**RESEARCH ARTICLE**

# Risk factors related to recurrence after surgical excision procedure for cervical dysplasia

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**Abstract**

**Background:** This study aimed to identify the risk factors of recurrence following surgical excisional procedures for cervical intraepithelial lesions (CIN).

**Methods:** We conducted a retrospective cohort study of women with cervical pathology treated surgically over seven years. All patients with surgical interventions of the cervix were recorded and analyzed according to the recurrence following their primary treatment. We utilized the Chi-square test and the multivariate regression analysis to identify recurrence risk factors. We also employed the Kaplan-Meier survival analysis for disease recurrence.

**Results:** In total, 83 patients were reported; 81 (97.6 %) were treated with loop electrosurgical excision, and two (2.4 %) with cold knife conization. The histopathological results of the treatment approach showed one case (1.2 %) of low-grade intraepithelial lesions, 70 (84.4 %) high-grade intraepithelial lesions (HSIL), five (6 %) with IA1, and two (2.4 %) with IA2, cervical cancer, while in five patients (6 %) the results were negative for cervical pathology. Recurrence was diagnosed in 23 cases (27.7 %), and HSIL was diagnosed in 10 patients (12 %). The median time of recurrence was 11.6 months. Positive endocervical margins [odds ratio (OR): 52.478; 95 % confidence interval (CI): 8.315-331.203; p <0.001], excision of the cone in multiple specimens (OR: 8.793; 95 % CI: 1.854-41.693; p =0.006), and depth of cone less than one cm (OR: 21.225; 95 % CI: 3.176-141.863; p =0.002) were identified as independent risk factors for recurrence.

**Conclusions:** Positive endocervical margins, multiple loop passes, and depth of the cone less than one cm were the most significant risk factors for recurrence. HIPPOKRATIA 2023, 27 (4):132-140.

**Keywords:** Cervical pathology, dysplasia, high-grade cervical lesions, recurrence, treatment

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## Introduction

Cervical cancer remains the fourth most common cancer in women worldwide, even though there is a substantial decrease in its rate in high-income countries due to increased access to screening and prevention[1]. Cervical cancer can be prevented and treated at early stages; secondary prevention of cervical cancer includes cervical cytology and molecular biology in detecting human papillomavirus (HPV) DNA or mRNA[2]. Furthermore, colposcopy of high magnification field with or without punch biopsies can be used to diagnose the cervical lesion[3]. On the other hand, HPV vaccination, which is the primary prevention against HPV infection, should start as early as possible, starting at nine through 14 years of age, according to American Society of Clinical Oncology (ASCO) guidelines[4].

The United States Preventive Services Task Force (USPSTF) recommends screening for cervical cancer every three years in women aged 21 to 29 years with cervical cytology alone and either every three years in women aged 30 to 65 years with cervical cytology alone or every five years with high-risk HPV (hrHPV) testing alone or every five years with hrHPV testing combined with cytology (co-testing)[5]. Furthermore, the USPSTF recommends against screening in women younger than 21 years and those older than 65 with adequate prior screening[5]. The American Cancer Society recommends screening in women aged 25 to 65, either with a Papanikolaou (PAP) smear test every three years or with hrHPV testing or a co-testing every five years[6].

According to the World Health Organization (WHO) and the Lower Squamous Terminology (LAST), histopathology reports of HSIL (High-Grade Squamous Intraepithelial Lesion) should include CIN2 and CIN3 (Cervical Intraepithelial Neoplasia), while LSIL (Low-Grade Squamous Intraepithelial Lesion) includes CIN1[3]. HPV 16 and 18 infections pose the highest risk for CIN3 and occult cancer; thus, additional evaluation with colposcopy and biopsy is mandatory for these cases[7]. On the other hand, surveillance should be preferred to treatment

in LSIL lesions. Moreover, continued surveillance solely with HPV testing or co-testing at three-year intervals for a minimum of 25 years is recommended after treatment of HSIL or adenocarcinoma in situ (AIS)[8]. When HPV testing or co-testing is not offered, surveillance with cytology alone every six or 12 months is recommended.

