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ORIGINAL ARTICLE

LEEP Excision of High-grade Cervical Intraepithelial Lesion to Achieve Appropriate Excision Margins

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Abstract

Background: **In the case of cervical intraepithelial neoplasia (CIN), cervical conization is the treatment of choice. If resection is only partial, the remaining disease remains a risk. Yet, so far, there have been very few studies looking at how to optimize LEEP resection by removing more of the lesion.**

Aims and objectives: **The goal of loop electrosurgical excision techniques (LEEP) for high-grade cervical intraepithelial lesions is to remove as much of the tumor as possible without leaving any healthy tissue behind.**

Subjects and methods: **The Department of Obstetrics and Gynecology of Al Zahraa University Hospital, Al-Azhar University, Cairo, Egypt, participated in this cross-sectional, analytical study. Our study took part in and lasted for two and a half years. Sixty women with high-grade cervical intraepithelial lesions diagnosed with Pap smear undergone LEEP conization. Six subjects were excluded due to having benign lesions on their LEEP biopsies. So, 54 subjects continued to be analyzed. All patients were subjected to the following: history taking, general examination, and local examination.**

Results: **68.5% of subjects had negative biopsy margins, while 31.5% had positive margins, with a statistically significant association with obesity, high-grade lesions, and carcinoma.**

Conclusion: **It was concluded that the 7 mm cone depth for treating high-grade cervical intraepithelial lesions was appropriate for a higher negative cone margin after LEEP biopsy. Ki-67 staining is beneficial in confirming the histopathological diagnosis.**

Keywords: Cervical intraepithelial neoplasia, Cold-knife conization, Ki-67, Loop electrosurgical excision procedure

1. Introduction

According to the World Health Organization, more than 270 thousand women lose their lives to cervical cancer each year. Thus, it is crucial to take measures to prevent and detect dysplastic lesions of the cervix as early as possible.[1]

According to the Bethesda system 2022, high-grade squamous intraepithelial lesion (HSIL), also known as cervical intraepithelial neoplasia (CIN) 2–3, is a well-defined precursor lesion of cervical invasive squamous cell carcinoma. The cervix uteri has a transformation zone where these lesions tend to occur. Precursor lesions can lie dormant for as long as ten years before developing malignant invasion capabilities.[2]

The likelihood of invasive growth is proportional to the severity of the dysplasia and the time it is discovered. Cervical preinvasive lesions and cancer continue to be significant public health concerns.[3]

The risk of future complications during pregnancy is decreased when the loop electrosurgical excision process (LEEP) is combined with intraoperative colposcopy, resulting in substantially smaller cone specimens without sacrificing margin status. It also facilitated access to the endocervical squamocolumner junction. The best reliable specimens for histology with the least amount of morbidity appear

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to be obtained through large loop excision of the transformation zone.[4]

The advantages of LEEP over other techniques are that it is associated with lesser blood loss, lower morbidity rates, a lower obstetric complications rate, a lower cost, and favorable surgical, oncologic, and obstetric outcomes.[5]

To provide the highest level of patient safety, cervix uteri dysplastic lesion excisions must show negative margins (i.e., without dysplasia). Yet, the deeper the resected cone, the greater the risk for premature delivery.[2]

As a result of this conundrum, definitive guidelines for the ideal depth of cone size in the treatment of high-grade dysplastic lesions of the cervix uteri are lacking. Cone specimen depth is often decided during surgery and is influenced by the patient's age, parity, fertility goals, and early colposcopic results.[6]

2. Patients and methods

This research was a cross-sectional analytical study performed at Al Zahraa University Hospital, Al-Azhar University's Department of Obstetrics and Gynecology, on 60 women with high-grade cervical dysplasia diagnosed on a Pap smear and colposcopic examination undergoing LEEP conization. Our study lasted for two years and a half.

2.1. Type of study

Cross-sectional analytical study.

2.2. Inclusion criteria

Patients with abnormal Pap smear tests (high-grade cervical dysplasia), confirmed high-grade cervical dysplasia by colposcopic biopsy (CIN II and III), and patients with endocervical biopsy showing negative pathology.

2.3. Exclusion criteria

Patients with colposcopic biopsies showing no or low-grade cervical lesions; previous cervical surgeries; patients with confirmed invasive cervical carcinoma; and pregnant patients.

2.4. Methods

Two sections of 4 m were provided by cutting all tissue blocks. Using the automated IHC Dako staining system, one slice was used for H&E staining, and the other slice was used for immunostaining with mouse monoclonal antibodies against Ki-67 antigen.

