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Precise Measurement of the Thickness of Vaginal Intraepithelial Neoplasia

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Objectives: Although carbon dioxide laser vaporization is frequently used for treating vaginal intraepithelial neoplasia (VaIN), the optimal depth of epithelial destruction with laser vaporization requires elucidation. We aimed to evaluate VaIN depth and better illustrate epithelial destruction during laser vaporization.

Materials and Methods: We included 246 women diagnosed with VaIN (low-grade VaIN [VaIN 1], 123 women; high-grade VaIN [VaIN 2/3], 123 women) using colposcopy-directed biopsy at our hospital from January 1, 2019, to April 30, 2020. The thickness of the noninvolved epithelium, if available, was determined. All available data, including cytology and histological information, were recorded. The *t* test and Pearson χ^2 test were used for statistical analysis. Statistical significance was set at $p < .05$.

Results: The involved epithelial thicknesses in VaIN 2/3 and VaIN 1 were 0.41 ± 0.21 and 0.40 ± 0.19 mm, respectively, which were both greater than their noninvolved epithelial thickness values (0.17 ± 0.10 and 0.17 ± 0.08 mm, $p < .01$ and $p < .01$, respectively). In subgroup comparisons between the VaIN 2/3 and VaIN 1 groups, the involved epithelial thickness did not differ between premenopausal patients, postmenopausal women receiving estrogen, and postmenopausal women who did not receive estrogen ($p > .05$). In the VaIN 2/3 group, the lesion thickness in premenopausal was greater than that in postmenopausal women receiving estrogen ($p = .016$) and those who were not receiving estrogen ($p = .017$).

Conclusions: The thickness of VaIN is generally less than 1 mm for women of all ages, except in rare cases of visible lesions with papillary hyperplasia.

Key Words: vaginal intraepithelial neoplasia, carbon dioxide laser vaporization, vaginal, depth

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The diagnosis rate of vaginal intraepithelial neoplasia (VaIN) has been increasing steadily because of the widespread application of cytology/high-risk human papilloma virus (hrHPV) cotesting and colposcopy in cervical cancer screening.[1–3] Vaginal intraepithelial neoplasia is defined by the presence of atypical stratified squamous cells in the mucosa of the vagina without invasion. Low-grade VaIN (VaIN 1) or low-grade squamous intraepithelial lesion is a benign manifestation of human papillomavirus (HPV) infection and is managed by close surveillance rather than treatment. In contrast, VaIN 2/3 or high-grade squamous intraepithelial lesion is a precancerous lesion that requires treatment.[4]

The current treatment methods for VaIN include surgical excision, ablative therapy, and topical treatment. Surgical excision is performed for lesions with suspected invasion or lesions that cannot be fully visualized. Ablative therapy is one of the most common treatment methods for VaIN if invasive disease has been excluded by colposcopy-directed biopsy and the lesion can be fully visualized. Carbon dioxide (CO_2) laser vaporization is generally well tolerated, shows satisfactory healing, and results in minimal sexual dysfunction.[5] Topical therapy is a common next-line option for treatment of VaIN, especially for recurrent and multifocal lesions. Topical therapy can apply the agents to the entire vaginal mucosa and providing good coverage in cases involving multifocal VaIN and VaIN in the folds and recesses of the vagina. Imiquimod is a reasonably effective and well-tolerated option for the treatment of VaIN. In addition, 5% imiquimod cream can be applied to the vaginal lesions 3 times a week for 8 weeks.[6] The most common adverse events are local burning and soreness, which are generally not severe enough for patients to discontinue treatment. Compared with imiquimod, topical fluorouracil is less commonly used and also reported in the literature with failure rates comparable with other techniques.[7] Several dosing protocols have been suggested, ranging from twice-daily application for 14 days to once weekly for 10 weeks. Complications of topical FU include vaginal irritation or burning and ulcerations.[7,8] Besides, intravaginal estrogen therapy offers an alternative to standard therapies with a success rate that is comparable with that previously reported with other more potentially morbid therapies for postmenopausal VaIN women.[9]

In laser surgery, a high-energy light beam is used to vaporize the VaIN. With an appropriate ablation depth, the procedure rarely causes adverse reactions and can be repeated several times. Thus, the ablation depth is a crucial aspect of laser therapy. Nevertheless, few studies to date have investigated the optimal depth of epithelial destruction with laser vaporization. Benedet et al[10] reported that epithelial destruction to a depth of 1.5 mm, including the zone of thermal necrosis, should be sufficient to destroy the epithelium containing VaIN without damaging the surrounding structures. However, their small sample size of 63 biopsy specimens may have caused bias and resulted in a lower level of evidence for the conclusions. Thus, a large-sample study with VaIN lesions of more precise thickness is needed to help guide clinical laser treatment.