HPV infection is necessary for the development of cervical cancer; however, other co-factors may increase the risk[9,10]. According to the literature, the most significant factors for recurrence of the cervical lesions after surgical treatment are the positive surgical margins of excision and the depth of the cone[11-14]. Greece has a population of about 4.6 million women, with cervical cancer being the 3rd most common female cancer in women aged 15 to 44 years[15]. Feedback from data collected from the first phase of the pilot implementation of the national screening program showed that 439 women were referred for colposcopy, and biopsies detected 138 cases of CIN II+ lesions, out of which 17 with cervical cancer[16].

The ongoing effort to optimize cervical cancer prevention in our country renders the investigation of possible risk factors of recurrence, as well as the adequate treatment of the lesions. This study aimed to present and analyze possible risk factors for recurrent cervical dysplasia after primary surgical excision procedures in patients with cervical intraepithelial pathology.

## Material and methods

In this retrospective cohort study, we included patients with cervical pathology surgically treated over seven years (2014-2020) in the Cervical Pathology Unit of the Third Department of Obstetrics and Gynecology, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece. An Institutional Review Board was obtained from the Hippokration Hospital for the present study (decision No 56442, date: 07/12/2021). All the participants consented for the use of their data in research.Patients mainly from Northern Greece attended the Unit for a screening test, primary diagnosis, second opinion, or treatment of pathology of the lower genital tract (cervix, vagina, vulva). All the patients with surgical interventions, including loop electrosurgical excision procedure (LEEP) or cold knife conizations (CKC) of the cervix, were recorded and analyzed according to the recurrence following their primary treatment. Of note, pregnant women with cervical pathology, as well as those with a surgical history of hysterectomy or cancer under hormonal treatment, were excluded. The follow-up included cervical cytology and colposcopy ± punch biopsy within six-month intervals at the Cervical Pathology Unit. The follow-up continued in some cases until the normalization of the cytology and colposcopy. All the colposcopies were performed by two obstetricians-gynecologists certified in colposcopy (IK, AT).

The main endpoint of the current study was to identify the risk factors for the recurrence of patients with surgical excision procedures for cervical pathology in our Unit. Patient age (reference: 35 years)[17], age of first sexual intercourse (reference: 18 years)[18], number of sexual partners (reference: five partners)[19], severity of the cervical disease according to the LEEP histology (reference: CIN2)[7], surgical endocervical margins following the primary treatment (free vs involved), cone depth (reference: one cm)[11] and number of passes during conization (reference: single pass)[20] were investigated as potential risk factors for recurrence.

We present the qualitative variables as frequencies and percentiles, while the median and range describe the quantitative variables. We used the Shapiro-Wilk test to check for the normality of distribution and the chi-square test to evaluate the associations between categorical variables. Using statistically significant variables obtained by univariate analyses, we conducted a multivariate logistic regression analysis (enter method) to identify independent risk factors for recurrence. We employed the Kaplan-Meier survival analysis to estimate the recurrence rate between the different parameters of the study, such as histology of the lesion, number of passes/specimens (1 or >1), depth of the cervical cone (cutoff: one cm) and clear endocervical margins of the cervix (yes/no). The Odds Ratios (OR) with 95 % Confidence Intervals (CI) were estimated. Statistical significance was considered at 5 %. The statistical program IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA) was used for the analyses.

## Results

In total, 83 patients with cervical pathology were treated during the study period by surgical excision procedures. The median follow-up of the study population was 24 months (range: 12-54 months) (Table 1).

