Prevalence of high-grade squamous intraepithelial lesion within two years of large loop excision of the transformation zone at a tertiary hospital colposcopy clinic in Cape Town

by
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DECLARATION BY CANDIDATE

I, Victor Olujobi, hereby declare that the work on which this dissertation is based is my original work and that neither the whole work nor any part of it has been or is to be submitted for another degree in this or any other university.

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I have supervised the research which Victor Olujobi has undertaken and presented in this dissertation.

I am satisfied that this dissertation should be submitted in partial fulfilment of his requirements for the CMSA certificate examination in Obstetrics and Gynaecology since it is his original work.

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LIST OF ABBREVIATIONS

ASC-H	Atypical squamous cells, cannot exclude HSIL
ASC-US	Atypical squamous cells of undetermined significance
CI	Confidence interval
CIN	Cervical intra-epithelial neoplasia
CKC	cold knife conisation
DNA	Deoxyribonucleic acid
EFC	European Federation of Colposcopy
HIC	High-income countries
HIV	Human immunodeficiency syndrome
HPV	Human papillomavirus
HREC	Human research ethics committee
Hr-HPV	High-risk human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
LBC	Liquid based cytology
LEEP	Loop electrosurgical excision procedure (also LLETZ)
LLETZ	Large loop excision of the transformation zone
LSIL	Low-grade squamous intraepithelial lesion
LMIC	Low-middle-income countries
NHLS	National Health Laboratory Service
NILM	Negative for intraepithelial lesion or malignancy
OR	Odds ratio
P	Probability
Pap	Papanicolaou
RCT	Randomised Controlled Trial

SIL	Squamous intraepithelial lesion
TBH	Tygerberg Hospital
TZ	Transformation zone
WHO	World Health Organisation

ABSTRACT

Background

Large loop excision of the transformation zone (LLETZ) has become widely accepted as the technique of choice for the treatment of cervical intraepithelial neoplasia. Despite its high efficacy in preventing cervical cancer, variable rates of post-LLETZ residual/recurrent high-grade squamous intraepithelial lesion (HSIL), also known as treatment failure is reported globally. This study was conducted to determine the prevalence of treatment failure within two years of LLETZ treatment for HSIL at Tygerberg hospital.

Aim

To determine the proportion of women treated for high-grade cervical intraepithelial neoplasia who developed cytological HSIL within two years of LLETZ.

Materials and Methods

In this retrospective cohort study, the electronic medical records of the first 139 consecutive women who underwent LLETZ treatment in 2016 and had a final diagnosis of HSIL as well as at least one follow-up cytology within two years of LLETZ were reviewed.

Setting: Tygerberg Hospital colposcopy clinic, one of two tertiary referral hospitals in Cape Town, South Africa.

Results: The rate of recurrent HSIL at the first follow-up cytology after LLETZ was 17.3% (95% confidence interval [CI] 11.4 to 24.6). 68.3% of study participants had a normal cervical cytology at the first follow-up visit after LLETZ. LLETZ margins were positive for CIN2/CIN3 in 58.3% (81/139) of biopsies, with involvement of the endocervical margin in almost half of these cases. Age 40 and above was significantly associated with post-LLETZ HSIL recurrence (odds ratio [OR] = 2.7, 95% CI 1.03 to 7.07, p = 0.04). There was nonsignificant increase in the odds of post-LLETZ residual/recurrent HSIL among women living with HIV, (OR =2.0, 95% CI 0.68 to 6.10, p = 0.2). Also, a nonsignificant increase in treatment failure was found when cases

with positive margins were compared with those with clear margins (OR = 2.3, 95% CI 0.69 to 7.53, p = 0.18), as well as when uncertain margin status was compared with clear margin status (OR = 1.27, 95% CI 0.20 to 8.10, p = 0.80). 16.7% (4/24) of treatment failure occurred among women with clear LLETZ margins, and no treatment failure was detected at follow-up when the ectocervical margin was the only involved margin. The rate of loss to follow-up for a second cytology within two years of LLETZ was 74.8%.

Conclusions: Even though LETTZ is an effective modality for the treatment of CIN, one in six treated women develop treatment failure within two years of LLETZ. Women aged 40 and above at the time of LLETZ are at a higher risk of developing treatment failure. There is a high rate of loss to follow-up for a second cervical cytology in the study population.

Key words: High grade squamous intra-epithelial lesion, large loop excision of the transformation zone, LEEP, residual, recurrent, cervical intraepithelial neoplasia, margin status, complete excision, recurrent, treatment failure, post-treatment disease.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Background (Epidemiology, cervical screening, treatment, treatment failure)

1.1 Introduction:

1.1.1 Epidemiology and aetiology

Globally, cervical cancer is the fourth commonest cancer in incidence and mortality amongst women of all ages. It is sadly the leading cause of cancer related mortality among women in many low-middle income countries (LMICs) and low resourced communities.[1,2] It is well established that persistent infection of the cervical transformation zone (TZ) by oncogenic strains of Human Papilloma virus (HPV) is the essential underlying cause of cervical cancer and cervical cancer precursor, also known as cervical intraepithelial neoplasia (CIN).[3,4]

CIN is graded from 1-3 depending on the degree of cellular abnormality and extent of epithelial involvement.[4] The natural history of CIN involves regression, persistence, or progression to higher grades of CIN or invasive cancer, and this natural history is dependent on the persistence or clearance of HPV infection.[5] 90% HPV infection is spontaneously cleared within two years of acquisition, especially in young women with an intact immune system.[6] While the majority of CIN1 lesions regress spontaneously, the regression rate for untreated CIN2 is about 50%, with higher regression rates among women younger than 30 years.[5] CIN2/CIN3 can arise de novo without progression from CIN1.[4,7] CIN3 lesions have the least chance of spontaneous regression and is recognised as the true pre-invasive precursor of cervical cancer.[5] A third of women with untreated CIN3 will develop invasive cervical cancer if the lesions are left untreated.[8] Fortunately, in most cases, precancerous lesions of the cervix may exist for years before progression to invasive cancer.[9,10] This latency of CIN offers a window of opportunity for screening and treatment. Women living with HIV are at a six-fold higher risk of developing cervical cancer than HIV negative women.[11]

