

## RESEARCH ARTICLE

# Predictors of residual disease after loop electrosurgical excision procedure

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## ABSTRACT

The study aims to evaluate the importance of resection margins in the risk of residual disease (RD) and to investigate other factors that could potentially predict RD before patients engage in follow-up. Eighty-six women with a histologically confirmed diagnosis of cervical intraepithelial neoplasms (CIN) treated by loop electrosurgical excision procedure (LEEP), were included in this retrospective study, between January 2015 and May 2016. Age, smoking habit, menopause status, and LEEP margins were evaluated as possible predictors of RD. The mean age at diagnosis was 35.8 years (range 18-61). The mean follow-up period was 12 months. 11.6% of patients (09/86) were lost in follow-up. 64% of patients (55/86) had clear margins in the specimen and 34.8% of patients had positive surgical margins (30/86). In 1.2% of patients (01/86) the resection margins were uncertain. RD was demonstrated by positive Pap Smear and by colposcopy-guided biopsy in 26.7% of patients (23/86). We found significant differences in the frequency of RD depending on the status of margins: 65.2% of cases with positive margins vs. 24.5% of cases with negative margins ( $p < 0.0001$ ). Multivariate analysis showed that only high-grade squamous intraepithelial lesion (H-SIL) detection in cervical biopsy and status of the LEEP margins were significantly predictive of RD (OR 5.4, 95%CI 1.08-27.7,  $p < 0.05$  and OR 7.05, 95%CI 2.1-23.1,  $p = 0.001$ ; respectively). The combination of histological examination of resection margins plus H-SIL detection in cervical biopsy would help to classify LEEP-treated patients into categories of different risk levels of residual disease.

**Keywords:** Cervical intraepithelial neoplasia; Minimal residual disease; Conization; Papillomavirus infections.

## OPEN ACCESS

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## INTRODUCTION

Uterine cervical carcinoma is one of the most common cancers in the female population in Brazil and worldwide [1]. The infection caused by human papillomavirus (HPV) is involved in the pathogenesis of this type of cancer, which has as precursor lesions cervical intraepithelial neoplasms (CIN), particularly those of a high degree. There are several options in the treatment of this type of pre-invasive disease, specifically the high-grade squamous intraepithelial lesion (H-SIL) or recurring low-grade squamous intraepithelial lesion (L-SIL), which include among others: cold knife conization (CKC) and loop electrosurgical excision procedure (LEEP) [2].

LEEP was introduced in 1989 by Prendiville et al. to treat lesions that could be visualized by colposcopy [3]. The procedure involves excision of cervical tissue through the thermal effect of alternating electric current with high frequency with the use of a small loop. It is an outpatient procedure, safe, conducted under local anesthesia and has a lower bleeding rate when compared to CKC, and is therefore increasingly preferred [4, 5]. Moreover, it is related to lower obstetric adverse outcome rates, ensuring the preservation of women's fertility and proper evaluation of the resection margins, and can have healthy healing rates in up to 95% of cases [6, 7].

The literature is still controversial about the influence of margin compromise on the occurrence of residual disease (RD). However, most studies show that there is a positive relationship [6-9]. There is also divergence in the literature on what would be the predictors of RD, with some authors pointing to age [8-11] and others looking at persistent HPV infection [9-11] associated with a higher risk of persistent disease. On the other hand, studies are rare in which the LEEP is performed only by resident physicians, enabling the assessment of whether the experience and skill of the surgeon contribute to the increase of the compromised margin (CM) and RD prevalence.

Moreover, few studies have been conducted in Brazil on the prevalence of CM and RD after LEEP [12]. It is not well-defined in this country which factors are related to the higher risk of the development of RD and how prevalent CM and RD are in Brazilian women.

The objective of this study is to evaluate the prevalence of compromised margins and residual disease post LEEP and check the predictors of RD in a reference cervical pathology center.

## MATERIALS AND METHODS

This is a retrospective analysis of 86 women with histologically confirmed CIN 1, 2 or 3 who were treated by LEEP in the Cervical Pathology Department of a Federal University Hospital in Brazil between January 2015 and May 2016. Approval was obtained from the institutional review board.

Conization was performed exclusively by gynecology residents using loop diathermy with a blend setting and a power output of 40 W. A 5-mm cautery ball with a power setting of 50 W was used to achieve hemostasis. Monsel solution was applied as needed. All specimens were marked for orientation with a delayed absorbable suture at the 12-o'clock position for pathology examination.

