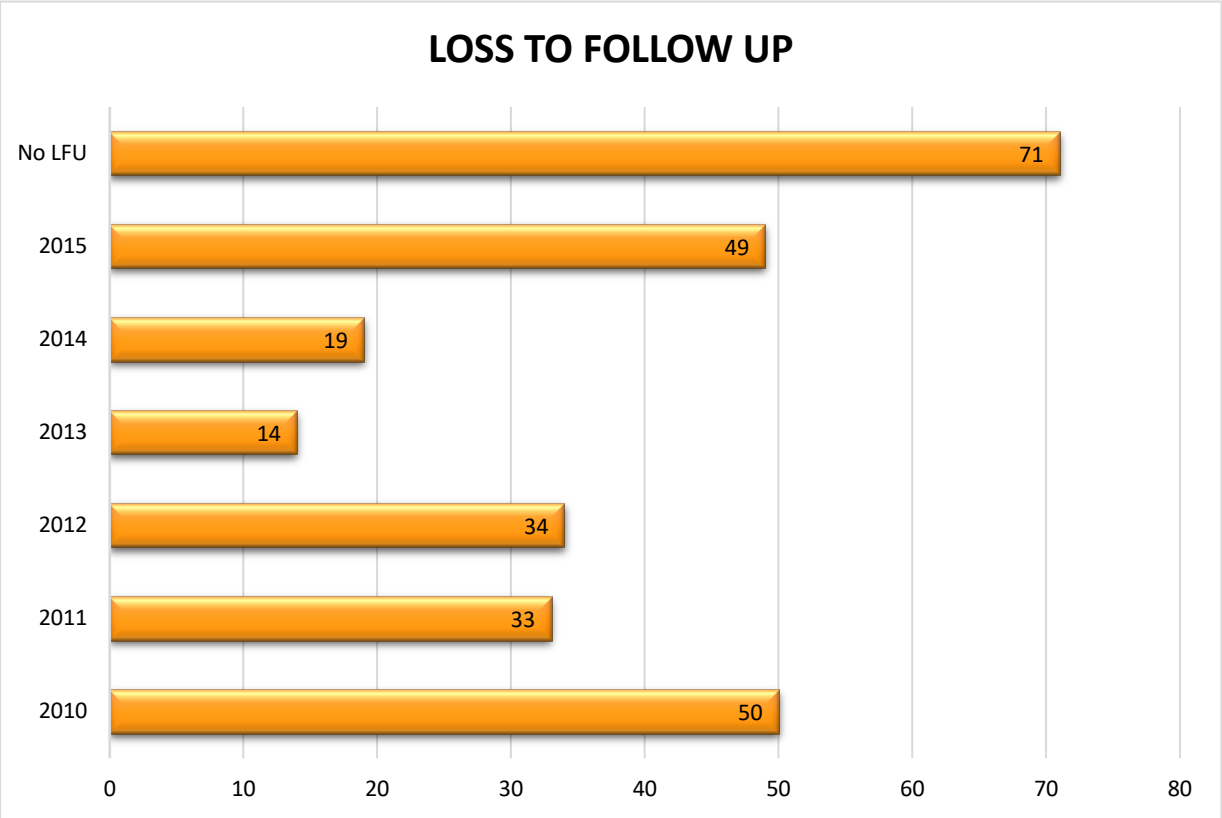
Table 3: Recurrence statistics (N=155)

	Recurrence/Persistence		Statistics		
	Yes n = 30	No n = 125	X^2	p	V
Endo-margin involvement			17.31	<.001*	0.33
Yes	19 (38.8%)	30 (61.2%)			
No	11 (10.4%)	95 (89.6%)			
Ecto-margin involvement			7.31	.007*	0.22
Yes	10 (38.5%)	16 (61.5%)			
No	20 (15.5%)	109 (84.5%)			
Dual-margin involvement			13.80	<.001*	0.30
Yes	9 (52.9%)	8 (47.1%)			
No	21 (15.2%)	117 (84.8%)			
Crypt-involvement			3.61	.058	0.15
Yes	28 (22.4%)	97 (77.6%)			
No	2 (6.9%)	27 (93.1%)			

Note. Data presented with proportions in parentheses. V = Cramer's V (effect size for a chi-square test).

Patients were followed up for 5 years and were lost to follow up at similar rates over the years. There were only 26% followed up consistently for the whole 5 years.

Figure 3: Loss to follow up



Illustrated above are the number of patients that did not attend their follow-up appointments to the clinic.

Ten women had repeat LLETZ procedures, 9 had a repeat Cone biopsy, 8 had a hysterectomy. The majority (n = 243) of patients had either no repeat procedure or were lost to follow-up

DISCUSSION

There were 281 women who had excisional procedures of the cervix in our clinic in 2010. Of these 11 (4%) had evidence of micro-invasion .In the remaining 270 specimens, 46 women (17%) were overtreated with no evidence of CIN2 or 3 and 224 (83%) had premalignant disease.

