Depth of Cervical Intraepithelial Neoplasia Grade 3 in Peruvian Women: Implications for Therapeutic Depth of Necrosis

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Objective: To determine the involvement of cervical intraepithelial neoplasia grade 3 (CIN3) in a population of women in a lower-resource setting.
Methods: One hundred twelve consecutive cone excision specimens with histological diagnosis of CIN3 were retrieved from the National Institute of Neoplastic Diseases in Lima Peru. Two pathologists independently evaluated each specimen microscopically and confirmed 107 cases that could be measured by optical micrometry. Depth and breadth of the lesions were measured microscopically.
Results: The mean maximal depth of cervical involvement by CIN3 was 2 ± 0.13 mm; depth was less than 3.5 mm in 89.7% of cases and less than 5 mm in 93.5%. Mean breadth of CIN3 was 7.3 ± 4.4 mm; breadth was less than 15.9 mm in 95% of cases and less than 20.5 mm in 99.7%. The correlation coefficient between breadth and depth of CIN3 was 0.61. No significant correlation was found between age and depth.
Conclusions: Depth of CIN3 involvement in a developing country is significantly deeper than that reported in the United States. Treatment selection for women with CIN3 and risk of treatment failure may vary between developing and developed countries because of the difference in the depth of lesions. Countries with underscreened populations need to consider the increased disease severity in devising treatment strategies.

Key Words: cervical intraepithelial neoplasia, cervical pathology, cervical cancer, cervical cancer screening, tissue necrosis, ablation, cryotherapy

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Cervical cancer is the fourth most common cancer in women worldwide, with an estimated 528,000 new cases reported in 2012. Approximately 85% of this cancer burden is in low-and middle-income countries (LMICs), where 90% of the estimated 266,000 cervical cancer deaths occurred.[1] Based on data from newly screened women in developing countries showing that at least 1% of women in these groups have histologically confirmed cervical intraepithelial neoplasia (CIN) grade 2 or more severe abnormalities (CIN2+),[2] we can assume that there are at least 10 million women worldwide with cervical cancer precursor lesions.

There are limited data on the extent of CIN involvement in developing countries. Studies of depth of CIN involvement have primarily been conducted in high-resource countries on populations that are screened frequently.[3–5] It is assumed that lesions present in highly screened populations are predominantly incident, whereas those found in LMICs, where underscreened or unscreened populations predominate, are more likely to be long-standing. Scant data exist on the long-term cure rates of women when treated with conventional ablative methods such as gas-based cryotherapy, and data are even more limited in developing countries.

Determining the depth and breadth, or linear extent, of prevalent lesions in women in LMICs would help establish the depth of necrosis required to eradicate CIN2+ lesions. Although the minimum tissue depth necessary to achieve destruction of CIN2+ has not been established, and there could be other factors, such as inflammation or immune response, that could influence the success of treatment, identifying mean depth of involvement of CIN2+ lesions seems to be a necessary first step in evaluating therapeutic strategies. In a London-based study of 343 biopsies, mean depth of CIN3 was 1.24 mm (SD, 0.85 mm); depth was 2.92 mm or lesser in 95% and 3.8 mm or lesser in 99.7% of patients.[6] Another study in the United States found that 99.7% of CIN3 would be eradicated if cervical tissue was destroyed up to a depth of 4.8 mm.[3]

The current study was conducted at the National Institute of Neoplastic Diseases (INEN) in Peru, a middle-income country. Most women evaluated and treated in that hospital are referred from other institutions in Lima or other parts of the country. The primary objective was to establish mean extent of cervical epithelial involvement of CIN3 measured in women who had excision of the uterine cervix.

MATERIALS AND METHODS

The study was designed as a retrospective analysis of conization or hysterectomy specimens from January 1 to December 31, 2013. We initially identified 112 cases received consecutively at the surgical pathology laboratory at INEN and processed with a diagnosis of high-grade squamous intraepithelial lesions (CIN 2-3). For inclusion in the study, cases required a diagnosis of CIN3 on loop electrosurgical excision procedure (LEEP) or cold knife cone. Each case had to have all of the original glass slides and all paraffin blocks available for examination. Exclusion criteria included cases not confirmed with a diagnosis of CIN3 during the study review or cases that did not have all slides and all blocks available for review.

