

Factors Associated With the Persistence/Recurrence of CIN2/3 in Women Submitted to Loop Electrosurgical Excision Procedure in a Teaching Hospital in Northeastern Brazil: A Case-Control Study

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Abstract: Objective: This study aimed to identify risk factors associated with the persistence/recurrence of cervical intraepithelial neoplasia (CIN) 2/3 in women submitted to loop electrosurgical excision procedure (LEEP) in a hospital in northeastern Brazil.

Materials and Methods: A case-control study included 50 women with and 50 women without persistence/recurrence of CIN2/3 after LEEP at the Instituto de Medicina Integral Prof. Fernando Figueira (IMIP) between 2004 and 2011. Data were collected from June to December 2011. Inclusion criteria were diagnosis of CIN2/3 during follow-up (cases) and women free of CIN2/3 after a follow-up of 2 years or longer (controls). Exclusion criteria (cases/controls) were LEEP performed at another hospital, LEEP performed for persistent CIN1, invasive carcinoma in the cone specimen or at cytology, and/or histopathology within a 2-year follow-up period. Persistence was defined as residual disease detected in the first year after LEEP, and recurrence was defined as the reappearance of a lesion more than 1 year after surgery. Bivariate analysis was performed for biological, sociodemographic, sexual, reproductive, lifestyle, and clinical variables. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated, and a multiple logistic regression analysis was performed to control for potential confounding factors. The study was approved by IMIP's internal review board.

Results: Multiple logistic regression analysis showed a significant association between persistence/recurrence of CIN2/3 and living outside the capital city (OR = 3.11, 95% CI = 1.14–8.41), smoking (OR = 4.22, 95% CI = 1.18–15.05), and positive endocervical margins (OR = 6.58, 95% CI = 2.37–18.28).

Conclusions: Women with persistence/recurrence of CIN2/3 are more likely to live outside the state capital, be smokers, and have positive endocervical margins, so these women should be followed up more closely.

Key Words: conization, cervical intraepithelial neoplasia, local recurrence of neoplasia

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The natural history of cervical cancer begins with a human papillomavirus (HPV) infection and continues with persistence of the infection, progression to a precursor lesion (high-

grade cervical intraepithelial neoplasia [CIN] 2/3), and, finally, the development of invasive cancer.¹

The principal techniques used for treating CIN2/3 encompass destructive treatments such as cryotherapy, electrocautery, and laser therapy as well as excisional therapies such as the loop electrosurgical excision procedure (LEEP), cold knife conization, and hysterectomy.² The high rates of regression of low-grade CIN (CIN1/HPV) allow expectant management. Surgical techniques are indicated in cases of high-grade CIN (CIN2/3)^{2,3} or in cases of persistent CIN1.^{3,4}

The risk of recurrence or persistence of the disease after LEEP conization is about one third of the patients.⁵ The concept of persistence/recurrence of CIN after the established treatment remains controversial. Some authors define persistence or residual disease as the identification of CIN in the first year after LEEP and relapse or recurrence as the reappearance of CIN more than a year after treatment.^{6–8}

Possible risk factors for the persistence/recurrence of CIN2/3 are as follows: a positive endocervical margin,^{9,10} the number of quadrants involved, the initial histological grade of the lesion,¹¹ lesions in the endocervical canal, incomplete excision of the lesion,^{9,10} older than 50 years,¹² HIV positivity,¹³ and persistence of HPV.¹¹

Identifying the factors predictive of persistence/recurrence and the target population would contribute toward improving the quality of care and consequently reducing the incidence of invasive cervical cancer in this population.

METHODS

In this analytical, observational, retrospective, case-control study, histopathological results from LEEP excision were revised. For each woman with persistence/recurrence (case) in her LEEP specimen, another examination belonging to a woman free of persistence/recurrence (control) was selected, comprising a total of 50 cases and 50 controls. Data were collected between June and December 2011. A total of 715 medical records of women with CIN2/3 were analyzed, 582 of whom had been submitted to LEEP. One hundred of these women were included in the study (see Figure 1).