In the colposcopy clinic of the largest obstetrics and gynecology tertiary teaching hospital in China, the average detection rate of VaIN among all lower genital tract intraepithelial lesions was 11% (1,923/16,732), with an increasing trend.[1–3] In this study, we aimed to explore the depth of the involved and noninvolved vaginal epithelium in women with VaIN and provide more data regarding epithelial destruction during laser vaporization.

MATERIALS AND METHODS

Patients

We included women diagnosed with VaIN by colposcopy-directed biopsy at the Obstetrics and Gynecology Hospital of Fudan

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Approval was obtained from institutional review board of the Obstetrics and Gynecology Hospital of Fudan University before data extraction was performed.

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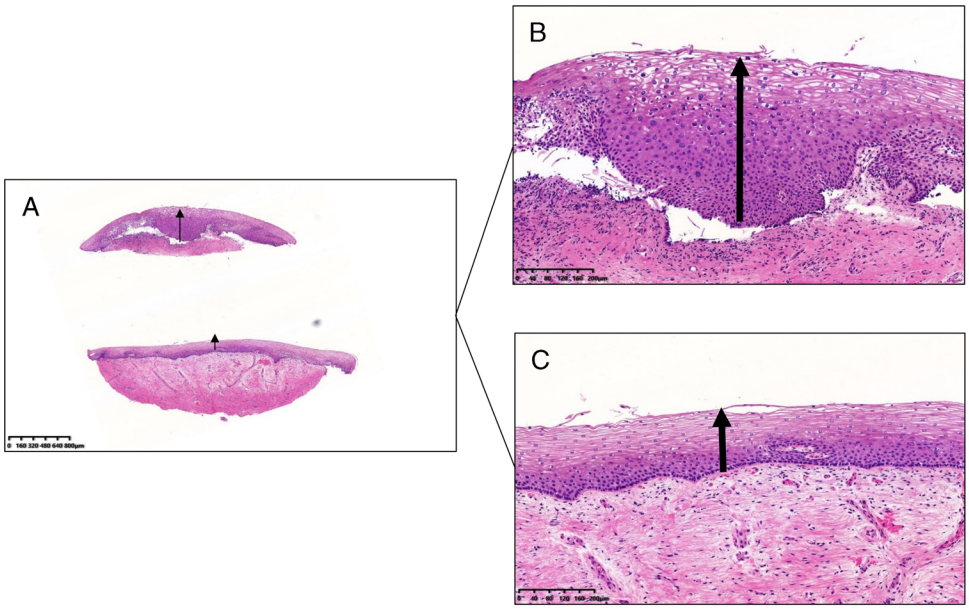

FIGURE 1. Digital pathology slides were scanned by K-scanner (KF-BIO-120, digital pathology slides scanner, KFBIO) and reviewed on K-viewer software. The epithelium showing involvement and non-involvement of VaIN1 was evaluated on the same slide HE × 2 (A). Measurement of depth from the basal layer to the surface of the squamous epithelium was obtained as the arrow was pulled at the locus of VaIN1. HE × 8 (B), with the thinnest uninvolved epithelium (C) serving as the normal control. HE × 8. (HE, hematoxylin-eosin staining).

University (OGHFU) from January 1, 2019, to April 30, 2020. More than 400 women were diagnosed with VaIN in the OGHFU. Women with an incomplete medical history or biopsy specimens and those who were lost to follow-up were excluded. Finally, 246 women were enrolled in the study. Among them, 123 were diagnosed with VaIN 1 and 123 were diagnosed with VaIN 2/3. The thickest lesion was used to define the final lesion thickness. Approval was obtained from the institutional review board of the OGHFU before data extraction was performed, and all women provided consent to participate in the study.