Cone biopsy samples obtained either by LEEP (n = 15) or by cold knife cone (n = 97) were originally radially sectioned at 3- to

4-millimeter intervals, and slices were placed into tissue cassettes for processing. After routine histological processing, 5-micron sections were cut, placed onto glass slides, and stained with hematoxylin and eosin. Slides were evaluated in a standard binocular light microscope outfitted with an optical micrometer.

Of the 112 CIN2-3 cases, four were found not to have CIN3, and one case had a specimen too small to be measured with the optical micrometer. The remaining 107 cases had measurable confirmed CIN3 and were included in the analysis. All available slides for the 107 cone biopsy specimens were reviewed by an INEN pathologist and a US-based pathologist, both with expertise in gynecologic pathology.

An initial subset of 20 cases was selected at random from the 107 specimens to establish the validity of using only the slides available in the archives. For this purpose, 5 deeper cuts were made from the paraffin block at 500-micron intervals (deeper sections), and these slides were evaluated for depth of CIN3 lesions. Measurements of this depth did not increase by more than 1% beyond the depth observed in any of the cases in the original set of slides, indicating that the existing slides in the archives provided reliable measurements of depth of involvement (data not shown). After this validation, the pathologists individually reviewed each case and recorded the maximum depth and breadth of involvement.

Discrepancies in the depth and breadth measurements recorded by the 2 pathologists were anticipated because of the subjectivity of assessing the dimensions of a cervical lesion histologically. Discrepant cases were recorded, and concordance of depth measurements was calculated. In anticipation that lesions of greater depth would yield the largest discrepancies, it was decided a priori that the 2 pathologists would simultaneously review the deepest lesions to reach a consensus depth of involvement. We arbitrarily chose the depth of 3.5 mm for inclusion in this evaluation. For the 15 cases in which either pathologist recorded a depth of 3.5 mm or greater, the slides were digitally scanned using a Leica SCN scanner (Leica Biosystems, Buffalo Grove, IL). The digitally scanned slides are available in a password-protected Web address allowing the simultaneous viewing of the tissue by two or more persons. Digital images of the 15 cases were reviewed by both pathologists simultaneously via Web conference. A consensus measurement of CIN3 depth was attained in all 15 cases.

Descriptive statistics for depth of CIN3 involvement in the 107 cases were calculated based on combining mean and consensus depths as follows: 1) the mean depth of the pathologists' measurements for the 92 cases in which both recorded a depth of less than 3.5 mm, and 2) the consensus depth reached by the pathologists for the remaining 15 cases in which the initial depth recorded by one or both was 3.5 mm or greater.

Concordance coefficients were calculated for the correlation between the pathologists' depth measurements and between breadth measurements. The significance level was set at 0.05, and all statistical analyses were conducted with SPSS version 24 (IBM, Armonk, NY).

A human subjects research exemption was requested and approved by the institutional review boards of the INEN in Peru, the Cleveland Clinic, and the University of Southern California. As the research included only analysis of archival slides and retrospective chart review, informed consent was not obtained.

RESULTS

A total of 107 cases of CIN3 were reviewed. Eleven cases were obtained via LEEP and 96 via cold knife cone biopsy. The mean age of women with CIN3 was 44.7 ± 12.0 years. The median number of children was three. One-quarter of women (25.2%, 27/107) reported a history of screening. Of the 22 women for whom the year of last screening was available, 54.5% (12/22) were screened within the past 5 years, and 27.2% (6/22) had not been screened in more than 10 years. Four women (3.7%) reported no history of screening, and the medical history record had no information about screening for 76 women (71.0%). None reported a history of smoking. Two women (1.9%) reported a history of immunosuppression.