2.5. Ki-67 index proliferation score

Score 0: assigned to nuclear Ki-67 staining in squamous epithelium cells if the staining pattern was normal (i.e., staining of nuclei in the basal layer).

Score 1: positive nuclei that are primarily found in the lowest third of the epithelium.

Score 2: positive nuclei that are predominately located in the lower two-thirds of the epithelium.

Score 3: positive nuclei in more than two-thirds of the epithelium.

2.6. Statistical analysis

Data were collected, revised, coded, and entered into the statistical package for social science (SPSS). Quantitative data are provided as percentages, whereas qualitative data are presented numerically as means, standard deviations, and ranges wherever parametric. Data were collected, updated, coded, and put into SPSS version 23.

Chi-square tests were used to compare groups on qualitative data, and P values were calculated for correlations.

We accepted a 5% margin of error within a 95% confidence zone. Hence, the P value was considered significant for the following reasons: $P > 0.05$: nonsignificant; $P > 0.05$: significant; and $P > 0.001$: highly significant.

3. Results

Table 1.

Table 1. Demographic data of the included subjects.

	$N = 60$			
	Min.	Max.	Mean	SD
Age (years)	28	77	48.444	11.062
BMI	18	46	30.436	8.987
Marriage duration (years)	3	55	23.944	12.169
	No. (%)			
Marital status				
1	56 (93.3%)			
4	3 (5%)			
5	1 (1.7%)			
Parity				
Nullipara	11 (18.3%)			
Multipara	49 (81.7%)			
1–2	9 (15%)			
3–5	30 (50%)			
>5	10 (16.7%)			
Menopausal status				
Nonmenopause	43 (71.6%)			
Menopause	17 (28.3%)			

The ages in this table varied from 28 to 77, with a mean (±SD) of 48.444±(11.06) years. Their body mass index (BMI) was in the range of 18–46, with a mean (±SD) of30.44±(8.987). Also, 93.3% of included subjects were married once, 81.7% were multiparous. However, 71.6% were nonmenopause, and 28.3% were menopause (Fig. 1, Table 2).

This table shows that 7 biopsies with high-grade pre-LEEP diagnosis were found to be low grade lesion by LEEP biopsy. However, 8 biopsies of high-grade pre-LEEP diagnosis (2 were CIN2 and 6 were CIN3) were diagnosed as micro-invasive lesions on LEEP biopsy. While 9 biopsies with high-grade pre-LEEP diagnosis show invasive carcinomas on LEEP biopsy. Also, 6 biopsies out of the 60 subjects found to have benign lesions on their LEEP biopsies (while they were CIN2 on their pre-LEEP diagnosis). Those 6 benign biopsies (condyloma and chronic cervicitis) were excluded from the following analysis (Fig. 2, Table 3).

This table shows that there is a statistically significant association (*P* 0.05) between HPV status and LEEP biopsy findings. In spite of the low number (17) of HPV-positive patients in the studied population, there is a positive correlation between the histopathology grade of LEEP biopsies and the positive HPV infection. 41.2%, 29.4%, and 17.6% were CIN3, microinvasion, and invasive carcinoma, respectively (Table 4).

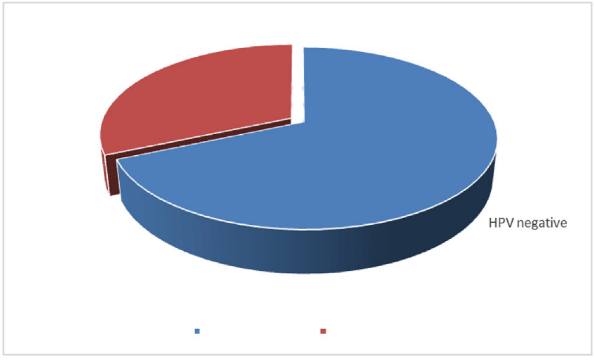

Fig. 1. HPV status of the studied population.

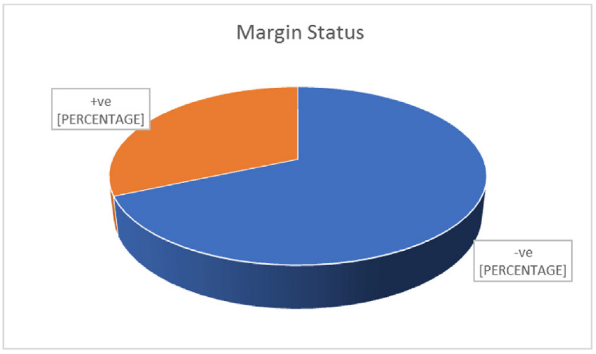

Fig. 2. The status of LEEP biopsy margins.