The inclusion criteria for both cases and controls were women with a histopathological diagnosis of CIN2/3 who had been submitted to LEEP for treatment of the lesion at the Women's Healthcare Center, Instituto de Medicina Integral Prof. Fernando Figueira (IMIP) between 2004 and 2011. The women had to have undergone at least 2 years of follow-up at the clinic (except for patients with persistence/recurrence of the disease). The exclusion criteria for both cases and controls consisted of women who had undergone LEEP in another institute, women submitted to LEEP but with no evidence of CIN2/3, and a histopathological diagnosis of invasive cervical carcinoma in the LEEP specimen or at cervical cytology and/or

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This study was conducted at the Instituto de Medicina Integral Prof. Fernando Figueira, Recife, Pernambuco, Brazil.

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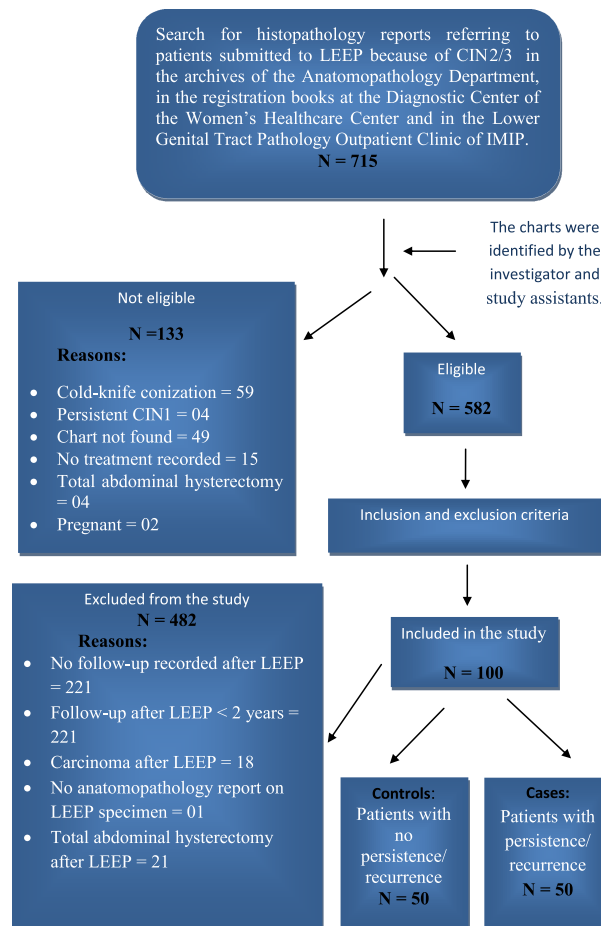


FIGURE 1. Flowchart depicting the selection of cases according to the data obtained from medical charts.

histopathology with a cervical biopsy performed within 2 years of surgery.

The internal review board of IMIP approved the study protocol under reference number 2168.

A pilot study was conducted in which the first 30 women (15 cases and 15 controls) were evaluated. The endocervical margin was affected in 60% of the women with persistence/recurrence, whereas in the group of women with no persistence/recurrence of the disease, the margin was involved in 30% of cases. On the basis of these data and using the STATCALC function of the Epi-Info software program, version 7.1.0.6 (CDC, Atlanta, GA), a sample size of 100 women was calculated.

Using the name and the registration number of the women submitted to LEEP, the patients' charts were then located in the institute's archives. Once a case was identified, the woman was included in the study. Controls were selected in sequence by including the woman submitted to LEEP immediately after the case if there was no persistence/recurrence of CIN2/3. Persistence was defined as residual disease identified in the first year after LEEP, and recurrence (or relapse) was defined as the appearance of a lesion more than 1 year after LEEP.⁶⁻⁸

The dependent or outcome variable was the persistence/recurrence of CIN2/3 and the independent variables were age, marital status, schooling, place of residence, age at sexual debut, number of partners, parity, condom use, alcohol consumption, smoking, cone margin status, glandular involvement, the use of antiretrovirals, and HIV status.