Regarding cervical cytology, we identified 12 (14.5 %) patients with atypical squamous cells of undetermined significance (ASCUS), 5 (6 %) with atypical squamous cells that cannot exclude high-grade intraepithelial lesions (ASC-H), 16 (19.3 %) with LSIL, and 50 (60.2 %) patients with HSIL. In total, we performed 45 HPV-DNA tests, of which 44 (97.8 %) were positive and 5 HPV-mRNA tests, all positive. Following colposcopic evaluation, according to the REID classification[21], three patients (3.6 %) were classified as LSIL (CIN1) and 80 patients (96.4 %) as HSIL [CIN2 n =41 (49.4 %), CIN3 n =39 (47 %)]. The colposcopic evaluation was unsatisfactory in two (2.4 %) of 83 cases (two cases of CIN 1 colposcopic evaluation, according to REID). Punch biopsy

**Table 1:** Characteristics of the 83 women with cervical pathology treated surgically over seven years who were included in this retrospective cohort study.

| Sample characteristics | Median | Range |
|---|---|---|
| Age (years) | 37 | 22 - 76 |
| Age of sexual initiation (years) | 18 | 13 - 25 |
| Number of partners | 5 | 1 - 50 |
| Follow-up (months) | 24 | 12 - 54 |

CI: Confidence Interval, IQR: Interquartile Range.

**Table 2:** Cervical pathology for the 83 women according to the diagnostic and therapeutic approach of the participants (n =83).

| Cervical cytology | n | Colposcopy (REID classification) | n | Histology (punch biopsy) | n | Histology (treatment)* | n |
|---|---|---|---|---|---|---|---|
| ASCUS | 12 (14.5) | CIN2 | 6 (7.2) | CIN2 | 9 (10.8) | CIN2 | 4 (4.8) |
| | | CIN3 | 6 (7.2) | CIN3 | 3 (3.6) | CIN3 | 8 (9.6) |
| ASC-H | 5 (6.0) | CIN2 | 3 (3.6) | CIN2 | 2 (2.4) | CIN2 | 1 (1.2) |
| | | CIN3 | 2 (2.4) | CIN3 | 3 (3.6) | CIN3 | 4 (4.8) |
| LSIL | 16 (19.3) | CIN1 | 1 (1.2) | CIN1 | 1 (1.2) | CIN1 | 0 |
| | | CIN2 | 9 (10.8) | CIN2 | 13 (15.7) | CIN2 | 7 (8.4) |
| | | CIN3 | 6 (7.2) | CIN3 | 2 (2.4) | CIN3 | 8 (9.6) |
| | | | | | | IA1 | 1 (1.2) |
| HSIL | 50 (60.2) | CIN1 | 2 (2.4) | CIN1 | 1 (1.2) | CIN1 | 1 (1.2) |
| | | CIN2 | 23 (27.8) | CIN2 | 25 (30.2) | CIN2 | 11 (13.3) |
| | | CIN3 | 25 (30.2) | CIN3 | 23 (27.8) | CIN3 | 27 (32.7) |
| | | | | IA1 | 1 (1.2) | IA1 | 4 (4.8) |
| | | | | | | IA2 | 2 (2.4) |
| | | | | | | NEG | 5 (6) |
| **Overall** | 83 (100) | LGSIL | 3 (3.6) | LGSIL | 2 (2.4) | LGSIL | 1 (1.2) |
| | | HGSIL | 80 (96.4) | HGSIL | 80 (96.4) | HGSIL | 70 (84.4) |
| | | | | IA1 | 1 (1.2) | IA1 | 5 (6) |
| | | | | | | IA2 | 2 (2.4) |
| | | | | | | NEG | 5 (6) |

Values are presented as frequencies with percentage in brackets. n: number, ASCUS: atypical squamous cells of undetermined significance, ASC-H: atypical squamous cells-cannot exclude high grade intraepithelial lesion, LGSIL: low grade squamous intraepithelial lesion, HGSIL: high grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia, NEG: negative, * loop electrosurgical excision procedure (LEEP) or gold knife

was performed in all cases; two patients (2.4 %) were diagnosed with LSIL (CIN1), 80 (96.4 %) with HSIL [CIN2 n =49 (59 %), CIN3 n =31 (37.3 %)], while one case (1.2 %) was diagnosed with IA1 cervical cancer, according to International Federation of Gynecology and Obstetrics (FIGO) classification (2009)[22] (Table 2). Of the two cases with CIN1 diagnosis from punch biopsies, in the first case, the PAP test showed HSIL, the colposcopy showed CIN2, and the final histologic diagnosis after surgical treatment was CIN3, whereas in the second case, the PAP test showed LSIL, HPV DNA test was positive for high risk, colposcopy was inadequate, punch biopsy revealed CIN1 and the final diagnosis following loop excision was also CIN3.