It was found that 31.5% of the specimens with premalignant disease had endo-margin involvement, 17% had ecto-margin involvement, and 8.9% had dual margin involvement. Crypt involvement was found in 78.9 % of specimens. Recurrence occurred in 19.4% of the patients that followed up. 43% were lost to follow-up.

The mean age of our population was 37.8 with a range of 20-60 years old. Almost half (48%) of this group was using injectable form of contraception. Implants were only introduced in the state sector in 2014 (33). In the 2016 South African demographic and health survey (34)

injectable contraception was the second most commonly known to women following the male contraception. In this survey of sexually active women between the ages of 15 and 49, injectables were the most commonly used form if contraception at 24.8% amongst the 60% that were using contraception.

Overall, there were 40% of women who were not using any form of contraception with is similar to our sample sitting at a 32% rate of non-users. Our study, in keeping, emphasizes more priority and education required regarding contraception to younger women in South Africa.

By the end of 2018, internationally there were 37.9 million people living with HIV. Of those, 20.6 million were living in Eastern and Southern Africa (35). According to STATS SA the prevalence of HIV in South Africa around the same time was 7.52 million (36). Our colposcopy clinic saw a disproportionately large number of HIV positive patients sitting at 62.6%. Colposcopy clinic is likely to see disproportionately higher numbers of HIV infected patients as it is shown that HIV positive patients are more likely to have higher numbers of abnormal pap smears even though that might not translate to a higher prevalence of invasive cancer of the cervix (37).

Adams et al had a self-reported HIV positive rate of 22.4% (266 positive out of 1186) in their study done at Chris Hani Baragwanath Academic hospital (CHBAH) colposcopy clinic (14). Our high HIV prevalence is consistent with a study done by Batra et al at the Groote Schuur hospital from 2007-2009 where the prevalence of HIV positive women in the colposcopy clinic was around 50% (1022/2031) (38). The Western Cape department of health at the time instructed that all HIV positive women should undergo annual pap smears from the time of diagnosis, which was different from the national screening guidelines that offered all women 3 free pap smears done at ten yearly intervals from the age of 30 (38)(39). This could explain the high prevalence of HIV in our study as more HIV positive women were being screened more frequently at that time. The current guidelines however state that HIV positive patients should have cervical cytology from time of diagnosis and repeated every 3 years if normal (40).

In 2010 there were new HIV guidelines that were released-(41) viral load testing was to be done 6 months after initiation, six months later and thereafter yearly viral load testing was to be done. As the guidelines were updated in 2010 when our study was initiated, there were very few patients with viral load results done and available. The burden on HIV in south Africa meant that the number of patients that needed testing was more than the national health laboratory system could accommodate.

It has been well documented that cigarette smoking as well as passive smoke have been associated with increased prevalence of cervical cancer (42) and thus considered a risk factor

for cervical cancer. We looked at the prevalence of cigarette smoking in our study population. At 19% the prevalence was lower that what was expected for the province of the Western Cape. Our low prevalence could have been as a result of under-reporting from the patients as in the 2012 National health and nutrition examination survey the prevalence amongst women in the western cape was 26.9%.

Patients seen in the GSH colposcopy clinic are managed by the see and treat protocol. Patients referred with an abnormal pap smear and a colposcopy that is in keeping with a high-grade lesion are offered immediate treatment with an excisional procedure. The positive predictive value of the colposcopy should be at least 65% for CIN 2 and above. Overtreating is the biggest concern with the see and treat method (43).

The see and treat strategy was introduced in 1990 with low voltage diathermy loop given in one visit. Overtreating has been quoted to be between 13-72% for normal smears and LSIL, and said to be reduced when see and treat is limited to HSIL and high grade colposcopy findings only (44). Overtreatment can be justified when see and treat is done in a low resource setting with a high prevalence of cancer and a low treatment complication (44). The alternative is to do a colposcopy guided biopsy followed by excisional procedure if necessary. Colposcopy guidelines (43)(45) recommend that overtreatment is acceptable in 10-15% of cases. In our study it was 17% which is slightly higher than recommended. Comparable studies of see and treat patients done at CHBAH and GSH had an overtreatment rates of 9.7% and 16.7% respectively (46).