Table 3. The correlation between histopathology of LEEP biopsies and HPV status among the 54 subjects with high-grade lesions.

	HPV-positive *N* = 17 N (%)	HPV negative *N* = 37 N (%)	Chi square test	
			X^2	*P value
CIN 1	0 (0%)	7 (17.2%)	11.179	0.025
CIN2	2 (11.8%)	12 (37.9%)		
CIN3	7 (41.2%)	9 (24.1%)		
Microinvasion	5 (29.4%)	3 (10.3%)		
Carcinoma invasion	3 (17.6%)	6 (16.2%)		

This table shows that there is a statistically significant association between histopathological findings and biopsy margin findings ($P < 0.001$). All cases of invasive carcinoma grade (9 cases) were associated with a positive biopsy margin (Table 5).

This table shows, based on Ki-67 score, that 50% of the studied biopsies have score 3, followed by 29.6% with score 2, 13% with score 1, and 7.4% with score 0 (Table 6).

There is a statistically significant association ($P < 0.05$) between the Ki-67 proliferating index score and the BMI of the studied subjects. A higher Ki-67 score was associated with a higher BMI (Table 7).

This table shows that there is a statistically significant association ($P < 0.05$) between Ki-67 proliferating index score and biopsy margin status. Higher scores were associated with positive margin biopsies (Figs. 3–5).

Table 2. Difference between LEEP Histopathology findings and pre-LEEP biopsy diagnosis (Pap smear & Colposcopic examination).

Pre-LEEP LEEP (*N* = 54)	CIN2 (*N* = 36)	CIN3 (*N* = 24)	Total (*N* = 60)
Bengin Lesion	6 (16.7%)	_____	
Low Grade	7 (19.4%)	_____	7
CIN 2	10 (27.8%)	4 (16.7%)	14
CIN 3	11 (30.6%)	5 (20.8%)	16
Microinvasion	2 (5.7%)	6 (25%	8
Invasive	_____	9 (37.5%)	9

Table 4. The correlation between histopathological findings and status of the LEEP biopsy margins.

	Negative margin $N = 37$ (68.5%) N (%)	Positive margin $N = 17$ (31.5) N (%)	Chi square test	
			X^2	*P value
CIN 1	7 (18.9%)	0 (0%)	26.060	**<0.0001**
CIN2	12 (32.4%)	2 (11.8%)		
CIN3	13 (35.1%)	3 (17.6%)		
Microinvasion	5 (13.5%)	3 (17.6%)		
Invasive Carcinoma	0 (0%)	9 (52.9%)		

Table 5. Ki - 67 proliferating index score of the 54 included subjects.

	No. (%)
Ki immune staining score	
Score 0	4 (7.4%)
Score 1	7 (13.0%)
Score 2	16 (29.6%)
Score 3	27 (50.0%)

Table 6. The correlation between Ki-67 proliferating index score and demographic data of included subjects.

	Ki-67 immune staining score	
	r	P value
Age	−0.140	0.314
Marital status	−0.026	0.854
BMI	0.338	0.032
Parity	−0.157	0.280

Table 7. The correlation between Ki-67 proliferating index score and status of LEEP biopsy margins.

	Negative margin $N = 37$ N (%)	Positive margin $N = 17$ N (%)	Chi square test	
			X^2	*P value
Score 0	4 (10.8%)	0 (0%)	8.843	**0.031**
Score 1	7 (18.9%)	0 (0%)		
Score 2	12 (32.4%)	4 (23.5%)		
Score 3	14 (37.8%)	13 (76.5%)		

Fig. 4. Score 1 nuclear expression of ki −67 in low grade squamous intraepithelial lesion (power × 100).

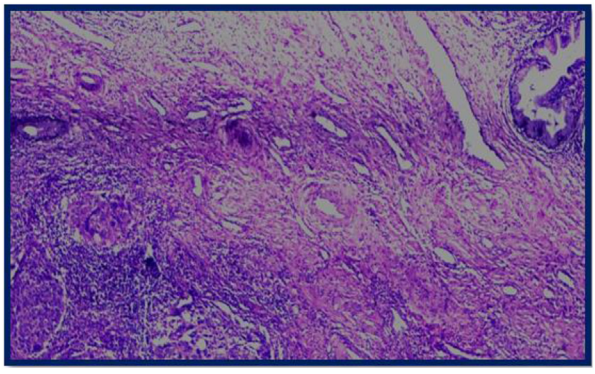

Fig. 5. Microinvasive carcinoma with negative endocervical margin by H &E stain power X40.

