OBJECTIVE: To investigate whether the size of the lesion correlates with the completeness of excision, and whether human immunodeficiency virus (HIV) infection was a risk factor for incompleteness of excision. We investigated the size of high-grade cervical intraepithelial lesions of loop electrosurgical excision procedure (LEEP) specimens to assess its effect on the status of the excision margins. Because of the possible negative effect of HIV infection on the completeness of excision, we compared HIV-positive with HIV-negative cases.

STUDY DESIGN: Cross-sectional study of 226 consecutive LEEP specimens with cervical intraepithelial neoplasia (CIN). There were 126 CINs ≥ 2; 59 (46.8%) were HIV positive. We measured the linear surface, the number of involved crypts, and the number of step sections involved by CIN ≥ 2.

RESULTS: The excision margins were involved in 65.1%. The measurable linear extent and the average number of involved crypts and sections were significantly higher with incomplete excision (p < 0.0001, p = 0.001, and p = 0.02, respectively). The endocervical excision margin was involved in 52.4% of incomplete excisions. The excision was incomplete in 43.9% of HIV-infected and 56.1% of HIV-uninfected cases ($\chi^2 = 0.03$, p = 0.88).

CONCLUSION: It appears that instead of the linear surface involvement, it is rather the location in the endocervix and the extension inside the crypts that affects the excision margin status. HIV infection did not affect the completeness of excision. (Anal Quant Cytopathol Histopathol 2014;36:183–188)

Keywords: cervical intraepithelial neoplasia, cervical lesions, endocervical excision margin, excision completeness, gynecologic surgery, histometry, HIV, human immunodeficiency virus, large loop excision of the transformation zone, LEEP, LLETZ, loop electrosurgical excision procedure, preinvasive lesion size, uterine cervix.

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Traditionally, when cold knife conization was the standard procedure to treat high-grade preinvasive cervical lesions, clinicians expected the histopathology report to provide information about the excision margin status. Free excision margins meant, so it was thought, that the procedure had hopefully been “therapeutic.” Currently, electrical devices, called loop electrosurgical excision procedure (LEEP) or large loop excision of the transformation zone (LLETZ), are more popular because they cause less bleeding. For the pathologist, however, because of burn artifacts they may make it more difficult to assess the status of the excision margins to identify residual disease.1

There has been and there still is debate about whether conization (cold knife or electrical) is effective, since what might matter more is the presence of high-risk human papillomavirus (HR-HPV). If that is the case, the concept of “therapeutic” conization needs reconsideration.2 However, this does not demean the notion that it is most likely more beneficial to achieve full excision with clear margins.3 Common sense would expect the completeness of excision to be to a large extent dependent on the size of the preinvasive lesion. Surprisingly, little research has been devoted to this concept.

Most studies focus on the correlation between size of cervical intraepithelial neoplasia (CIN) and risk of microinvasion.

Jarmulowicz et al4 (1989) reported the quantitative histology of 84 laser cone biopsies and found a significant correlation between the grade of cervical smear and the size of CIN. Tidbury et al5 investigated the linear surface involvement and perimeter of involved crypts of 39 cone biopsies for CIN 3 associated with microinvasion. They found that the mean size of CIN 3 showing microinvasion was 7 times greater than without invasion. To the best of our knowledge, Sherman et al6 were the first to measure the linear extent and gland involvement of CIN 3 lesions. They found a mean linear extent of 9.5 mm ± 9.1 (median, 6.5). It was not mentioned whether the excision margins were free.

The aim of the present study was to investigate whether the size of the lesion correlates with the completeness of excision. We also wished to find out whether human immunodeficiency virus (HIV) infection was a risk factor for incompleteness of excision.

Materials and Methods

This was a cross-sectional study of 226 consecutive cases of LEEP indicated by a cytologic diagnosis of high-grade squamous intraepithelial lesion in 83 (36.8%) HIV-infected and 143 uninfected patients. None had a previous cervical excision procedure. The histopathological diagnosis was classified as CIN 1 or less (CIN < 1), CIN 2 or more (CIN ≥ 2), and invasive cervical cancer. Since the scope was to correlate the size of CIN ≥ 2 and the state of the excision margins, cases of CIN < 1 (n = 90) and invasive cervical cancer (n = 10) were excluded from the study (Figure 1).

The majority of LEEPs consisted of multipasses (Figure 2). Step sections were made of each specimen along the entire length of the endocervix to the ectocervix. Four micrometer thin sections were stained with hematoxylin and eosin. We measured the horizontal length and vertical widths of each specimen on the histopathology sections with a ruler inserted in the eyepiece of the microscope (in millimeters). We counted the number of sections with or without CIN ≥ 2, the linear extent of CIN ≥ 2, and the number of involved crypts per section. The completeness of excision was assessed at the endocervical, ectocervical (i.e., the transition zone), and deep excision margins (i.e., the crypts). Negative margins were defined as the absence of CIN ≥ 2 at all cone margins by histopathological examination.