1.1.2 Cervical screening

Organised cervical cancer screening and treatment programmes have resulted in significant reduction in the incidence and mortality from cervical cancer in high-income countries (HIC).[10] South Africa launched a national cervical cancer screening programme in 2002. Asymptomatic women from age 30 are offered three free pap smears at a 10 yearly interval.[12] There is ongoing plans for the incorporation of HPV testing into the national cervical cancer prevention programme in the not-too-distant future.[12]

1.1.3 Treatment

Therapeutic options for the eradication of CIN can either be ablative (cryotherapy, electro-coagulation, diathermy and laser vaporisation) or excisional (laser conisation, LLETZ, cold knife conisation [CKC], hysterectomy).[13,14] LLETZ, also known as Loop electrosurgical excision procedure (LEEP), was first developed by Carter, and popularised by Prendiville et al. in the late 1980s.[15,16] It is an effective, minimally invasive, rapid, relatively inexpensive procedure, which is mostly performed in an out-patient setting under local anaesthesia.[13,17] It is at least as effective as ablative procedures, with the added advantage of availability of tissue for histopathologic analysis.[8,18] It has become the widely preferred technique for the treatment of cervical precancer,[18,19] with an estimated average efficacy of 90-95% in eradicating HSIL.[20,21] Women who have received treatment for CIN are at five times higher risk of developing invasive cervical cancer than women in the general population and this risk remains for up to 20 years after treatment.[21,22]

There are two recognised policy options in the management of HSIL. A traditional two-staged strategy where cervical biopsy is obtained after a positive screening test, and LLETZ performed following histological confirmation of HSIL. The second strategy is a single-step approach also referred to as “look-and-LLETZ” approach, in which screening and treatment are performed at a single visit.[10,13,23] The later approach has been associated with overtreatment, with some studies showing up to 5-20% absence of CIN in the final histology of LLETZ biopsies.[13,24] Despite this limitation, the “look-and-LLETZ” strategy is still preferred and justifiable in countries and communities with high rates of loss to follow-up.[24,25] The main argument in favour of the two-staged treatment approach is that it should minimise the rate of overtreatment.[13] However

Denny et al. found that the two-staged approach was associated with 25% false negative biopsies, did not show a reduction in the occurrence of over-treatment and was associated with significant loss to follow-up.[26]

1.1.4 Treatment failure, follow-up strategies

The majority of the residual or recurrent HSIL are detected within two years of treatment. Thus studies assessing treatment failure are mostly confined to the first two years after treatment.[8,27–29] Owing to the difficulty in differentiating residual from recurrent HSIL, most authors simply classify all occurrences of HSIL or CIN2/CIN3 within two years of index treatment as treatment failure.[8,19,29] The factors associated with treatment failure include incomplete CIN excision, positive HIV status, size, location and severity of lesion, older age, and more importantly, the presence of Hr-HPV after LLETZ.[13,30] At the moment, there is no standardised strategy for the follow-up of women who have received treatment for CIN.[31,32] Hr-HPV DNA testing six months after treatment is currently the most sensitive test of cure. Other methods of determining the efficacy of CIN treatment include cytology, colposcopy, and colposcopically directed cervical biopsies.[31–33]

1.1.5 Justification for this study

Currently in South Africa, HPV testing is not routinely available in the public health sector and LLETZ is the commonest modality in place for the treatment of women with cervical pre-invasive disease. There is a paucity of data on treatment efficacy after LLETZ in South Africa and many Sub-Saharan countries. Most of the available data on the efficacy of LLETZ in treating CIN and preventing cervical cancer is from HICs. This retrospective review of clinical records was therefore conducted to determine the efficacy of LLETZ in the treatment of HSIL as well as the prevalence of treatment failure after LLETZ.

1.1.6 Research question

What is the prevalence of high-grade squamous intraepithelial lesion within two years of large loop excision of the transformation zone in Cape Town?

1.2 Study Objectives

1.2.1 Primary Objectives

To determine the prevalence of high-grade squamous intraepithelial lesion within two years of large loop excision of the transformation zone at Tygerberg Hospital.

1.2.2 Secondary Objectives

1) To determine the efficacy of LLETZ in the treatment of HSIL
2) To determine adequacy of treatment by assessing the completeness of loop excision of the transformation zone.
3) To determine if the patient's age and HIV status were associated with HSIL recurrence after LLETZ.
4) To ascertain the rate of loss to follow-up after LLETZ.

CHAPTER 2
MATERIALS AND METHODS

This is a retrospective analysis of data from the colposcopy clinic database in 2016 at Tygerberg Hospital, Cape Town. The drainage area comprises roughly half of Cape Town Metro (East), with most of the women living in low-socioeconomic settings. A target of 139 patients was established based on sample size calculation. Colposcopy and LLETZ were performed in the colposcopy clinic under local anaesthesia. The procedures were mostly performed by trained obstetrics and gynaecology registrars and senior medical officers under the supervision of gynaecological oncologists. Follow-up assessment of treatment efficacy was by cervical cytology.

Extracted data included, Age, HIV status, Indication for LLETZ, histological diagnosis at LLETZ, margin status on LLETZ specimen, number of post treatment cytology within two years of LLETZ and the cytological diagnosis at follow-up. HIV status was ascertained by self-reporting and from referral information. Positive HIV status was verified from NHLS laboratory database. Margin status refers to the presence or absence of CIN2/CIN3 at either the endocervical or ectocervical excision margin.