4. Discussion

In the present research, the ages of the subjects varied from 28 to 77 years, with a mean value (±SD) of 48.444± (11.06) years. Their BMI ranged between 18 and 46, with a mean value (±SD) of 30.44± (8.987).

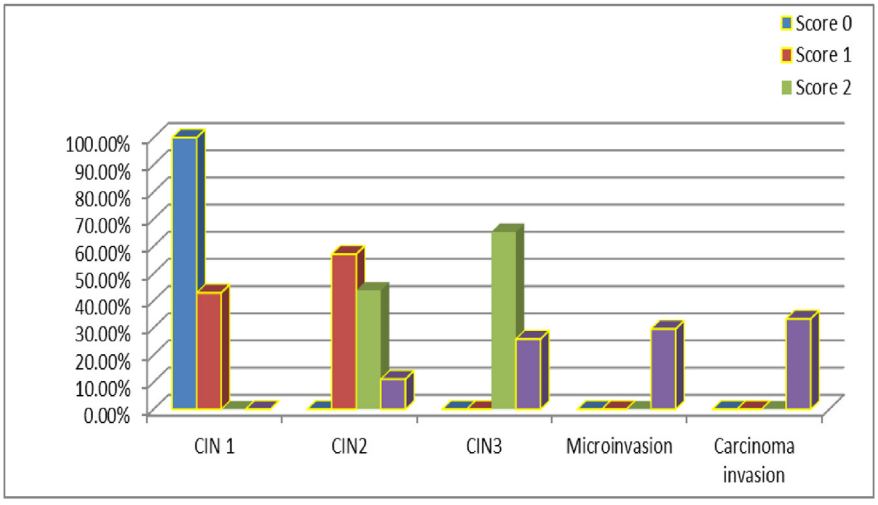

Fig. 3. Ki - 67 proliferating index score of the included subjects in relation to histopathological findings.

Also, 93.3% of the included subjects were married once, and 81.7% were multiparous. However, 71.6% were nonmenopausal and 28.3% were menopausal.

Conceding with these results, Moscicki et al.[7] study, done on 112 patients, found that the mean age (±SD) of their subjects was 49 ± 5.4 years, and 14% occurred in women under 35 years.

Clarke et al.[8] looked into the possibility that a higher body mass index makes it harder to spot cervical precancerous lesions, which increases the likelihood of developing cancer of the cervix. It was also determined that women who were overweight or obese were at a higher risk of developing cervical cancer, most likely due to a failure to detect cervical precancerous lesions. According to histopathology assessment of the LEEP biopsies taken in the present study, 26.7% of the studied biopsies have CIN3, and 23.3% have CIN2. However, 17 biopsies (31.5%) showed invasive lesion [with 8 biopsies (13.3%) having microinvasion and 9 biopsies (15.0%) having invasive carcinoma, while 7 biopsies (11.7%) showed low-grade lesion by LEEP biopsy]. and six (10.0%) biopsies showed benign lesions.

Women with conclusive results for CIN 2/3 who underwent cone biopsy using LEEP or Cold-Knife Conization did not have micro-invasive or invasive cervical cancer, according to research by Phaliwong et al.[9] Based on histopathology assessment of the taken LEEP biopsies, seven biopsies with a high-grade pre-LEEP diagnosis (by Pap smear and colposcopic examination) were found to have a low-grade lesion by LEEP biopsy. However, eight biopsies with a high-grade pre-LEEP diagnosis (2 were CIN2 and 6 were CIN3) were diagnosed with a micro-invasive lesion on the LEEP biopsy. While nine biopsies with a high-grade pre-LEEP diagnosis showed invasive carcinomas on LEEP biopsy, And just six pre-LEEP CIN2 biopsies had benign lesions (condyloma and chronic cervicitis) on their LEEP biopsy findings. In six subjects out of sixty, benign lesions were found on their cervical tissue biopsies excised by LEEP. So, 54 subjects continued to be analyzed.

These consequences were reinforced by Siegler et al.,[10] who found that regarding the LEEP indications, there were 1.9%.

The current study showed that there was a statistically significant association ($P > 0.05$) among HPV status and LEEP biopsy findings. Positive HPV cases (17 cases) were associated with high-grade lesions and carcinoma on histopathological diagnosis, as the majority of cases with HPV-positive status are associated with invasive carcinoma. However, the 37 negative HPV cases (68.5%) were associated with high-grade lesions and carcinoma. There were 6 biopsies (3 with positive HPV infection and 3 with negative HPV) that showed bengin pathology (chondyloma and chronic cervicitis).