Regarding treatment, 81 (97.6 %) cases were treated with LEEP and two cases (2.4 %) with CKC. The histology following conization, showed one (1.2 %) patient with CIN1, 23 (27.7 %) patients with CIN2, 47 (56.7 %) with CIN3, five (6 %) with IA1 cervical cancer, two with IA2 (2.4 %) (FIGO 2009), while five (6 %) patients had negative findings. In the case with a final histology diagnosis of CIN1, the woman initially presented with HSIL in the PAP test, while colposcopy and punch biopsies showed CIN2. Among the five cases with negative results after surgical treatment, three had an HSIL in the PAP test and CIN2 in both colposcopy and punch biopsies, the 4$^{th}$ case had an HSIL in the PAP test, colposcopy was inadequate and the punch biopsy showed CIN2, and the 5$^{th}$ case had an HSIL in the PAP test, CIN1 in colposcopy, CIN2 in punch biopsies and a positive high-risk HPV DNA test. All five cases with negative results after treatment were under close follow-up and remained negative for intraepithelial lesions. The two women with IA2 diagnosis underwent further treatment with radical abdominal hysterectomy plus lymphadenectomy. Two of the five patients with micro-invasive cervical cancer (IA1) underwent total abdominal hysterectomy (Table 2).

With regards to the cervical conization procedure, there was a single pass of the loop in 56 cases (67.5 %), whereas in 27 cases (32.5 %) more than a single pass was performed. The median depth of the cones was 1.4 (range 0.5-2.2) cm. In 19 (22.9 %) cases, the depth of the cone was up to one cm (≤1 cm), while in 64 cases (77.1%), the depth was more than one cm. There was only one case of positive lymph-vascular space invasion (LVSI) (1.2 %). In 22 (26.5 %) cases the endocervical margins of the cone specimen margins were not free of disease (Table 3).

During follow-up, six months following primary treatment, the PAP smear tests showed 70 cases (84.3 %) negative for intraepithelial lesion or malignancy (NILM), one patient with ASCUS (1.2 %), one ASC-H (1.2 %), four LSIL (4.8 %) and seven HSIL (8.4 %). A punch biopsy was performed in cases with an abnormal PAP smear test or a suspicious lesion identified during colposcopy.

Recurrence of the disease was identified in a median time of 11.6 (range 6-16) months in 23 (27.7 %) patients

**Table 3:** Cervical conization procedures that the 83 women with cervical pathology underwent.

| Histology following conization | n | Number of passes | n | Depth of cone | n | Endo-cervical margin * | n |
|---|---|---|---|---|---|---|---|
| LGSIL | 1 (1.2) | 1 | 0 | ≤1cm | 0 | – | 1 (1.2) |
| | | >1 | 1 (1.2) | >1cm | 1 (1.2) | + | 0 |
| HGSIL | 70 (84.4) | 1 | 47 (56.7) | ≤1cm | 17 (20.5) | – | 50 (60.3) |
| | | >1 | 23 (27.7) | >1cm | 53 (63.9) | + | 20 (24.1) |
| IA1 | 5 (6) | 1 | 4 (4.8) | ≤1cm | 1 (1.2) | – | 4 (4.8) |
| | | >1 | 1 (1.2) | >1cm | 4 (4.8) | + | 1 (1.2) |
| IA2 | 2 (2.4) | 1 | 1 (1.2) | ≤1cm | 0 | – | 1 (1.2) |
| | | >1 | 1 (1.2) | >1cm | 2 (2.4) | + | 1 (1.2) |
| NEG | 5 (6) | 1 | 4 (4.8) | ≤1cm | 1 (1.2) | – | 5 (6) |
| | | >1 | 1 (1.2) | >1cm | 4 (4.8) | + | 0 |
| **Overall** | 83 (100) | 1 | 56 (67.5) | ≤1cm | 19 (23) | – | 61 (73.5) |
| | | >1 | 27 (32.5) | >1cm | 64 (77) | + | 22 (26.5) |