2.1 Study outcomes measures

- Treatment failure rate
- Cure rate
- Rate of follow-up default

2.2 Participant's entry into the study

2.2.1 Pre-recruitment evaluations

The database was screened to exclude women who did not meet the inclusion criteria.

2.2.2 Inclusion criteria

Cytological HSIL, persistent low grade squamous intra-epithelial lesions (LSIL), final diagnosis of CIN2/CIN3 at LLETZ, at least one follow-up cytology within two years of LLETZ.

2.2.3 Exclusion criteria

- Nonavailability of cytology result before and after LLETZ
- LLETZ performed outside the study period
- Pregnancy
- Final LLETZ diagnosis showing CIN1 or absence of CIN
- Invasive disease on LLETZ histology

2.3 Statistics and data analysis

2.3.1 Sample size

The sample size was calculated using a 5% margin of error, 95% confidence level and expected proportion of 10% (as quoted in systematic review by Kalliala).[21] These variables were entered into a sample size calculator and a sample size of 139 was derived. The first 139 consecutive women who underwent LLETZ treatment at Tygerberg hospital colposcopy clinic in 2016, who met all inclusion criteria were enrolled.

2.3.2 Data analysis

Data analysis was done with Stats 17 (College Station, Texas 77845 USA) statistical package. Categorical variables were summarised using count (percent), and age using mean (standard deviation {SD}). Association between categorical variable were assessed using the X^2 test or the Fisher's exact test in a univariate analysis. Multivariate analysis to determine independent risk factors for treatment failure was performed using logistic regression. Odds ratio was reported as measures of association, with the corresponding 95% confidence interval. Statistical significance was set at $p<0.05$.

2.3.3 Regulatory issues

Ethics approval for the study was obtained from the Stellenbosch University Health Research Ethics Committee (HREC) with ethics reference number S20/08/215. Study approval was also obtained from Tygerberg hospital management. The study was conducted in accordance with the Declaration of Helsinki and the National Department of Health guidelines for Good Clinical Practice.

2.3.4 Confidentiality

Compliance with data protection legislation was ensured, and the confidentiality of women involved in the study was maintained. The colposcopy clinic database and all the computers used for the storage and analysis of data were password protected. All enrolled participants were given unique study number so that the data sheet cannot be used to trace the women.

2.3.5 Sponsor

This study was self-funded.

CHAPTER 3
RESULTS

3.1 Demographics:

In the process of identifying the first 139 consecutive women who underwent LLETZ treatment and met all the inclusion criteria, the records of 233 women were accessed. 94 of the women whose records were accessed did not have record of cervical cytology within two years of LLETZ. All 139 women included in this study had record of at least one cervical cytology within two years of LLETZ.

Clinical data extracted from medical records include age, HIV status, indication for LLETZ, and LLETZ histopathology (Table 3.1). The women's age ranged from 23-77 years, with a mean of 38.6 and standard deviation (SD) of 8.9. Close to two-thirds of the women (88/139) were living with HIV, while 35.3% (49/139) were HIV negative and two women had unknown HIV status at the time of undergoing LLETZ treatment. The indication for LLETZ (in descending order of frequency) were, cytological HSIL 87.0% (121/139), persistent cytological LSIL 5.8% (8/139), biopsy proven CIN2 5.8% (8/139), and biopsy proven CIN3 1.4% (2/139).

Table 3.1 shows that the final histological diagnosis at LLETZ were CIN3 (69.8%) and CIN2 (30.2%) lesions. 58.3% of LLETZ biopsies obtained were positive for CIN2/CIN3 at excision margins, while 30.2% of biopsies had clear margins, and 11.5% of biopsies had uncertain margin status. The most compromised margin was the endocervical margin (28.8%), with 22.3% ectocervical margin involvement.

Table 3.1: Clinical characteristics of participants

HIV status	n/N	%
Positive	88/139	63.3
Negative	49/139	35.3
Unknown	2/139	1.4
Indications for LLETZ		
CIN2	8/139	5.8
CIN3	2/139	1.4
Persistent LSIL	8/139	5.8
HSIL	121/139	87.0
Final histology		
CIN2	42/139	30.2
CIN3	97/139	69.8
Margin status		
Uncertain	16/139	11.5
Incomplete- ectocervical margin	12/139	8.6
Incomplete- endocervical margin	21/139	15.1
Incomplete- both margins	19/139	13.7
Incomplete- uncertain margin	29/139	20.9
Complete	42/139	30.2

3.2 Findings at first follow-up cytology

At the first follow-up cytology after LLETZ, 17.3% (95% CI 11.4 to 24.6) of the women had residual/recurrent HSIL, while 68.3% (95/139) had normal cervical cytology and 14.4% (20/139) had LSIL or ASCUS (Table 3.2).

Table 3.2: Findings at first cytology after LLETZ

1st cytology after LLETZ	n/N	%
NILM (normal)	95/139	68.3
ASC-US/LSIL	20/139	14.4
ASC-H/HSIL	24/139	17.3

3.3 Age and findings at first follow-up cytology

Table 3.2 shows that there was no detection of HSIL within two years of LLETZ among women aged less than 30 years. From age groups 30-34, 35-39 and 40-44 HSIL detection rate was constant at four per age group, and then peaked to 9 in the 45-49 age group, followed by a sharp decline from age 55 downwards. Two out of the three women who were aged above 60 in this study developed HSIL at follow-up. The rate of post-treatment HSIL detection increased with advancing age. Univariate analysis (Table 3.5) shows a significant increase in the odds of post-LLETZ HSIL detection among women who were aged 40 and above relative to women who were younger than 40 years (p=0.04).