The National Health System(NHS) in England recommend that margins should be clear in 80% of specimens(43). Margin involvement quoted in the literature were 37% (20), 20.1%(22), and 53.4% (24). Adams et al (28) in their Bara study found a positive margin rate of 50% in the patients they had follow up results for in their study. By a comparison of these, it shows our study had a relatively higher number of patients with positive margins at 57.4%. The Spanish association of cervical pathology and colposcopy (AEPCC) recommends that positive margins at conization shouldn't exceed 20% and positive endocervical margins shouldn't exceed 15% (45). Recurrence of pre-malignant lesions was found in those with positive margins, whether ecto ($P=0.007$), endo($P<0.001$), or dual margin($P<0.001$) involvement.

In addition the United Kingdom NHS guidelines of 2016, they suggest that the size of the specimen taken needs to be between 7mm -25mm depending on the type of transformation zone visible. Ideally this should be less for women of reproductive age to avoid complications such as cervical stenosis and cervical incompetence (32). We would have to look at the size of specimens that we excise to review if we are taking too small a specimen or if our population of patients have large lesions. Although residual disease at the margins is associated with increase recurrence of disease, it is currently not an indication for a repeat excisional procedure but instead warrants closer surveillance. The exception is for women over the age of 50 years old as they are at higher risk of persistent or recurrent disease (43).

Our study as well as the study in Bara has found a far higher rate of margin involvement than suggested by international literature. We are unable to comment on the size of our excisional procedures as this was not looked at in this study. Another consideration is whether the high use of injectable contraception in the South African population has an impact, as this causes atrophy of the cervix with potentially smaller cervices and hence smaller specimens.

Residual disease in the cervical crypts have also been shown to be an independent risk factor for recurrence of pre-malignant disease of the cervix (47). Documented numbers of crypt involvement quoted in the literature range between 15-58% (47) which would show that our numbers are much higher in comparison at 78.9%. Crypt involvement reflects the extent of the disease. This may be explained by the high prevalence of HIV positive patients in our clinic population. HIV positive patients with cervical dysplasia are prone to more severe disease. They have a higher incidence of disease, more rapid progression and higher recurrence rates (48). In addition it may also reflect the long periods for screening in our HIV negative population who are only screened every 10 years. We found a trend in the patients with crypt involvement having recurrent disease in comparison to those without crypt involvement (P=0.058)but this trend was not statistically significant.

In our clinic the standard of care was to follow-up all women 4-6 months after the initial visit and thereafter yearly for a total of 5 years regardless of margin status. At the five year mark there was a 26% follow-up rate. In the study conducted at CHBAH by Adams et al, they had a follow up rate of only 57% at the 4-6 months. HPV DNA testing has allowed for the shortening of the follow-up period when used in conjunction with cytology 6 months after excision. Those screening negative continue with routine screening (43). At the time of this study,-high-risk HPV testing was not yet available in the public sector in South Africa.

Research has shown that treatment of premalignant lesions of the cervix reduces the rate of cervical cancer by 90-94% (48). Studies, including our own, have also demonstrated that positive margins increases the chances of recurrence of disease. Follow up of these patients is difficult and requires repeated visits to the clinic and possible repeat procedures. Finding a way to stratify patients into high risk for recurrence using the information collected in the clinic would be helpful in streamlining those at higher risk. As demonstrated in the research patients with positive margins are at risk and should therefore be followed up closely with a high index of suspicion.

This risk stratification would assist in decreasing the large numbers of patients seen at the colposcopy clinic and may decrease the loss to follow up rate.

LIMITATIONS

The retrospective nature of the research as well as the very high loss-to-follow-up rate over the five years impacted on the ability to get the recurrence rates that are a true reflection of the population.

CONCLUSION

Women who have had excisional procedures of the cervix with margin involvement have a significantly higher risk of recurrence of premalignant disease. This study suggests that women attending a colposcopy clinic in limited resource settings could be triaged post procedure and those with no margin involvement could have surveillance at longer periods whereas those with positive margins should maintain intense surveillance. Currently crypt involvement should not be recommended to adjust triage of women post procedure.

SUGGESTED FURTHER RESEARCH

1. HPV DNA testing in women without margin and crypt involvement versus those with involvement

2. Comparing size of the histological specimens with and without margin involvement

3. Specifically looking at the size of the specimens of women using injectable contraception versus those using other methods

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DISCLOSURE

The author reports no conflict of interest in this work.

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20 August 2019

HREC REF NO: 552/2019

Dr Tracey Adams
Obstetrics and Gynaecology
Room 45, H Floor, Old Main Building
Groote Schuur Hospital
Observatory, 7925

Dear Dr Tracey Adams

PROJECT TITLE: THE IMPACT OF POSITIVE MARGINS AND CRYPT INVOLVEMENT IN EXCISIONAL PROCEDURES OF THE CERVIX ON RECURRENCE RATES OF PREMALIGNANT DISEASAS OF CERVIX (MASTER DEGREE - DR H ADDAE)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 August 2020.

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Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, *Dr Haleema Addae* will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

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