Contrary to our results, Wang et al.[11] showed that most CIN2 patients (158 (66.7%)) were positive for HPV, while 79 (33.3%) were negative.

Regarding the status of the studied LEEP biopsies margins in the current study, biopsy margins showed 68.5% having a negative margin (the dysplastic lesion was more than 1 mm distance from the margin), while 31.5% of the studied biopsies had a positive margin (the dysplastic lesion was less than 1 mm distance from the margin), all on LEEP wire of 7 mm depth.

According to Srijarusith and Rodpenpear's[12] study of 170 patients, the optimal cut-off points for cone depth were calculated and presented at a resection depth of 7.21 mm, which demonstrated proper cone depth with a sensitivity of 63.53% and a specificity of 71.76%. This finding is consistent with the depth of resections used in the current study. To produce a negative cone margin from LEEP, the optimal cone depth for treating high-grade precancerous lesions was at least 7.21 mm, which is similar to the depth of LEEP biopsies taken in the present investigation.

On the other hand, increasing cone depth was associated with a significant increase in the risk of preterm delivery, with an estimated 6% increase in the risk per additional millimeter of tissue excised (odds ratio 1.06, 95% confidence interval 1.03–1.09), according to a study by Noehr et al.[13]

The present research showed that there was a positive relationship between BMI and the status of the biopsy margins of LEEP biopsies. The obese patients showed positive margins more than the nonobese patients, which is statistically significant (P value < 0.05). However, there is no statistically significant difference ($P < 0.05$) among negative and positive margin biopsies regarding the other demographic data (age, multiple marriages, and menopausal status) of the studied subjects.

Okoro et al.[14] conceded that there was a link between obesity and abnormalities in the cervical epithelial cells. This highlights the importance of encouraging women to adopt healthier habits as a means of reversing this public health crisis.

In addition, the existing research showed that there was a statistically significant association ($P > 0.0001$) between histopathological findings and biopsy margin status. It showed that a higher carcinoma grade and invasion were associated with a positive biopsy margin. All nine subjects with invasive carcinoma showed positive LEEP biopsy margins, which gives an idea of the need for urgent referral and a more aggressive management plan.

Also regarding the Ki-67 proliferating index score of the 54 subjects included in this study, the highest reported score (score 3) was found in 50%, followed by score 2 in 29.6%, score 1 in 13%, and score 0 in 7.4%. Ki-67 proliferating index score showed a statistically significant link (P value < 0.05) with the BMI, biopsy margins. Therefore, higher scores were associated with a high-grade cervical intraepithelial lesion, invasive carcinoma, positive margin biopsy, and a high BMI. However, there was no statistically significant relationship between the Ki-67 proliferating index score and other demographic data.

With the findings of the current research,Kim et al.[15] showed that the more reported Ki-67 score was score 3 in (44.61%), followed by score 2 in (26.15%), then score 1 in (15.38%), and score 0 in (13.84%) among both CIN2 and CIN3. Similarly, the Ki-67 score of invasive SCC was 3 in (62.9%) and 2 in (37.1%). High Ki-67 immunostaining (more than 2+) was linked with a higher risk of positive resection margin involvement.

In addition, Lim et al.[16] analyze the correlation between HPV infection status and the use of the Ki-67 immunohistochemical marker for the precise interpretation of cervical biopsies. Among the HSIL group, the most often reported score was 3, with 26.2% of patients reporting this, followed by 2.1% reporting score 2, 3.9% reporting score 1, and 0% reporting score 0. In patients with a high-risk HPV infection status, the positivity of Ki-67 increased dramatically with the severity of the cervical lesion (P < 0.001). Ki-67 was found to be correlated with CIN grade in a study conducted by Gupta et al.[17] There is a linear relationship between the histological grading of cervical dysplasia and the Ki-67 proliferative marker, suggesting that the two are related. Ki-67 rises as the severity of CIN advances.

4.1. Conclusion

It was determined that a larger negative cone margin could be achieved with a cone depth of 7 mm, while performing LEEP on high-grade cervical intraepithelial lesions. Obesity and carcinoma are two major contributors to a positive cone margin (high-grade lesions and carcinoma). Histopathological diagnosis can be strengthened by Ki-67 staining.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Conflicts of interest

The authors declared that there were NO conflicts of Interest.

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