Standard follow-up in our department was a visit at 6 and 12 months after cervical conization with clinical examination, Pap smear (PS), colposcopy and eventual biopsy. However, if surgical margins were affected, the first check-up was performed at 3 months after conization. Cervical smears were stained using the Papanicolaou method and were evaluated following the 2014 Bethesda criteria [13]. All women with abnormal cytology and/or an abnormal transformation zone underwent a colposcopy directed biopsy. When the transformation zone was not visible or only partially visible or no colposcopic abnormality was identified, an endocervical curettage using a Kervokian curette was also performed.

Criteria for defining RD were based on positive surgical margins at conization and/or abnormal check-up at 6-12 months. The presence of RD was based on positive histology of colposcopy-directed biopsy or endocervical curettage. Histologic evidence of CIN of any grade was considered as RD. Women with two consecutive negative Pap smears and normal colposcopy were considered negative for RD.

Statistical analysis was performed using SPSS statistical software version 20.0 for Windows. Quantitative variables were compared using Student's t-test or Mann Whitney test. Categorical variables were compared using the chi-squared test or Fisher's exact test. Odds ratio and 95% confidence intervals (95% CI) were estimated by logistic regression analysis. Variables found to be significant by univariate analysis were examined by multivariate analysis using the Cox proportional hazards regression model. P values <0.05 were considered statistically significant for all statistical tests.

## RESULTS

In the study period, 93 women were treated with LEEP for the first time, however, only 86 could be included, since 7 were excluded from the analysis because they did not attend any follow-up visit, or because they were diagnosed with invasive cervical carcinoma.

The mean age of patients was  $35.8 \pm 9.5$  years (range 18-61). Among the patients, 15.1% were aged between 18 and 25 years, 75.6% between 25 and 50 years and 9.3% were older than 50. Regarding the number of pregnancies and childbirths, the majority had had between 1 and 3 (46.5%). Most women, more than 90%, were in the menopause. Clinically, most patients presented negative serologies for syphilis, HIV and hepatitis. Previously with the LEEP, 81.4% had a biopsy revealing H-SIL. Table 1 presents the sociodemographic and clinical characteristics of the patients.

**Table 1.** Sociodemographic and clinical characteristics of the patients.

| Variable        | Category    | n  | %    |
|-----------------|-------------|----|------|
| Age             | 18-25       | 13 | 15.1 |
|                 | 25-50       | 65 | 75.6 |
|                 | >50         | 8  | 9.3  |
| Color           | White       | 9  | 10.5 |
|                 | Black       | 2  | 2.3  |
|                 | Mestice     | 75 | 87.2 |
|                 | Yellow      | 0  | 0    |
| Parity          | Nulliparous | 15 | 17.4 |
|                 | Multiparous | 71 | 82.6 |
| Menopause       | No          | 79 | 91.9 |
|                 | Yes         | 7  | 8.1  |
| Cervical biopsy | Normal      | 2  | 2.3  |
|                 | L-SIL       | 9  | 10.5 |
|                 | H-SIL       | 70 | 81.4 |
|                 | Unknown     | 5  | 5.8  |
| Total           |             | 86 | 100  |

Abbreviations: L-SIL = low-grade squamous intraepithelial lesion; H-SIL = high-grade squamous intraepithelial lesion.

The LEEP histopathology was positive for H-SIL or higher in 58.1% of cases (50/86) and for L-SIL or lower in 41.9% of cases (36/86). After LEEP, a total of 64% patients (55/86) had clear margins in the operation specimens and the corresponding number of patients with positive surgical margins was 34.8% (30/86). In 1.2% of patients (01/86) the resection margins were uncertain. Of the overall group of patients with positive margins, the exocervical margin was apparent in 3.3% (01/30), the endocervical margin in 16.6% (05/30), and both margins in 80% cases (24/30) (Table 2).

**Table 2.** Anatomopathological characteristics of loop electrosurgical excision procedure specimens.

|                            | n %        | n RD %    |
|----------------------------|------------|-----------|
| Clear surgical margins     | 55 (64%)   | 8 (14.5%) |
| Positive surgical margins  | 30 (34.8%) | 15 (50%)  |
| Exocervical                | 01         | 01        |
| Endocervical               | 05         | 05        |
| Both                       | 24         | 09        |
| Uncertain surgical margins | 01 (1.2%)  | 01 (100%) |
| Total                      | 86 (100%)  |           |

Abbreviations: RD = Residual Disease.

**Table 3.** Multivariate analysis of residual disease risk.

|                          | OR   | 95% CI |      | p value |
|--------------------------|------|--------|------|---------|
|                          |      | L      | H    |         |
| Positive surgicalmargins | 7.05 | 2.1    | 23.1 | 0.001   |
| H-SIL cervical biopsy    | 5.4  | 1.08   | 27.7 | <0.05   |

Abbreviations: OR = Odds Ratio; CI = Confidence Interval; L = Lower; H = Higher; H-SIL = high-grade squamous intraepithelial lesion.