Cytology

All cervical or vaginal cytology samples were collected by gynecologists and were prepared using the SurePath platform (BD Diagnostics) according to the manufacturer's instruction. All cytology slides were prepared in the pathology laboratory of the OGHFU. The cytology tests were interpreted and reported by pathologists using the 2014 Bethesda System criteria and terminology.

Human Papillomavirus Testing

All HPV testing samples were collected by gynecologists separately from samples used for cytology. Human papillomavirus testing was performed using fluorescence-based multiplex real-time HPV DNA genotyping kit (Bioperfectus, Jiangsu, China), which can detect high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

Pathology

Biopsy specimens and excision specimens were fixed in buffered formalin and embedded in paraffin. Sections (0.004 mm) were stained with hematoxylin and eosin. All VaIN slides were scanned and reviewed in the form of digital slides by 2 gynecological pathologists. To compare the thickness of vaginal epithelium between before and after formalin fixation, 7 radical hysterectomy samples of International Federation of Gynecology and Obstetrics phase I cervical cancer patients were selected, each of which has a frozen section diagnosis of vaginal margin and their corresponding formalin-fixed and paraffin-embedded (FFPE) samples. All margin diagnoses were negative for intraepithelial lesion or invasive cancer. In total, 28 paired sections were retrieved from those cases with 4 from each, except 1 lost epithelium in the FFPE section, which was excluded. The measurement of the thickness of epithelium was processed on the whole-slide image on the similar sites of paired sections.

Vertical measurements were started at the surface of the epithelium and extended to the basal layer. Measurements of multiple foci were performed, and the maximum values were recorded (see Figure 1). The thicknesses of the uninvolved epithelium were also obtained if available in the same set of slides.

All available data, including patient history, cytology, hrHPV test results, and histological information, were recorded. The Bethesda System terminology was used to report cytology results.

TABLE 1. Clinical Characteristics of the Women With Vaginal Intraepithelial Neoplasia

	VaIN 2/3 (n = 123)	VaIN 1 (n = 123)	p
Age, mean ± SD, y	50.01 ± 11.41	45.67 ± 12.54	<.001
hrHPV positivity, %	96.60	92.40	.12
Cytology			
≤LSIL	78	116	<.001
≥HSIL	41	3	
Involved epithelial thickness, mean ± SD (range), mm	0.41 ± 0.21 (0.10–1.20)	0.40 ± 0.19 (0.05–1.38)	.70

HSIL, high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; ≤LSIL: atypical squamous cells of undetermined significance, no intraepithelial or malignant lesions, or low-grade squamous intraepithelial lesion.

TABLE 2. Involved and Noninvolved Vaginal Epithelial Thickness in Patients of Different Ages

		Epithelial thickness, mean ± SD, mm						
		VaIN 2/3				VaIN 1		
Age group y	No. patients	Involved	Noninvolved	*p*	No. patients	Involved	Noninvolved	*p*
≤40	27	0.45 ± 0.23	0.25 ± 0.09	<.01	46	0.40 ± 0.22	0.19 ± 0.07	<.01
41–50	35	0.48 ± 0.22	0.18 ± 0.11	<.01	28	0.41 ± 0.18	0.17 ± 0.08	<.01
51–60	38	0.37 ± 0.18	0.15 ± 0.09	<.01	32	0.37 ± 0.18	0.14 ± 0.09	<.01
≥61	23	0.30 ± 0.15	0.11 ± 0.06	<.01	17	0.42 ± 0.17	0.14 ± 0.06	<.01
Total	123	0.41 ± 0.21	0.17 ± 0.10	<.01	123	0.40 ± 0.19	0.17 ± 0.08	<.01

Statistical Analysis

The *t* test and Pearson χ^2 test were performed using SPSS (version 16.0; SPSS, Inc, Chicago, IL) for statistical analyses. Statistical significance was set at $p < .05$.