Values are presented as frequencies with percentage in brackets. n: number, LGSIL: low grade squamous intraepithelial lesion, HGSIL: high grade squamous intraepithelial lesion, CIN: cervical intraepithelial neoplasia, IA1: micro-invasive cervical cancer, -: free endocervical margins, +: involved endocervical margins.

[13 (15.7 %) with LSIL, 10 (12 %) with HSIL], while 60 (72.3 %) patients were free of disease. Of note, in one out of the 13 patients diagnosed with LSIL in follow-up, the recurring lesion was at the vaginal cuff as this patient had been previously treated by radical hysterectomy and pelvic lymphadenectomy because of an original diagnosis of IA2 cervical squamous cancer. All the patients with LSIL were under close follow-up for at least 24 months (cytology & colposcopy). The entire subgroup of the LSIL recurrences was free of disease at the end of the follow-up. Regarding the treatment of patients with HSIL (n =10) recurrences, a second LEEP was performed in four patients; one patient was lost to follow-up despite the consultation for a second LEEP; total abdominal hysterectomy was performed in two (2.4 %) patients (due to poor compliance and patient's wish, respectively), while three patients (3.6 %) were under close follow-up. These three patients were young women (<30 years) who had not achieved their reproductive goals; therefore, a conservative management with strict follow-up was recommended over performing a second LEEP. Finally, at the end of the follow-up period of the current study, 80 out of the 83 patients (96.4 %) had no signs of disease; of the three affected patients: a) one patient had recurrence of HSIL, 16 months after the primary LEEP performed for HSIL (current management: consultation for second LEEP, but requested a second opinion), b) one patient had LSIL, 26 months after the primary LEEP performed for HSIL (current management: close follow-up), and c) one patient with relapse HSIL at the vaginal wall, 20 months following a total abdominal hysterectomy (performed 10 months after her primary LEEP due to HSIL) (current management: local vaginal excision with knife, under close follow-up).

Further analyses were conducted to identify the potential risk factors and their association with the recurrence of the disease after primary treatment (Table 4). Using multivariate logistic regression, involved surgical margins (OR: 52.478; 95 % CI: 8.315-331.203; p <0.001), cone depth ≤1cm (OR: 21.225; 95 % CI: 3.176-141.863; p =0.002) and more than a single pass of the cone (>1) (OR: 8.793; 95 % CI: 1.854-41.693; p =0.006) were identified as independent risk factors for recurrence (Table 5).

Following Kaplan-Meier analysis, the recurrence rate was time-related to the group of patients with positive endocervical margin, more than a single pass of the loop (multiple specimens) during the excision procedure, and a depth of cone less than one cm (Figure 1, Figure 2, Figure 3). The mean recurrence time was higher in the group of patients with free surgical margins, compared to those with positive surgical margins (49.0 vs 15.3 months; p <0.001). In the group of patients with multiple specimens at the conization, the mean recurrence time was lower than those with a single pass (one specimen) (19.3 vs 46.9 months; p <0.001). Patients with a depth of cone ≤1cm had a lower mean recurrence time compared to those with a depth of cone more than one cm (18.6 vs 45.0 months; p =0.001).

## Discussion

*Main findings*

According to the results of the present study, i) in almost 85 % of the patients the final histology after conization was HSIL, ii) relapse was identified in about 25 % of the patients, although the rate of HSIL recurrence was restricted to 10 % of the patients, in a median of approximately 12 months after the primary treatment, iii) the involved endocervical margins, the depth of cone ≤1 cm and >1 pass during conization were identified as independent risk factors for recurrence, and iv) the mean recurrence time was less in cases of involved endocervical margins, >1 pass at the excision procedure and depth of cone ≤1cm.