Table 3.3: Findings at first cytology after LLETZ for each age groups

Age group	NILM		ASC-US/LSIL		HSIL		TOTAL	
	N	%	N	%	N	%	N	%
20-24	2	1.4	1	0.7	0	0	3	2.2
25-29	9	6.5	4	2.9	0	0	13	9.4
30-34	32	23	4	2.9	4	2.9	40	28.8
35-39	18	12.9	1	0.7	4	2.9	23	16.5
40-44	16	11.5	3	2.2	4	2.9	23	16.5
45-49	12	8.6	6	4.3	9	6.5	27	19.4
50-54	4	2.9	1	0.7	1	0.7	6	4.3
55-59	1	0.7	0	0	0	0	1	0.7
60-64	1	0.7	0	0	0	0	1	0.7
65-69	0	0	0	0	0	0	0	0
70-74	0	0	0	0	1	0.7	1	0.7
75-79	0	0	0	0	1	0.7	1	0.7
Total	**95**	**68.3**	**20**	**14.4**	**24**	**17.3**	**139**	**100**

Table 3.4 shows a higher detection of HSIL at follow-up among women living with HIV (17/24) than among HIV negative women (5/24). Positive HIV status was associated with nonsignificant doubling of the odds of treatment failure (OR=2.0, CI 0.68 to 6.10, p = 0.20) (Table 3.5).

Table 3.4: HIV status and first post-LLETZ cytology

HIV status	NILM	LSIL/ASC-US	HSIL n/N (%)	Total	%
Unknown	0	0	2/24(8.3)	2/139	1.4
Negative	42/95	2/20	5/24(20.8)	49/139	35.3
Positive	53/95	18/20	17/24(70.8)	88/139	63.3
%	68.3	14.4	**17.3**	139	100

Three out of five participants were living with HIV at the time of LLETZ treatment and 17 of the 24 treatment failures occurred among women living with HIV (table 3.4/figure 3.1).

Figure 3.1: Distribution of cytological findings at follow-up and HIV status

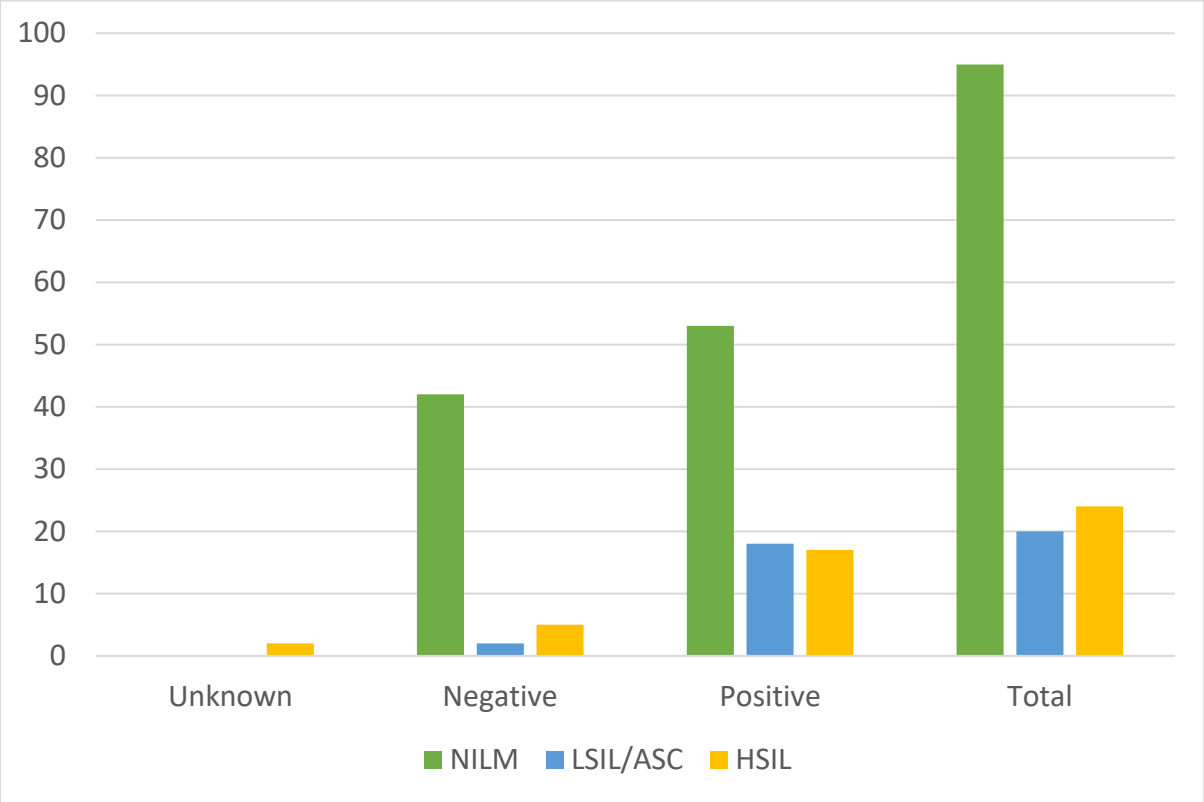


Table 3.5: Logistic analysis of factors contributing to treatment failure

Characteristics	Crude odds ratio	95% CI	P value	*Adjusted Odds ratio	95% CI	P value
Age > 40	3.23	1.28-8.17	0.01	2.70	1.03-7.07	0.04
Age ≤ 40	1 (reference)			1 (reference)		
HIV						
Positive	2.11	0.73-6.12	0.17	2.04	0.68-6.10	0.20
Negative	1 (reference)			1 (reference)		
Margin Status						
Complete	1 (reference)			1 (reference)		
Uncertain	1.36	0.22-8.25	0.74	1.27	0.20-8.10	0.80
Incomplete	2.71	0.85-8.62	0.09	2.28	0.69-7.53	0.18