The study was approved by the institutional research ethics board. Statistical evaluation was done using column statistics, Student’s t test, and contingency table analysis of proportions. The statistical software GraphPad (Prism, San Diego, California, U.S.A.) was used. The level of statistical significance was set at p < 0.05.

Results

The excision margins were clear in 44 (34.9%) and involved in 82 (65.1%). A little more than half of incomplete excision was at the endocervical margin. Only 2.4% of incomplete excisions were of the deep margin (Table I). The proportion of involved margins was 56.1% in HIV-uninfected and 43.9% in HIV-infected patients ($\chi^2 = 0.03, p = 0.88$).

Table II shows the details of comparative measurements of clear and involved margins. There was a similar number of sections per case in both groups ($p = 0.28$). The linear extent of CIN ≥ 2, the number of involved crypts, and the number of involved sections was greater ($p < 0.001$, $p = 0.001$, and $p = 0.02$, respectively) in the cases with involved margins. This, however, needs a proviso...
since the linear extent with involved margins is, by definition, unknown. Therefore, only the measurable linear extent of cones with clear margins could be assessed. The linear extent of CIN ≥ 2 of cones with clear margins was 5.2 mm ± 2.6 (median, 6.0) in HIV-uninfected and 4.4 ± 2.4 (median, 4.0) in HIV-infected cases (t = 1.1, p = 0.30).

**Discussion**

Although excision of cervical high-grade preinvasive lesions is an accepted treatment procedure, it raises a number of issues, among which the main ones are the importance of or lack of clear excision margins and the pretherapeutic or posttherapeutic presence of HR-HPV. The relatively high rates of recurrence/persistence after conization with clear margins raises the question of whether, despite clear margins, one or more foci were not excised. Furthermore, even though the “bulk” of HR-HPV may have been removed with the cone, there may be persistent HR-HPV as well as reinfection.

Incomplete excision of CIN exposes the patient to a high risk of high-grade posttreatment disease that requires a close follow-up. On the other hand, recurrence or persistence of high grade cannot be ruled out even with negative excision margins, especially when the cone exhibited a high-grade

<table>
<thead>
<tr>
<th>No. of involved excision margins</th>
<th>Endocervical</th>
<th>Ectocervical</th>
<th>Endocervical/ectocervical</th>
<th>Crypts</th>
<th>Three margins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>43 (52.4%)</td>
<td>11 (13.4%)</td>
<td></td>
<td>2 (2.4%)</td>
<td></td>
</tr>
<tr>
<td>Double</td>
<td></td>
<td></td>
<td>15 (18.3%)</td>
<td>1 (1.2%)</td>
<td></td>
</tr>
<tr>
<td>Triple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 (12.2%)</td>
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lesion, crypt involvement, and multiple quadrants. As already mentioned, two reasons might explain this: either there was residual disease beyond the cone, and/or there was persistent/recurrent (reinfection) HR-HPV infection. As indicated by several studies, the most important predictor of recurrence is the presence of HR-HPV, followed by positive endocervical margins and pretherapeutic presence of HR-HPV. It has been suggested that conization might clear the presence of HR-HPV in up to two-thirds of the infected cases. Whatever the case, it shows the need for postconization follow-up with cytology and/or HPV testing regardless of the status of the excision margins.

It would make sense that multifocality and multiglandular involvement increase the size/extent of the dysplastic lesion and, therefore, the rate of incomplete excision. Furthermore, the higher the endocervix is involved, the higher would be the risk of incomplete excision. The endocervical margin has been shown to be involved in around 40% of cones. The “top-hat” technique, however, with a mean depth of excision of 21 mm (versus 12 mm with conventional LLETZ) did not decrease the incidence of endocervical margin involvement.

In areas of high cervical cancer and HIV co-endemicity, the treatment outcomes of cervical neoplasia with LEEP/LLETZ is of special concern and raises the question of the need for specific management guidelines. One study found a 33.3% rate of margin involvement in HIV-infected and uninfected patients. Other reports found that immunodepression and positive margins were associated with a high risk of recurrence of high-grade CIN, and that positive margins were more common in HIV-infected women.

In conclusion, the comparative histometry of cones shows that margins are more likely to be involved if the cones show that dysplasia is multifocal and where a high number of crypts are involved. Endocervical margin involvement occurred in more than half of the cases, hindering the assessment of the linear extent of CIN ≥ 2. In cases with clear margins, the linear extent and the rate of involved margins was comparable regardless of the HIV serostatus. Because clear excision margins are no guarantee of the completeness of excision, postexcision follow-up is mandatory for all cases treated for CIN ≥ 2.
References