RESULTS

The clinical characteristics of the women with VaIN are shown in Table 1. The mean ± SD patient age was 50.01 ± 11.41 and 45.67 ± 12.54 years in the VaIN 2/3 and VaIN 1 groups, respectively ($p < .001$); 96.6% of the patients in the VaIN 2/3 group and 92.4% of those in the VaIN 1 group showed positive results for hrHPV; more than 90% of the women had HPV 16 infection in both groups; and 34.5% and 2.5% of women in the VaIN 2/3 and VaIN 1 groups, respectively, showed high-grade squamous intraepithelial lesion cytology or severer. The involved epithelial thickness was 0.41 ± 0.21 and 0.40 ± 0.19 mm in the VaIN 2/3 and VaIN 1 groups, respectively. More than 99% of the VaIN 2/3 cases (122/123) did not show thickness greater than 1 mm (≤0.93 mm), with only one case showing thickness greater than 1 mm (1.2 mm); coincidentally, more than 99% of the VaIN 1 cases (122/123) also did not show thickness greater than 1 mm (≤0.89 mm), with only one case showing thickness greater than 1 mm (1.4 mm).

Table 2 shows the epithelial thickness of the VaIN in patients of different ages. The involved epithelial thickness in both VaIN 2/3 and VaIN 1 groups was consistently greater than the noninvolved epithelial thickness across all age groups (0.41 ± 0.21 vs. 0.17 ± 0.10 mm, $p < .01$; 0.40 ± 0.19 vs. 0.17 ± 0.08 mm, $p < .01$). The thickness of the noninvolved vaginal epithelium showed a decreasing tendency with age in both groups. The average involved epithelial thickness was 0.41 ± 0.21 and 0.40 ± 0.19 mm in the VaIN 2/3 and VaIN 1 groups, respectively. The involved epithelial thickness in the VaIN 2/3 group showed a decreasing tendency after 50 years of age, while no decreasing tendency appeared in the VaIN 1 group (see Figure 2).

In the VaIN 2/3 group, the lesion thickness in premenopausal women, postmenopausal women receiving estrogen, and postmenopausal women not receiving estrogen was 0.47 ± 0.23, 0.36 ± 0.17, and 0.34 ± 017 mm, respectively (see Table 3). The lesion thickness in premenopausal women was greater than those of postmenopausal women receiving estrogen ($p = .016$) and not receiving estrogen ($p = .017$); however, lesion thickness did not significantly differ among the 2 subgroups of postmenopausal women ($p = .53$).

In the VaIN 1 group, the lesion thickness in premenopausal women, postmenopausal women receiving estrogen, and postmenopausal women not receiving estrogen was 0.41 ± 0.21, 0.41 ± 0.19, and 0.32 ± 0.15 mm, respectively. The lesion thickness in premenopausal women was not significantly different from that in postmenopausal women receiving estrogen ($p = .87$) and those not receiving estrogen ($p = .11$), and lesion thickness did not significantly differ among the 2 subgroups of postmenopausal women ($p = .08$).

Noninvolved epithelial thickness in premenopausal women in both VaIN 2/3 and VaIN 1 groups was greater than the corresponding values in postmenopausal women not receiving estrogen (0.22 ± 0.10 vs. 0.09 ± 0.06 mm, $p < .001$; 0.18 ± 0.07 vs. 0.13 ± 0.07 mm, $p = .010$), respectively.

The involved and noninvolved epithelial thickness values are compared between the VaIN 2/3 and VaIN 1 groups in Table 3. In comparisons between the VaIN 2/3 and VaIN 1 groups, the differences in the involved epithelial thickness were not statistically significant for any of the subgroups, namely, premenopausal women, postmenopausal women receiving estrogen, and postmenopausal women not receiving estrogen ($p > .05$ for all comparisons). Comparisons of the noninvolved epithelial thickness also showed no subgroup-level significant differences between the VaIN 2/3 and VaIN 1 groups ($p > .05$ for all comparisons).

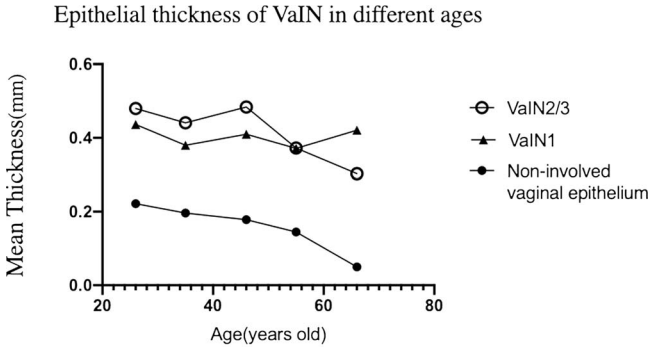

FIGURE 2. Thickness of the involved epithelium in VaIN2/3 and VaIN1 lesions as well as the thickness of the non-involved epithelium in patients of different ages.