*Odds ratios were adjusted for variables in the model

Table 3.6 shows that there was no residual/recurrent HSIL detection when the only compromised margin is the ectocervical margin and 16.7% (4/24) of post-LLETZ HSIL was detected among women with clear LLETZ margins. 20.8% (5/24) of persistent/residual HSIL was detected in cases with uncertain margin status. 29.2% (7/24) of residual/recurrent HSIL was detected among women with endocervical margin involvement, while 33.7% (8/24) of recurrent HSIL was detected in women with involvement of both the endocervical and ectocervical margins. Endocervical margin involvement (alone and in combination with the ectocervical margin) accounted for 62.5% (15/24) of all treatment failures. There was positive LLETZ margin in 75% (18/24) of all cases in which treatment failure occurred (table 3.7). Univariate analysis shows that the there was a 2.7-fold odds of treatment failure when LLETZ margin involvement was compared with clear margins at follow-up, but the difference was not statistically significant (OR = 2.71, CI 0.85 to 8.62, p = 0.09) (Table 3.5).

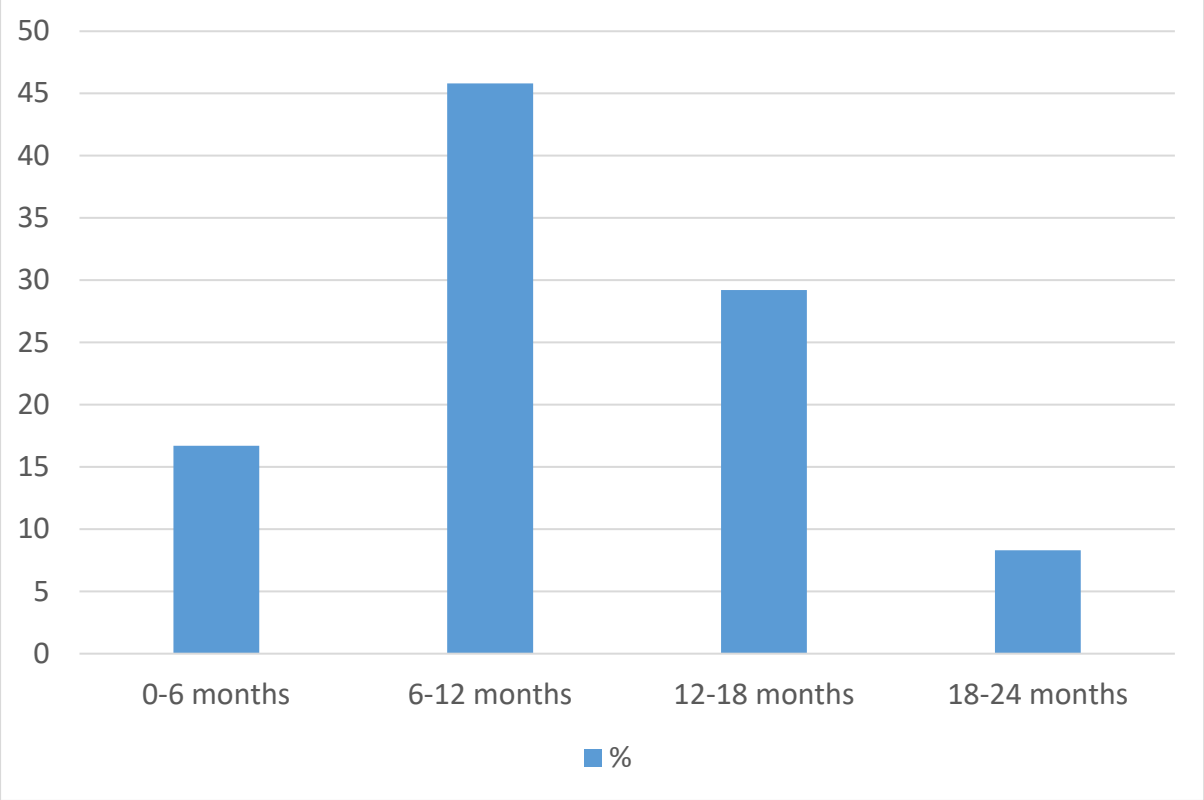
Table 3.6: Margin status and HSIL detection at first cytology after LLETZ

Margin Status	HSIL n (%)
Uncertain	2 (8.3)
Incomplete: uncertain margin	3 (12.5)
Incomplete: endocervical margin	7 (29.2)
Incomplete: both margins	8 (33.3)
Incomplete: ectocervical margin	0 (0)
Complete	4 (16.7)
Total	24

Table 3.7: Margin status and HSIL detection

Margin Status	HSIL n (%)
Uncertain	2 (8.3)
Involved margin	18 (75)
Complete	4 (16.7)
Total	24

Figure 3.2: Percentage HSIL detection at intervals after LLETZ



3.4 Timing of first follow-up and HSIL detection intervals

Table 3.8 show the interval between LLETZ and first follow-up cytology. Only 13.7% of women in this study had their first post-LLETZ cytology within six months of LLETZ. Majority of the women (57.6%) had their first follow-up cytology between 6 and 12 months of LLETZ, while 19.4% had theirs between 12 and 18 months, and the remaining 9.4% had theirs between 18 and 24 months.

Figure 3.2 shows that 16.7% (4/24) of post-LLETZ HSIL detection occurred within 6 months of LLETZ, with a peal post-LLETZ HSIL detection of 45.8% (11/24) at 6-12 months, followed by a decline to 29.2% (7/24) at 12-18 months, with the least post-LLETZ HSIL detection of 8.3% (2/24) at 18-24 months.