First, a bivariate analysis was performed to test the association between persistence/recurrence (the dependent variable) and the various biological, sociodemographic, sexual, reproductive, lifestyle, and clinical variables. The χ^2 test of association and Fisher exact test were used, as appropriate. Significance was established at 5%. To determine the strength of the association, the odds ratios (ORs) were calculated together with their respective 95% confidence intervals (95% CIs).

All the variables used in the bivariate analysis were included in the multivariate analysis using the stepwise multiple logistic regression procedure, based on the theoretical hierarchical model.¹⁴ In this model, the variables were divided into blocks: distal (age and place of birth), medial (age at sexual debut, number of sexual partners, parity, condom use, smoking, and alcohol consumption), or proximal levels (immunosuppression, glandular involvement, and involvement of the margins) in accordance with their association with persistence/recurrence. In each block, the variables that remained associated with the outcome variable at a significance level of 20% were selected and a new regression analysis was performed, selecting the variables in the block that remained associated with the outcome at a significance level of 5%.

RESULTS

The age of the women in the present study ranged from 17 to 70 years. In the group with persistence/recurrence of the disease, 27 of the women (54%) were older than 40 years

TABLE 1. Biological, Sociodemographic, Sexual, and Reproductive Characteristics of Women Submitted to LEEP

Variable	Persistent or recurrent disease		No persistent or recurrent disease		OR	95% CI	<i>p</i>
	<i>n</i>	%	<i>n</i>	%			
Age > 40 y	27	54	11	22	4.16	1.74–9.93	.0009
Living outside the capital city	26	52	16	32	2.30	1.02–5.19	.0427
≥5 sexual partners	12	25	07	14.9	1.90	0.67–5.36	.2182
Sexual debut at ≤16 y	34	68	23	48.9	2.21	0.97–5.05	.0566
Parity ≥ 4	14	28	07	14	2.40	0.87–6.55	.0856
No condom use	43	86	45	90	0.68	0.20–2.31	.5382

95% CI indicates 95% confidence interval; LEEP, loop electrosurgical excision procedure; OR, odds ratio; *p*, χ^2 test.

compared to 11 women (22%) in the control group (OR = 4.1, 95% CI = 1.7–9.9, *p* = .0009) (see Table 1).

The bivariate analysis showed that women with persistence/recurrence of CIN2/3 were around twice as likely to come from a part of the state other than the capital city (OR = 2.3, 95% CI = 1.02–5.19, *p* = .0023). No association was found between persistence/recurrence and having had 5 or more sexual partners, having given birth to 4 or more children, having been 16 years of age or younger at the time of first sexual intercourse, or the non-use of condoms (see Table 1).

Analysis of the association between persistence/recurrence of the disease and the women's behavioral and clinical variables showed an approximately 7-fold increased likelihood of the women in the recurrence/persistence group being smokers (OR = 7.04, 95% CI = 2.18–22.72, *p* = .0003) and consuming alcohol (OR = 7.04, 95% CI = 2.18–22.72, *p* = .0003). No association was found with the use of antiretrovirals or corticosteroids or with HIV positivity (see Table 2).

Furthermore, it was found that in the women with persistence/recurrence of CIN2/3, the risk of the endocervical margin being affected was 6.5 times higher (OR = 6.5, 95% CI = 2.62–16.11, *p* = .00002), the risk of the ectocervical margin being affected was 6 times higher (OR = 6.0, 95% CI = 2.45–14.67, *p* = .00004), the risk of having glandular involvement was around 4 times higher (OR = 3.6, 95% CI = 1.55–8.41, *p* = .0022), and the risk of finding CIN2/3 in the surgical specimen was 15 times higher (OR = 15.4, 95% CI = 1.92–124.0, *p* = .001; see Table 3).

After hierarchical multiple logistic regression, the following variables remained significantly associated with persistence/

recurrence in the final model: coming from somewhere in the state other than the capital city of Pernambuco (OR = 3.11, 95% CI = 1.14–8.41), being a smoker (OR = 4.22, 95% CI = 1.18–15.05), and having positive endocervical margins (OR = 6.58, 95% CI = 2.37–18.28; see Table 4).