Mean depth of CIN3 involvement was 2.0 ± 1.3 mm, and median depth of involvement was 1.8 mm. Overall, 16.8% (18/107) of lesions were less than 1.0 mm in depth, and 2.8% (3/107) were less than 0.5 mm in depth. Mean breadth of CIN3 was 7.3 ± 4.4 mm, and median breadth was 6.5 mm. In 89.7% of cases, maximum depth of involvement was less than 3.5 mm, and in 93.5% of cases, maximum depth was less than 5 mm.

Figure 1 shows the relationship between depth and breadth of CIN3, and further analysis is presented in Table 1. A correlation coefficient of 0.61 was found between depth and horizontal extent ($p < .001$). Of lesions with a depth less than 2 mm, 62.3% of cases had a breadth of 6 mm or lesser. Of those 2 to 3.50 mm in depth, 74.3% had a breadth greater than 6 mm, and of those 3.50 mm or greater in depth, 100% were greater than 6 mm in breadth. Table 2 presents depth and breadth of CIN3 lesions by age categories. No significant correlation was observed between advancing age and depth and breadth of CIN3 involvement.

Mean depth measured by the US-based pathologist was 2 ± 1.4 mm, and the median was 1.7 mm. As measured by the INEN pathologist, mean depth was 2 ± 1.1 mm, and the median was 1.8 mm. The mean difference between the pathologists' measurements of depth was 0.059 mm. This difference was not statistically significant (95% confidence interval, −0.088 to 0.205). Concordance between the INEN and US-based pathologists' depth measurements was 0.82 ($p < .001$) (Figure 2).

Mean breadth measured by the US-based pathologist was 6.5 ± 4.1 mm, and the median was 5.5 mm. As measured by the INEN pathologist, mean breadth was 8.2 ± 5 mm, and the median was 7.4 mm. The mean difference between the pathologists' measurements of breadth was −1.77 mm (95% confidence interval, −2.33 to −1.22). Concordance between the INEN and US-based pathologists' breadth measurements was 0.81 ($p < .001$).

DISCUSSION

The results of our study suggest that involvement of the cervical epithelium with CIN3 lesions is deeper in women in LMICs than has been reported for women in high-income countries.[3,6] We theorize that this increase in depth is due to less frequent or absent

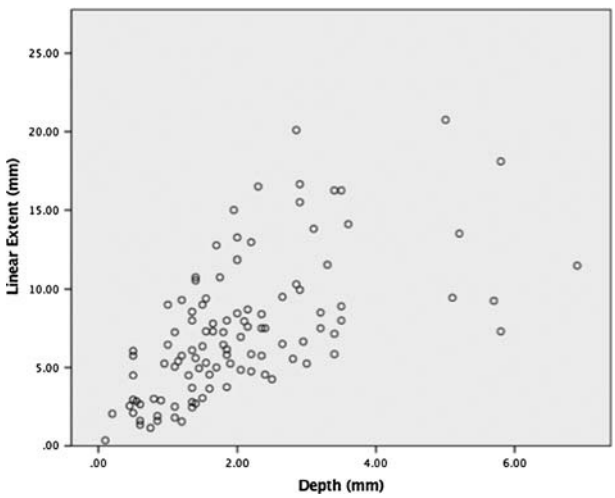

FIGURE 1. Relationship between CIN3 depth and breadth.

TABLE 1. Correlation of Depth and Breadth of CIN3

Depth (mm)	Mean age[a] (yr)	Length in mm (%)				Total (%)
		0–3	3.01–6	6.01–9	>9.01	
<2	44.4 ± 12	20 (31.7)	18 (30.6)	16 (26.2)	7 (11.5)	61 (100)
2–3.49	45.4 ± 12.6	0	9 (25.7)	13 (37.2)	13 (37.1)	35 (100)
≥3.50	44.6 ± 11.8	0	0	3 (27.3)	8 (72.7)	11 (100)
Total	44.7 ± 12	20 (18.7)	27 (25.2)	32 (29.9)	28 (26.2)	107 (100)

[a]Mean ± SD.

screening of women in LMICs than in women in high-income countries where screening is more readily available. Because the efficacy of cryotherapy has been correlated with size of CIN3 lesions, it is reasonable to assume that the larger, deeper lesions of women in LMICs may place them at greater risk of therapeutic failure.