**Table 4:** Comparison of the characteristics between disease-free and relapse groups of patients during the follow-up period.

| Characteristics | Disease free group (n =60) | Relapse group (n = 23) | Univariate Analysis | | |
|---|---|---|---|---|---|
| | | | p-value | ORs | 95 % CI |
| **Age** | | | | | |
| ≤35 | 28 (46.7) | 11 (47.8) | 1.000 | 1.048 | 0.400-2.743 |
| >35 | 32 (53.3) | 12 (52.2) | | | |
| **Age of first sexual intercourse** | | | | | |
| ≤18 | 36 (60) | 16 (69.6) | 0.459 | 1.524 | 0.545-4.257 |
| >18 | 24 (40) | 7 (30.4) | | | |
| **Number of partners** | | | | | |
| ≤5 | 35 (58.3) | 10 (43.5) | 0.325 | 1.820 | 0.689-4.806 |
| >5 | 25 (41.7) | 13 (56.5) | | | |
| **Endocervical margin** | | | | | |
| Free | 54 (90) | 7 (30.4) | <0.001 | 20.571 | 6.044-70.018 |
| Involved | 6 (10) | 16 (69.6) | | | |
| **Number of passes** | | | | | |
| 1 | 47 (78.3) | 9 (39.1) | 0.001 | 5.624 | 1.991-15.887 |
| >1 | 13 (21.7) | 14 (60.9) | | | |
| **Cone depth** | | | | | |
| >1cm | 51 (85) | 13 (56.5) | 0.009 | 4.359 | 1.470-12.927 |
| ≤1cm | 9 (15%) | 10 (43.5) | | | |
| **Gravity of cervical lesion*** | | | | | |
| <CIN2 | 6 (10) | 0 (0) | 0.180 | 0.426 | 0.233-1.650 |
| ≥CIN2 | 54 (90) | 23 (100) | | | |

Values are presented as frequencies with percentage in brackets. n: number, OR: odd ratio, CI: confidence interval, * according to loop electrosurgical excision procedure (LEEP) histology.

**Table 5:** Multivariate logistic regression analysis of the significant factors identified in the univariate analysis (endocervical margin, number of passes, cone depth).

| Characteristics | Multivariate Analysis | | |
|---|---|---|---|
| | p-value | ORs | 95% CI |
| **Endocervical margin** Free vs Involved | <0.001 | 52.478 | 8.315-331.203 |
| **Number of passes** 1 vs >1 | 0.006 | 8.793 | 1.854-41.693 |
| **Cone depth** >1 cm vs ≤1 cm | 0.002 | 21.225 | 3.176-141.863 |

OR: odd ratio, CI: confidence interval.

*Interpretation of the findings*

In clinical practice, the recurrence of HSIL after the primary surgical intervention is not uncommon. Depending on the series, two years after the primary surgical treatment, 5-15 % of high-grade lesions recur[13,23]. In our study, the recurrence rate was almost 27 %, however, the HSIL recurrence rate in the present data was restricted to 12 %. Our findings agree with previously published data with a similar population with a reported recurrence of up to 30 %[24-26]. The higher recurrence rate in this study could be explained by the fact that our Cervical Pathology Unit belongs to a tertiary center, dealing with many patients with HSIL, as well as cases with resistant cervical pathology.