DISCUSSION

In the present study, after hierarchical multiple logistic regression analysis, the following variables remained as risk factors for the persistence/recurrence of CIN2/3: being from somewhere in the state other than the capital city, being a smoker, and having a histopathology report of endocervical margin involvement.

There was an approximately 3-fold greater likelihood of the women in the persistence/recurrence group residing outside the capital city of the state of Pernambuco. This finding may reflect difficulties in accessing health care services, which tend to be poorly structured and poorly distributed throughout the state. This is a factor that generates inequalities, delays in initiating the appropriate treatment, and a consequent aggravation of the disease.¹⁵ According to the incidence estimates produced by the National Cancer Institute for 2012, the rate of new cases of cervical cancer in the state of Pernambuco is 917 per 100,000 women, while in the capital city Recife, this rate is 190 cases per 100,000 women, highlighting the difference of 727 cases per 100,000 women between the women living in the capital city and those living elsewhere in the state.¹⁶

Women with persistence/recurrence of CIN2/3 were 4.2 times more likely to be smokers. This finding may be explained by the direct effect of smoking in reducing the cells of the

TABLE 2. Behavioral and Clinical Characteristics of Women Submitted to LEEP

Lifestyle-related variables	Persistent or recurrent disease		No persistent or recurrent disease		OR	95% CI	<i>p</i>
	<i>n</i>	%	<i>n</i>	%			
Smoking	19	38	4	8	7.04	2.18–22.72	.0003 ^a
Alcohol consumption	19	38	4	8	7.04	2.18–22.72	.0003 ^a
Use of antiretrovirals	3	6	1	2	3.12	0.31–31.14	.3086 ^a
Use of corticoids	1	2	—	—	—	—	—
HIV positivity	3	8.8	2	6.7	1.35	0.21–8.70	.5603 ^a

95% CI indicates 95% confidence interval; LEEP, loop electrosurgical excision procedure; OR, odds ratio; *p*, χ^2 test.

^aFisher exact test.

TABLE 3. Histopathological Characteristics of the LEEP Specimens

Histological pattern in the LEEP specimen	Persistent or recurrent disease		No persistent or recurrent disease		OR	95% CI	<i>p</i>
	<i>n</i>	%	<i>n</i>	%			
Positive ectocervical margin	7	14.6	2	4.1	4.01	0.78–20.40	.07469 ^a
Positive endocervical margin	30	62.5	10	20.4	6.50	2.62–16.11	.00002
Positive surgical margins	30	60.0	10	20.0	6.00	2.45–14.67	.00004
Indeterminate endocervical margin	6	12.5	5	10.2	1.25	0.35–4.43	.72143
Indeterminate ectocervical margin	1	2.1	1	2.0	1.02	0.06–16.80	.74742 ^a
Glandular involvement	37	74.0	22	44.0	3.62	1.55–8.41	.00229
Presence of CIN2/3	49	98.0	38	76.0	15.47	1.92–124.0	.00107

95% CI indicates 95% confidence interval; LEEP, loop electrosurgical excision procedure; OR, odds ratio; *p*, χ^2 test.

^aFisher exact test.

immune system, notably the Langerhans cells, thus contributing to the persistence of HPV infection in the cervix.¹⁷ Nevertheless, other authors have failed to identify smoking as a factor associated with the persistence/recurrence of CIN2/3.^{13,18,19} Although these studies were appropriately designed to evaluate factors predictive of the persistence/recurrence of CIN2/3, only one of them¹³ conducted a logistic regression analysis to exclude potential confounding factors, which may in part justify the differences in findings. Another possible explanation may lie in the fact that the present study is retrospective and smoking was considered as a single fact in a woman's life, whereas this fact may not have coincided with the time of the disease. This may have introduced a bias and the question should be investigated further in future studies designed to evaluate this association.