Table 3.8: Timing of first follow-up cytology and findings

Month group	NILM	ASCUS/LSIL	HSIL (%)	Total	%
0-6 months	13	1	4/24 (16.7)	18	12.9
6-12 months	58	12	11/24 (45.8)	81	58.3
12-18 months	15	5	7/24 (29.2)	27	19.4
18-24 months	9	2	2/24 (8.3)	13	9.4
Grand Total	**95**	**20**	**24**	**139**	**100.0**

Table 3.9: Findings at second follow-up after LLETZ

2nd Follow-up cytology	Count (%)
No follow-up cytology	104/139 (74.8)
NILM	24/35 (68.6)
LSIL	6/35 (17.1)
HSIL	5/35 (14.3)

3.5 Findings at second follow-up cytology

104 participants did not have a second follow-up cytology within two years of LLETZ, representing 74.8% loss to a second follow-up. Residual/recurrent HSIL was detected among 5 out of the 35 women who had a second cytology during the study period. All the HSIL detected at the second follow-up were previously detected at the first post-LLETZ cervical cytology but had not been re-treated prior to the second cytology. There was no HSIL detection at the second follow-up among participants who had normal cervical cytology or LSIL/ASC-US at the first post-LLETZ cervical cytology.

CHAPTER 4
DISCUSSION

Key findings

The aim of the present study was to determine the prevalence of treatment failure within two years of LLETZ at Tygerberg Hospital, a tertiary hospital in Cape Town, South Africa. In this study treatment failure was defined as the cytological detection of ASC-H or HSIL within two years of LLETZ, and treatment efficacy was described as cervical cytology that was negative for intraepithelial lesion or malignancy (NILM) at the first follow-up within two years of LLETZ.

About one in six women developed treatment failure within two years of LLETZ, while 68.3% of the first post-LLETZ cervical cytology were reported as NILM. Close to two-thirds of women in this study were living with HIV at the time of undergoing LLETZ treatment. This study did not show a statistically significant difference in the rate of residual/recurrent HSIL between the participants who were living with HIV and those who were HIV negative. However, being aged 40 and above at the time of treatment was associated with a significant risk of developing treatment failure. There was margin involvement in more than half of all LLETZ procedures performed, and three out of four women who developed residual/recurrent HSIL had positive LLETZ margins. However, the association between treatment failure and positive LLETZ margin was not statistically significant.

Loss to follow-up

94 of the 233 women whose clinical records were accessed in the process of identifying the 139 study participants did not have record of follow-up cervical cytology within two years of LLETZ, representing a 40.3% loss to follow-up after LLETZ. The rate of loss to follow-up for a second cervical cytology after LLETZ was also high. By local protocol, patients were expected to have their first follow-up cytology 6 months after LLETZ and a repeat cytology 1 year later. However, four in five women did not have record of a second cytology within 2 years of LLETZ. Only 12.9% of participants had their first post-LLETZ cytology within 6 months of treatment, and the rate of loss to follow-up for a second post-LLETZ cervical cytology was also high. Four in five of the participants had no record of a second follow-up as required by institutional

protocol. A small proportion of women seen at Tygerberg Hospital colposcopy clinic are referred from private health facilities. It is probable that some of these women might have returned to their referring private facilities for their post-LLETZ follow-up.

A significant population of migrant workers and visitors from other parts of the South Africa as well as immigrants and international students from other parts of the world reside in Cape Town. It is probable that a small proportion of women included in this study could fall within this category of temporary residents who might have relocated during the follow-up period, thus resulting in failure to track their follow-up records. Furthermore, the hospital number assigned to patients treated in the public health sector in South Africa varies from province to province, and in some instances these numbers are hospital specific, making result tracking a challenge. While the record of laboratory tests carried out in the public health facilities are centralised in the National Health Laboratory Service (NHLS) database, those performed in the various private health facilities are stored in the individual database of the specific laboratories. In the instances where cervical cytology records were not readily available using the hospital numbers, efforts were made to track results on the database of the NHLS by matching patients' date of birth with surname, first and middle names and gender before a conclusion was made that there was no record of cervical cytology. Centralisation of laboratory results irrespective of where the tests were performed will enable access by clinicians who manage the patients, reduce test duplicity, and serve as a database for future research.

Treatment failure

The 17.3% treatment failure rate found in this study is higher than the average treatment failure rate of 5-10% quoted in a recent systematic review and meta-analysis of 27 studies in which the incidence of cervical cancer after treatment for CIN was assessed.[21] Depending on the series, the rate of recurrence of HSIL after treatment can be quite heterogeneous. Even studies from HICs have shown variable rates of treatment failure. For instance, while a study conducted in France found a 3.6% residual/recurrent high grade precancer among 204 women who had received LLETZ treatment for HSIL,[17] another study in neighbouring Netherlands found a 17.5% residual/recurrent HSIL among 435 women who were previously treated with LLETZ (82%) or cold knife conisation CKC (18%).[19] Such findings suggest that the variation in the prevalence of post-treatment HSIL might not be attributed to treatment adequacy

alone. Differences in the definition of cure, the duration of follow-up, HIV prevalence, variation in inclusion and exclusion criteria between studies, differences in methods used in assessing treatment efficacy have been identified as factors contributing to variation in treatment failure rates.[27,33,34]

Prevalence of post-LLETZ treatment failure in South Africa

There is paucity of data from LMICs on the efficacy of excisional treatment for HSIL, even though these countries carry most of the global burden of cervical cancer.[1,2] Only a few published studies have reported on disease recurrence after treatment for CIN in South Africa. A study on the outcomes of treated HSIL among 1,213 women conducted at Groote Schuur Hospital (GSH) and published by the University of Cape Town in 2018 showed a 17.0% residual/recurrent HSIL at the four-month follow-up.[35] Another study at a tertiary hospital in Soweto which assessed the rate of recurrent cytological abnormalities among 575 women who received LLETZ treatment for HSIL, showed a 22.6% rate of residual/recurrent HSIL.[25] Furthermore a RCT conducted in South Africa, which compared the efficacy of LLETZ with cryotherapy within one year of treatment among 166 HIV-seropositive women showed that 18.5% of women in the LLETZ arm developed residual/recurrent HSIL, while treatment failure in the cryotherapy arm was 27.2%.[36] Findings from this study, as well as from the studies highlighted above suggest that the true prevalence of treatment failure after LLETZ in South Africa could be somewhere between 17% and 22%.