TABLE 3. Comparison of Epithelial Thickness Between the VaIN 2/3 and VaIN 1 Groups

	Involved, mm			Noninvolved, mm		
Epithelium status	**VaIN 2/3**	**VaIN 1**	***p***	**VaIN 2/3**	**VaIN 1**	***p***
Premenopausal	0.47 ± 0.23	0.41 ± 0.21	.12	0.22 ± 0.10	0.18 ± 0.07	.06
Postmenopausal	0.35 ± 0.17	0.39 ± 0.18	.33	0.14 ± 0.09	0.14 ± 0.09	.89
Postmenopausal with estrogen	0.36 ± 0.17	0.41 ± 0.19	.22	0.16 ± 0.09	0.15 ± 0.09	.56
Postmenopausal without estrogen	0.34 ± 0.17	0.32 ± 0.15	.54	0.09 ± 0.06	0.13 ± 0.07	.09

Post with hormone, postmenopausal women on hormone therapy; post without hormone, postmenopausal women without hormone therapy.

To compare the thickness of vaginal epithelium before and after the treatment of FFPE, we enrolled 27 pairs of frozen section and corresponding FFPE-treated section. The thickness of epithelial was 0.39 ± 0.18 and 0.38 ± 0.17 mm on frozen and FFPE section, respectively, resulting in no significant difference in the changes in size caused by the tissue fixation ($p = .56$).

DISCUSSION

In comparison with cervical intraepithelial neoplasia, VaIN has been studied in less detail. Low-grade VaIN is often the result of infection with nononcogenic subtypes of HPV and often regress spontaneously. According to the existing guidelines and the consensus of experts, VaIN 1 is managed with close surveillance rather than treatment, while VaIN 2/3 has a higher risk of progression to squamous cell carcinoma of the vagina and is therefore treated.[11,12] A broad range of treatment options are available for cases with VaIN 2/3, including ablation, topical therapy, excision, and radiation therapy. Among them, ablation techniques such as carbon dioxide laser therapy offer the advantages of less trauma and easy operation and can also achieve therapeutic effects with minimal scarring and sexual dysfunction.[13,14]

The depth of treatment is an essential factor in carbon dioxide laser treatment. However, the existing studies on this topic have been conducted with small sample sizes. Anatomical site contributes to the degree of shrinkage. For lower limb skin tumors, it was showed that the pathology specimen is 28.6% smaller than the specimen marked for excision.[15] Excision and FFPE treatment can make the skin tumors shrink to various extents depending on the original liquid content in the sample.[15] However, temporal artery biopsies contract upon surgical excision but do not shrink further during formalin fixation.[16] However, the squamous cells construct a firm layer on the mucosa without blood vessels than the soft tissue beneath them. In addition, we demonstrated that FFPE sections of VaIN were reliable to reflect the true depth of VaIN in this study.

In 1992, Benedet et al[10] conducted a study with FFPE sections of 56 patients to evaluate the depth of carbon dioxide laser