There is limited evidence on the association between the depth of the cone specimen and recurrence rate. A retrospective Korean cohort study suggested that the cone depth should be adjusted according to age and the lesion's grade (<40 years and CIN2 ≥9mm, 40-50 years and CIN2 ≥12mm, <50 years and CIN3/IA1 ≥ 18mm)[12]. According to the same study, a cone length of >18 mm is required to achieve endocervical resection with a clear margin in 86 % of women over 50 years with CIN2+ and in 88 % of women less than 40 years with CIN2+[12]. Another observational study of 1,558 treated women found a significantly higher rate of positive endocervical margins in excised specimens measuring <10 mm in length than in larger excised specimens in both younger and older women, leading to an increased risk of recurrence in women >35 years but not in younger ones[11]. However, another report showed no significant impact on relapse rate after a deep pass of the excision procedure. Another

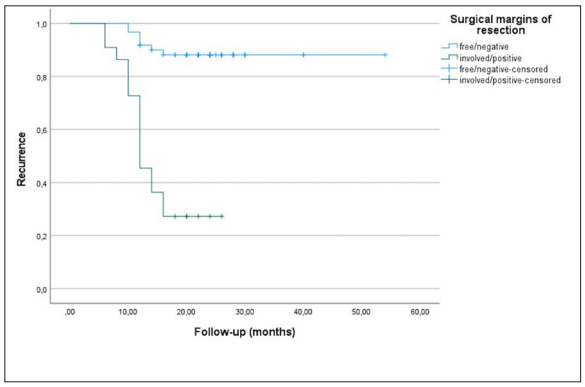


**Figure 1:** Kaplan-Meier curve demonstrating the relation of recurrence over follow-up time based on surgical margins of the cone (p <0.001).

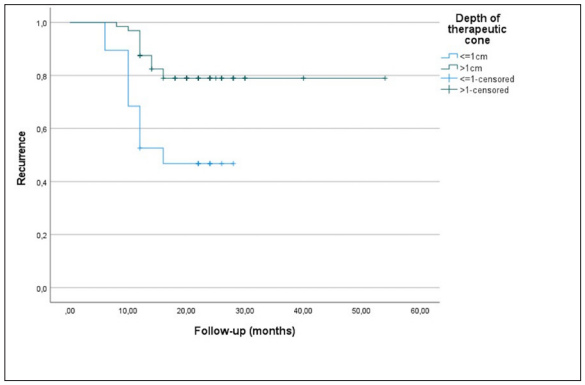


**Figure 3:** Kaplan-Meier curve demonstrating the relation of recurrence over follow-up time based on the depth of cones removed at the primary treatment (p=0.001).

observational study suggested that the depth of the cone (<8 mm vs >9 mm) was not associated with the treatment failure[27]. However, in that study, only 14.6 % of the women had an HSIL diagnosis (CIN2 or CIN3), whereas the rest had a CIN1 final diagnosis. In our study, 52.2 % of the patients were >35 years old, 98 % were CIN2+ after punch biopsy and the mean depth of the cone was 14 mm. Depth of the cone <10 mm was significantly related to treatment failure according to our data (OR: 21.225), which agrees with previous reports[11,12,28]. The depth of the cone is crucial to achieve a clear surgical margin, avoiding simultaneously potential adverse obstetrical outcomes in reproductive-age women, without compromising the oncological standards[28].

Unanimously, positive margins are significantly associated with recurrent disease according to the current literature[13,14,29]. In addition, a previously published study confirmed that the only factor independently associated with recurrence was the involved endocervical margin after LEEP excision[30]. Similarly, positive surgical margins after conization were associated with an increased risk of recurrence in our study (OR: 52.478). Notably, age and fertility status are important factors that should be taken into consideration when a LEEP procedure is to be performed in women of childbearing age, to minimize the risk of preterm delivery[11].

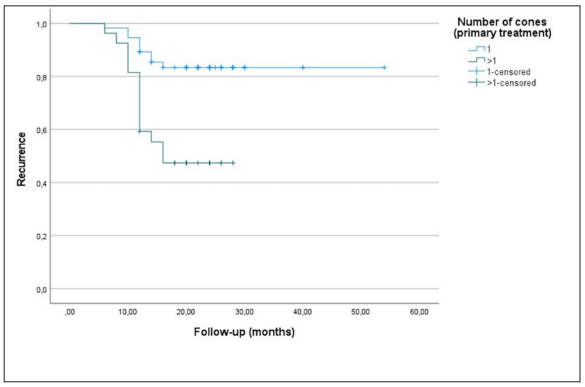


**Figure 2:** Kaplan-Meier curve demonstrating the relation of recurrence over follow-up time based on the number of cones removed at the primary treatment (p <0.001).