The total number of patients with residual disease (RD) was 26.7% (23/86). The mean age of patients was  $38.2 \pm 8.7$  in the RD group and  $34.6 \pm 10.4$  in non-recurrent cases. In our series, age is not predictive of RD ( $p=0.075$ ). Significant differences in risk of RD depending on the involved margin were observed: 65.2% (15/23) of cases with positive margins vs. 24.5% (13/53) of cases with clear margins ( $p<0.0001$ ). Patients with high-grade lesions in the cervical biopsy had a higher percentage of RD compared to low-grade lesions or lower ( $p<0.05$ ).

In multivariate analysis, only previous cervical biopsy with H-SIL and positive cervical margins were significant predictive factors of residual disease (OR 5.4; 95% CI 1.08-27.7;  $p<0.05$  and OR 7.05; 95% CI 2.1-23.1;  $p=0.001$ ) (Table 3). Only cases with complete data (77 patients) were included in the analysis.

In the follow-up, 27.9% of cases (24/86) underwent surgery a second time in response to an abnormal smear test or colposcopy. Of these 24 reintervention cases, 14 patients were treated with LEEP and 10 with CKC.

## DISCUSSION

In managing women with CIN, the goal is to prevent possible progression to invasive cancer while avoiding over-treatment of lesions that are likely to regress. In a previous study it was seen that the risk of cervical cancer is elevated for at least 20 years after the initial treatment of CIN [14], which underlines the importance of detecting factors that can predict, prior to follow-up, the eventual development of residual disease (RD). In our study, 26.7% of RD was observed.

The status of resection margins has been shown to be a predictor of RD, but the frequencies quoted in the literature are extremely variable, with values ranging from 11.9% to 53.4% [2, 8]. In this study, loop electrosurgical excision procedures were performed exclusively by residents and the frequency of positive margins after LEEP was 34.9%, which is comparable to the average for cases described in the literature. For the present research, the relationship between compromised margins and RD was statistically significant, according to the results found by Cejtin et al. [15], which corroborated findings of meta-analysis with more than 35 thousand women undergoing treatment for CIN [7].

Other predictive indicators of RD such as age, lesion severity and smoking have been described [16]. In our study, we found no significant difference with respect to age and smoking between patients with normal follow-up and those who had a RD. However, there was a statistically significant relationship between the lesion severity, represented by previous LEEP cervical biopsy with H-SIL, and RD.

This is a retrospective observational study that aims to provide an analysis of risk factors for RD after LEEP. There are certain limitations to be considered about our study. First, the retrospective nature of our case-series data unfortunately does not warrant a very high level of evidence. Secondly, the surprisingly large proportion of cases with positive or non-free margins suggests that the surgical technique was not optimal, which probably occurred because the procedure was performed exclusively by resident physicians. Moreover, although LEEP margins in pathology reports may be positive, the patient may in fact have no residual disease. This can be attributed to the thermal effect of the loop during surgery at the margins of the remaining cervix and to the use of diathermy for hemostasis, which both eradicate any remaining dysplastic cells.

Finally, after the LEEP it was not possible to perform, apart from Pap smear, colposcopy and cervical biopsy, a molecular method - PCR for detection of HPV in tissues, which may have generated a prevalence of RD underestimated in our analysis. Reliable data on the prevalence of HPV types is important for determining the types that should be included in a screening and follow-up program, since women with negative PS considered without RD, but infected with high-risk viral types should be more closely monitored because infection with a high oncogenic potential virus such as HPV 16 or 18 significantly increases the risk of cervical cancer in the future.

Therefore, the precise number of HPV-infected women is unknown in this study, and it is difficult to determine accurately, even with close follow-up with colposcopy/cytology. However, probably if the viral serotype research was performed in the patients, most would be found with persistent infection with HPV 16 and 18 as well as women from other developed countries, since a study with 97 women in the same region of Brazil where this study was performed demonstrated that approximately 40% had PCR positive for both serotypes [17].

In conclusion, we have shown that the most important prognostic markers for RD in patients with CIN treated with LEEP conization are affected surgical margins and H-SIL in cervical biopsy. Therefore, the combined evaluation of surgical margins status and cervical lesion severity could allow for the subdivision of patients treated with LEEP into categories of different risk levels of residual disease.

## AUTHORS' CONTRIBUTION

RNC conceived the idea for and designed the study. ACH and AFS were involved in data collection and performed the research. ACH and TDF analyzed the data, conducted the literature search, study selection and prepared manuscript. RNC, AKG and CAJ revised the manuscript for final submission. The final manuscript was read and approved by all authors.

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