Ablative treatments are based in the use of extreme temperatures (heat or cold) to destroy the cervical epithelium. Immediate necrosis after treatment is one of the factors presumed to be involved in the cure of CIN3 cases. Although there could be other factors such as late necrosis of cells damaged during treatment, inflammatory response, or an effective immune response, depth of initial necrosis is likely an important determinant of cure. Whereas previous research findings demonstrate the effectiveness of cryotherapy in treating CIN3 in a low-resource setting,[7] our results provide the only obtainable, objective information about the depths of necrosis required to destroy most CIN3 lesions. This information can serve as an initial basis to guide the development of therapeutic devices designed as alternatives to conventional cryotherapy.

In our study, 2 pathologists separately measured the involvement of CIN3 in the cervix, blinded to each other's findings, to establish the reproducibility of these measurements. The concordance between depth recorded by the pathologists was good, with the greatest discordance noted at the deepest depths recorded. Importantly, when cases were categorized by depth of involvement, the difference in depth measurements between the 2 pathologists did not alter the percentile categorization of any case.

Mean breadth was not significantly different from those previously reported in a screened population (7.60 ± 4.32 mm).[3] The difference in the pathologists' measurements of maximal breadth was statistically significant; these discrepancies also altered the percentile rank of several women with the largest lesions. The reason for this is not clear. Measurement of maximal breadth was made using the slide containing the greatest depth of involvement; however, it is possible that the selected slide did not also contain the maximal breadth for each case, likely leading to the discrepancies. The higher variability in this determination can explain the lack of significance from previous studies. Regardless of the variability, maximal breadth in our study correlated strongly with maximal depth of involvement.

TABLE 2. Correlation of CIN3 Involvement by Age

Age (yr)	No. cases	Depth (mm)		Length (mm)	
		Mean[a]	Median	Mean[a]	Median
21–30	9	1.73 ± 0.99	1.70	7.78 ± 5.45	6.05
31–40	37	2.21 ± 1.41	1.85	8.33 ± 4.43	7.50
41–50	30	1.92 ± 1.26	1.63	7.39 ± 4.88	7.23
≥50	31	2.06 ± 1.26	1.85	6.01 ± 3.05	5.60
Total	107	2.00 ± 1.32	1.80	7.35 ± 4.35	6.50

[a]Mean ± SD.

Because maximal breadth is a parameter that can and must be visually determined at the time of treatment, via visual examination with acetic acid, we consider it an extremely important value for the selection of women eligible for ablative therapy. Breadth is currently being used by treating clinicians at the time of visual triage. Women with larger lesions are triaged to an excisional procedure such as LEEP or cold knife cone rather than ablation. By doing so, they would likely be selecting women with the deepest involvement who would be more likely to fail ablative therapy. Breadth can therefore be a surrogate of depth of involvement, again acting as a safeguard in ablative treatment.

We did not find an association between advancing age and maximal depth of involvement by CIN3. This finding differs from previous work done in a screened population.[3] We hypothesize that in high-income countries, where women are screened frequently, lesions are discovered and treated at a high rate and thus are primarily smaller and incident in nature. In LMICs, screening is less frequent or nonexistent, and younger women may have lesions of comparably more similar duration and extent as women in developed countries.

This study has several strengths. We evaluated only CIN3, the best predictor of invasive cervical cancer and the lesion most likely to deeply involve the cervix.[8] We also evaluated more cases of CIN3 than have earlier studies. By using 2 pathologists, we established that microscopic evaluation of depth of involvement of CIN3 is reproducible and likely a valuable pathological parameter.