In large lesions, excision of the transformation zone can be performed in two or more separate loop passes. Similarly, for patients with a deep endocervical lesion (transformation zone type 2 & 3), more than a single pass of the loop is often required to receive an uninvolved endocervical margin[31]. It is known that multiple surgical specimens influence the accuracy of the histopathologic assessment. Evaluation of multiple specimens may also underestimate the average depth of the excisional procedure. In our study, in 32.5 % of the cases, the excision was performed in more than a single pass, which is in concordance with previously published data[32]. It is not always feasible to perform conization using a single pass, especially in cases of extended ectocervical or deep endocervical lesions, patients with increased body mass index, or patients with scarred cervix after a complicated vaginal delivery[31]. According to our findings, more than a single pass of the loop significantly impacts treatment failure (OR: 8.793), which contrasts with previous evidence[25]. Although the optimal surgical treatment includes a single pass of the LOOP excision (single specimen)[33], the evidence concerning relapse rates in multiple passes is equivocal. Kim et al reported a similar rate of relapse after a single vs second pass of the loop[30]. According to their analysis, the performance of the second cone specimen has not affected the treatment failure compared to a single cone. However, the authors underlined the significance of the single pass technique on the histopathologic evaluation of the cone specimen and on further management[30].

There is a discrepancy in the literature regarding the impact of patients' age on the recurrence rate. Menopause has been reported as a predictive factor of relapse, mainly due to deep endocervical lesions and types 2 or 3 transformation zone[34,35]. Furthermore, age over 35 years was reported as a risk factor for relapse[36]. On the other hand, it has been described that age and menopausal status were risk factors for residual lesion but not for recurrence[37,38]. We found that age was not a prognostic factor of relapse, which is in agreement with some previous studies[37,38].

Furthermore, according to the literature, smoking, age of first sexual intercourse, number of sexual partners,

parity, immunosuppression, the age of menarche, and conization type were not associated with recurrence[39]. Similarly, in this study, the age of first sexual intercourse and the number of partners were not found to contribute to higher recurrence rates.

Last, the mRNA test is reported to be a potential predictor of residual and/or recurrent cervical abnormalities after treatment. Including a molecular marker in a follow-up, protocol has been proposed in the literature and seems to reduce overtreatment in younger patients[40]. Moreover, available evidence suggests a potential reduction of recurrent disease after treatment for CIN if women are offered prophylactic HPV vaccines[41]. Finally, the hrHPV DNA test is an important predictive factor of recurrence, especially in positive surgical margins. It is widely recommended for use in the context of follow-up after treatment[42]. In the current study, the hrHPV DNA test, although not performed as a routine, was used in more than half of the women.

The present study investigates the risk factors of relapse after conization. Detailed epidemiological, diagnostic, and therapeutic characteristics were evaluated under a long-term median follow-up of 24 months. A major limitation of the study is its retrospective nature. Another limitation is that HPV-DNA testing was not routinely used during the follow-up period, which was the reason why it was not included in the analysis of the risk factors. However, in more than 50 % of our patients, HPV-DNA testing was performed following their primary treatment.

**Conclusions**

According to the presented data, we identified a positive endocervical margin after conization, more than a single pass of the loop, and the low depth of the cone as the most important risk factors for cervical pathology recurrence. On the other hand, the woman's age, the age of first sexual intercourse, and the number of partners were not associated with an increased risk of recurrence. At the end of our follow-up period, only 3 % of our population remained with evidence of disease, albeit under close follow-up. More studies are required to further highlight the risk of recurrence and associated factors to reduce the rates of cervical dysplasia.

**Conflict of interest**

The authors have no conflict of interest to declare.

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