Margin status at LLETZ

58.3% of the LLETZ procedures performed showed biopsy margins that were positive for CIN2/CIN3, with the endocervical margin being the most compromised margin. The biopsies with uncertain margin status were mostly due to tissue fragmentation and cautery artifacts. Despite the high rate of margin involvement in this study, a statistically significant association between positive margins status and treatment failure was not found. Furthermore, one in six treatment failures occurred among women with clear LLETZ margins, and most women with positive margins did not develop post-treatment HSIL. This further buttresses the widely accepted position that margin status is not a reliable predictor of treatment failure.

The percentage of margin involvement found in this study is almost three times higher than the 20% target recommended by the European Federation of Colposcopy (EFC)

Quality Standards Delphi Consultation.[26] A systematic review and meta-analysis in 2017 however showed that close to 60% of studies did not achieve the 20% positive margin target recommendation.[6] Aiming for 80% margin-free excision at LLETZ might promote the tendency for larger biopsies and inadvertently result in more complications and obstetric harm like preterm labour and the associated neonatal consequences in women of reproductive age who desire future fertility.[6,14,27]

The utility of margin involvement alone in determining the risk of post-treatment disease is controversial and has been a subject of considerable debate. Whilst some studies showed that margin status could be used to predict post-treatment disease recurrence, other authors found no association or limited association between margin status and post-treatment disease.[8,29,39] Furthermore, the practice of extensive cauterisation of the treatment crater to compensate for incomplete excision does not decrease obstetric harm in subsequent pregnancies and is therefore not recommended.[29] The recommendation from most authors is that margin status on its own should not be used for surveillance or as indication for reflex retreatment.[29,33]

Colposcopic experience and quality of LLETZ biopsy

LLETZ is a minor surgical procedure which can easily be learned. The LLETZ procedures in this study were performed by trained Gynaecology registrars, senior medical officers, gynaecological oncology fellows and consultants. There is constant supervision and training of junior doctors who perform colposcopy at Tygerberg hospital. Sparic and colleagues showed that surgeon characteristics, and the level of colposcopic experience is associated with the quality of LLETZ specimen sent for histological assessment. They found more thermal artifacts, positive margins, and tissue fragmentation in LLETZ specimens obtained by less experienced operators.[40] These findings are consistent with what other studies show.[8] However, in this study the profile and characteristics of the surgeon was not matched with the quality of LLETZ biopsy obtained.

Results also showed that age 40 and above at the time of LLETZ is an independent risk factor for the development of treatment failure. This finding supports the finding from previous studies that older women are at a higher risk of developing treatment failure as well as at a higher risk of more frequent incomplete excision.[8,18,29] Some studies suggest that older women might have a longer duration of exposure to HPV

and therefore tend to have larger lesions and higher grade of CIN.[41,42] Furthermore type 3 transformation zone is commoner among older women and a second pass "top hat" excision with a smaller loop directed towards the endocervix might be required to prevent incomplete excision at the endocervical margin in such case.[39] To mitigate the risk of incomplete CIN excision, larger biopsies are justifiable in older women.

The increase in the rate of treatment failure among women living with HIV in this study was not statistically significant. Furthermore, data on the viral load and CD4 count of participants living with HIV was not collected. A recent systematic review and meta-analyses of 40 studies showed a significant increase in the prevalence of treatment failure among women living with HIV when compared with HIV negative women. HIV-seropositive women have been shown to be less likely to clear HPV, have larger and multi-focal CIN, which are more likely to be incompletely excised at LLETZ.[36,43]

Role of adjuvant HPV vaccine at LLETZ in reducing treatment failure rate

Adjuvant HPV vaccination is a promising strategy for reducing the rate of residual/recurrent disease after treatment for CIN. Two recently published meta-analyses show that the administration of HPV vaccine either as part of a prophylactic regimen or as adjuvant to surgical treatment of CIN was associated with close to 50% reduction in the risk of recurrence of HSIL.[44,45] However, a double-blind, randomized clinical trial involving 180 HIV-seropositive women conducted in Johannesburg, and published in 2021, found that there was no benefit in adjuvant HPV vaccine in these women. These authors therefore did not support adjuvant HPV vaccination of women living with HIV as a measure to prevent post-LLETZ treatment failure.[43]

HPV vaccination in South Africa was rolled out in April 2014 through a national school-based campaign targeted at young girls aged between 9 and 13 years. It is thus unlikely that participants in this study could have received HPV vaccination through this campaign. Information about prior HPV vaccine exposure was not collected in the current study, hence the possible impact of HPV vaccination on post-LLETZ treatment failure among participants is unknown. This can be investigated in future studies.

Post-LLETZ follow-up

Since cervical cancer remains the leading cause of cancer related mortality among women in LMICs like South Africa, it is important to have a clear follow-up strategy after treatment for CIN, so that recurrent disease can be detected early, and

appropriate re-treatment promptly instituted. A systematic review and meta-analysis, published in 2020 shows that the relative risk of developing cervical cancer among women treated for CIN was three times higher than the general population, and that this risk remains raised for at least 20 years after the index treatment.[21] It is a sad reality that women in LMICs are at 18 times higher risk of mortality from cervical cancer than women in HICs.[2] It is therefore hoped that the findings and recommendations from this study will result in improvement in the care of women with pre-invasive cervical cytology in South Africa and other LMICs.