Concerning the histopathological characteristics of the cone margins, the only variable identified as a risk factor for the persistence/recurrence of CIN2/3 in the multivariate analysis was involvement of the endocervical margin. This finding is in agreement with the reports of other authors.^{18,20} Studies suggest that involvement of the endocervical margin is an independent predictive factor of a risk of persistence/recurrence of CIN2/3.^{11,21} This is biologically plausible because CIN2/3 in the endocervical cone margin may be indicative of an increased probability of the existence of a lesion further inside the canal, hence not completely eradicated in view of the more superficial nature of the LEEP excision.^{11,21} The present study found involvement of the endocervical margin in 62.5% of the women with persistence/recurrence of CIN2/3. After logistic regression analysis, women with persistence/recurrence of CIN2/3 were found to be 6.5 times more likely to have endocervical margin involvement. This finding is in agreement with reports from the previously mentioned studies.^{12,21}

Some studies have shown LEEP to be associated with rates of persistence/recurrence that range from 1.9% to 39%, with the

risk being higher when the surgical margins are affected irrespective of whether it is the endocervical or ectocervical margin that is involved. However, the predictive value of this variable alone is low.^{6,22–24} Other studies have suggested that endocervical glandular involvement,¹⁹ multifocal lesions, and persistent HPV infection may explain recurrences in patients with disease-free margins.^{12,25,26}

The fact that endocervical gland involvement did not remain associated with recurrence/persistence of CIN2/3 after logistic regression analysis in the present study contradicts findings from previous studies. The variable, however, was statistically significant in the bivariate analysis, with an approximately 4-fold greater risk of persistence/recurrence of CIN2/3 after LEEP. A retrospective study conducted in Barcelona to evaluate predictive factors of persistence/recurrence of CIN2/3 reported glandular involvement in 28.6% of cases; however, this variable also failed to remain statistically significant in the logistic regression analysis.²⁷

In the present study, no statistically significant association was found between endocervical gland involvement and/or narrow margins and persistence/recurrence of CIN2/3. One explanation for this finding may be the result of thermal destruction of the peripheral ectocervical lesion during electrical cauterization after LEEP. This hypothesis may not apply equally to endocervical lesions because the colposcopist generally avoids cauterization very close to the endocervical os to minimize the risk of endocervical stenosis after LEEP.^{11,22}

This was a retrospective study in which data were collected from medical charts; therefore, there are limitations that result from having to use stored data. Consequently, we were unable to conduct a statistical analysis of all the biological and sociodemographic variables proposed in the initial project. We therefore suggest that a further prospective cohort study be conducted to obtain data in a more methodologically appropriate way.

TABLE 4. Factors Associated With Persistence or Recurrence of CIN2/3 After Multivariate Regression Analysis

Variables	OR	95% CI	Coefficient	SE	<i>p</i>
Not living in the capital city	3.11	1.14–8.41	1.134	0.508	.0255
Smoker	4.22	1.18–15.05	1.441	0.648	.0261
Positive endocervical margin	6.58	2.37–18.28	1.885	0.520	.0003
Constant	*	*	–1.5551	0.426	.0003

95% CI indicates 95% confidence interval; CIN, cervical intraepithelial neoplasia; OR, odds ratio; *p*, χ^2 test; SE, standard error.

In countries where the access of the population to molecular biology tests is restricted, acquiring further knowledge on the clinical variables that constitute factors predictive of a risk of persistence/recurrence of CIN2/3 is of fundamental importance. This study suggests that women with CIN2/3 who come from regions in which access to health care services is difficult, who are smokers, or in whom histopathology of the specimen reveals involvement of the endocervical cone margin are at a greater risk of developing persistence/recurrence of CIN2/3. Consequently, differentiated management is required in these cases. These women should be reevaluated at 4- to 6-month intervals by cervical cytology and colposcopy and should be encouraged to stop smoking. If follow-up is difficult because the woman is unable to travel to the reference center, immediate surgery may be indicated. Options would include a new LEEP, cold knife conization, or hysterectomy and should take the woman's age and parity into consideration.

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