One limitation of this study is that we used a retrospective approach to select our study population. Also, although we attempted to minimize bias by analyzing consecutive cone specimens with a diagnosis of CIN2-3, we could not control for any bias involved

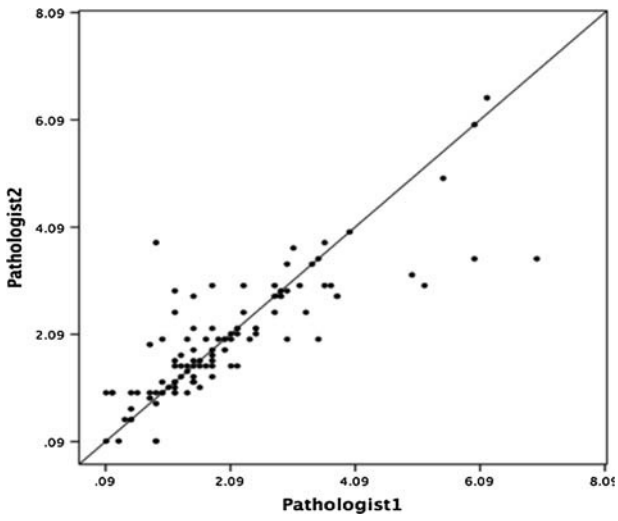

FIGURE 2. Concordance between pathologists' CIN3 depth measurements.

with which women were selected for LEEP or conization. Therefore, we excluded women who were treated with hysterectomy and could potentially have larger lesions. Similarly, we did not include women who had cryotherapy and are more likely to have smaller lesions.

Another limitation of this study is that we did not examine a comparable number of contemporaneous cone biopsy specimens in women in high-resource countries. Direct comparisons between these groups are extremely challenging because today most women in wealthy settings are treated with LEEP. The few cases selected for cold knife cone biopsies are mainly due to anatomical features of the vagina and cervix that make LEEP unfeasible but not due to the belief that the lesion is more severe. This selection bias would arguably make a comparison to contemporaneous cone biopsies less reliable than a comparison to historical data, particularly as we demonstrated that such measurements have high interobserver reliability. Although accruing a comparable number of CIN 3 cases in such different populations would be a daunting task, further studies could potentially assess within-population differences based on socioeconomic status (although differences in treatment may also apply to low- and high-income groups within LMICs).

In conclusion, women in LMICs who are likely to be underscreened or unscreened have deeper involvement of CIN3 than that reported for women in well-screened populations. Deeper involvement of CIN3 in these populations should prompt studies investigating long-term cure rates using conventional gas-based cryotherapy, the standard of care in LMICs. Finally, the data obtained in this study can be used as a guide for the development of new therapeutic strategies for women at risk of deep lesions.

REFERENCES

1. GLOBOCAN 2012. v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. *International Agency for Research on Cancer*; 2013. Available at: http://globocan.iarc.fr. Accessed April 22, 2014.
2. Jeronimo J, Bansil P, Lim J, et al. A multicountry evaluation of careHPV testing, visual inspection with acetic acid, and papanicolaou testing for the detection of cervical cancer. *Int J Gynecol Cancer* 2014;24:576–85.
3. Abdul-Karim FW, Fu YS, Reagan JW, et al. Morphometric study of intraepithelial neoplasia of the uterine cervix. *Obstet Gynecol* 1982;60: 210–4.
4. Gordon HK, Duncan ID. Effective destruction of cervical intraepithelial neoplasia (CIN) 3 at 100 degrees C using the Semm cold coagulator: 14 years experience. *Br J Obstet Gynaecol* 1991;98:14–20.
5. Loobuyck HA, Duncan ID. Destruction of CIN 1 and 2 with the Semm cold coagulator: 13 years' experience with a see-and-treat policy. *Br J Obstet Gynaecol* 1993;100:465–8.
6. Anderson MC, Hartley RB. Cervical crypt involvement by intraepithelial neoplasia. *Obstet Gynecol* 1980;55:546–50.
7. Luciani S, Gonzales M, Munoz S, et al. Effectiveness of cryotherapy treatment for cervical intraepithelial neoplasia. *Int J Gynaecol Obstet* 2008; 101:172–7.
8. Sauvaget C, Muwonge R, Sankaranarayanan R. Meta-analysis of the effectiveness of cryotherapy in the treatment of cervical intraepithelial neoplasia. *Int J Gynaecol Obstet* 2013;120:218–23.