CHAPTER 5
CONCLUSION, RECOMMENDATIONS, LIMITATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

5.1 Conclusion

LLETZ is an effective modality in the treatment of CIN. The rate of treatment failure after LLETZ is relatively high. Findings from this study reinforces the need for the close surveillance of women who have undergone treatment for CIN. Cytology is an acceptable option in the absence of HPV DNA testing as test of cure after LLETZ.

5.2 Recommendations

We recommend a follow-up protocol for women who have been treated for CIN that includes colposcopy and cytology six months after LLETZ and, assuming this to be normal, then yearly cytology for two years, followed by a three yearly cytology. These screening intervals can be increased where a negative hr-HPV result is available.

Regular colposcopy training and re-training of operators coupled with strengthening of the follow-up strategies, roll-out of mobile colposcopy units for outreach purposes to remote communities and reducing the turn-around time for cytology and histopathology results will go a long way shortening the interval between the detection of HSIL and treatment, possibly result in reduction in the current high rate of loss to follow-up after treatment, and ultimately lead to a decline in the incidence of invasive cervical cancer in the Western Cape of South Africa.

5.3 Limitations

This study is not immune from the limitations associated with retrospective studies. Cervical cytology was the only method available and used for the assessment of treatment efficacy, despite its relatively low sensitivity in detecting CIN in contrast to HPV DNA testing. Also, most of the women treated for HSIL in this study did not follow-up at the recommended interval after treatment, thus the peak timing of disease recurrence could not be accurately determined. Lastly, the high loss to follow-up for a second cytology within two years of LLETZ is another limitation.

Despite these limitations, the rate of post-treatment disease recurrence found in this study compares with the prevalence rate reported in studies conducted in some HICs.[19]

5.4 Further research

- Prevalence of invasive cervical cancer among women previously treated for HSIL.
- Survey of cervical screening pattern between women who have been treated for CIN and those with no prior abnormal cervical cytology
- Long term prevalence of invasive cervical cancer among women treated for HSIL who had adequate follow-up and those who defaulted follow-up
- Prevalence of abnormal cervical cytology between women who have received HPV vaccine and those who have not been vaccinated
- Correlation between the colposcopic skills of clinician performing LLETZ and treatment outcomes.

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ANNEXURES

APPENDIX 1: HREC APPROVAL LETTER

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Approval Notice

New Application

06/10/2020

Project ID: 17256

HREC Reference No: S20/08/215

Project Title: PREVALENCE OF ABNORMAL CERVICAL CYTOLOGY TWO YEARS AFTER LARGE LOOP EXCISION OF THE TRANSFORMATION ZONE (LLETZ)

Dear Dr Victor Olujobi

The **Response to Modifications** received on 01/10/2020 18:49 was reviewed by members of **Health Research Ethics Committee** via **expedited** review procedures on 06/10/2020.

Thank you for attending to the requested modifications, your research protocol is now finally approved.

Please note the following information about your approved research protocol:

Protocol Approval Date: 06 October 2020

Protocol Expiry Date: 05 October 2021

Please remember to use your Project ID 17256 and Ethics Reference Number S20/08/215 on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review

Translation of the informed consent document(s) to the language(s) applicable to your study participants should now be submitted to the HREC.

Please note you can submit your progress report through the online ethics application process, available at: Links Application Form Direct Link and the application should be submitted to the HREC before the year has expired. Please see *Forms and Instructions* on our HREC website (www.sun.ac.za/healthresearchethics) for guidance on how to submit a progress report.

The HREC will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility, permission must still be obtained from the relevant authorities (Western Cape Departement of Health and/or City Health) to conduct the research as stated in the protocol. Please consult the Western Cape Government website for access to the online Health Research Approval Process, see: https://www.westerncape.gov.za/general-publication/health-research-approval-process. Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.

For standard HREC forms and instructions, please visit: Forms and Instructions on our HREC website https://applyethics.sun.ac.za/ProjectView/Index/17256

If you have any questions or need further assistance, please contact the HREC office at 021 938 9677.

Yours sincerely,

Mrs. Brightness Nxumalo
HREC 2 Coordinator

National Health Research Ethics Council (NHREC) Registration Number:

REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by theWorld Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects;the South African Department of Health (2006). Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes andStructures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

APPENDIX 2: TBH APPROVAL LETTER

Western Cape Government
Health

TYGERBERG HOSPITAL
REFERENCE:
Research Projects
ENQUIRIES: **Dr GG Marinus**
TELEPHONE:**021 938 5752**

Project ID: 17256

Ethics Reference: S20/08/215

TITLE: **PREVALENCE OF ABNORMAL CERVICAL CYTOLOGY TWO YEARS AFTER LARGE LOOP EXCISION OF THE TRANSFORMATION ZONE (LLETZ)**

Dear Dr Victor Olujobi

PERMISSION TO CONDUCT YOUR RESEARCH AT TYGERBERG HOSPITAL.

1. In accordance with the Tygerberg Hospital Health Research Policy and Protocol of **April 2018,** permission is hereby granted for you to conduct the above-mentioned research here at Tygerberg Hospital.

2. Researchers, in accessing Provincial health facilities, are expressing consent to provide the Department with an electronic copy of the final feedback within six months of completion of research. This can be submitted to the Provincial Research Co-Ordinator (Health.Research@westerncape.gov.za).

DR GG MARINUS
MANAGER: MEDICAL SERVICES

Date: 17/Nov/2021

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