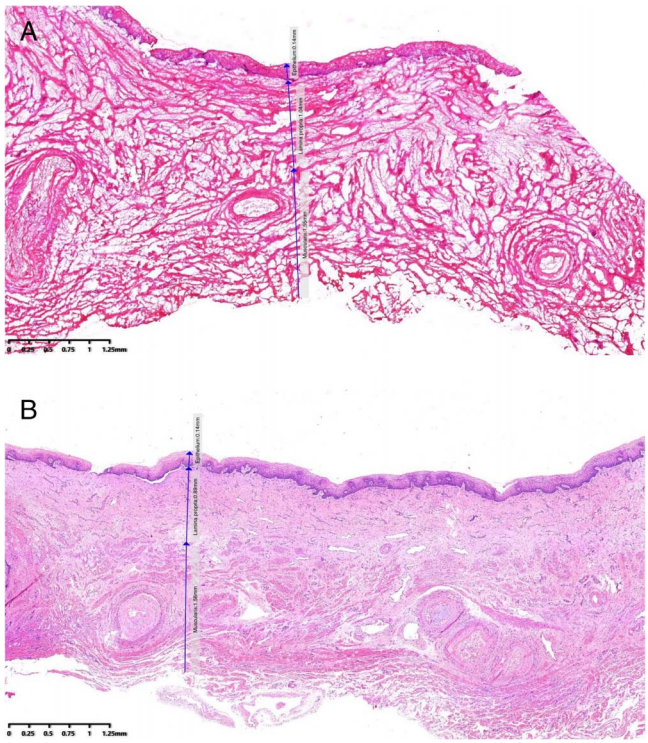

FIGURE 3. Vaginal wall consists of mucosa, lamina propria, and muscularis. The mucosal lining is a stratified squamous epithelium. The lamina propria lies beneath the squamous epithelium and consists of fibrovascular stroma. The vaginal muscularis is composed of smooth muscle bundles. Two sections from a same vaginal margin of a patient with International Federation of Gynecology and Obstetrics phase I cervical cancer who underwent radical hysterectomy. A, Shows the frozen section of vaginal margin and B, shows it's corresponding FFPE sample.

treatment for VaIN, and their findings showed that a laser vaporization depth of 1.5 mm was sufficient to clear the lesion. However, another study recommended a vaporization depth of 2–3 mm.[17] Thus, the recommended vaporization depth shows a wide variation from 1.5 to 3 mm. Because VaIN is limited to the epithelia, ablation of the involved epithelia is sufficient for treatment. However, excessive ablation can result in decreased elasticity, stenosis, and bleeding during vaginal examination.

The vaginal wall consists of 3 layers: the mucosa, muscularis, and adventitia. The mucosa consists of stratified squamous epithelium and lamina propria. The lamina propria contains many elastic fibers as well as a dense network of blood vessels and lymphatic and nerve supply. The muscularis consists of autonomically innervated smooth muscle fibers arranged into outer longitudinal and inner circular layers. The adventitia is rich in collagen and elastic tissue, providing structural support to the vagina (see Figure 3).

Song et al[18] measured the vaginal mucosal and muscle thickness in 7 fresh Korean cadavers aged 66.6 ± 12.1 years. In their study, the thickness of the mucosa ranged from 1.18 ± 0.20 to 3.50 ± 2.06 mm; the thickness of muscle ranged from 2.98 ± 0.3 to 5.59 ± 2.74 mm; and the full thickness of the vaginal wall ranged from 4.37 ± 0.82 to 9.99 ± 4.81 mm. Thus, the wide variations in the previously recommended vaporization depths ranging from 1.5 to 3 mm can result in excessive damage to the mucosa and even muscle.

In our study, the involved epithelial thickness was no more than 1 mm in 99.2% of both VaIN 2/3 and VaIN 1 cases, while one case of VaIN 2/3 and one case of VaIN 1 with papillary hyperplasia showed thickness greater than 1 mm (1.20 and 1.38 mm, respectively). The thickness of VaIN is generally less than 1 mm for women of all ages, except in rare cases of visible lesions with papillary hyperplasia. Therefore, we hypothesize that the vaporization depth of 1 mm is sufficient for the vast majority of VaIN. For VaIN with papillary hyperplasia, the raised lesion should be vaporized first and then vaporized to the depth of 1 mm.

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REFERENCES

1. Cong Q, Wang Q, Gao SJ, et al. Detection trend of vaginal intraepithelial neoplasia diagnosed by colposcopy guided biopsy from 2013 to 2015. *Zhonghua Fu Chan Ke Za Zhi* 2017;52:239–43.
2. Zhang L, Wang Q, et al. Clinical features of vaginal precancer and cancer: a retrospective study of 4562 cases in China. *Prog Obstet Gynecol* 2021;30: 15–9.
3. Cong Q, Song Y, Wang Q, et al. A retrospective study of cytology, high-risk HPV, and colposcopy results of vaginal intraepithelial neoplasia patients. *Biomed Res Int* 2018;2018:5894801.
4. Darragh TM, Colgan TJ, Cox JT, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *J Low Genit Tract Dis* 2012;16:205–42.
5. Piovano E, Macchi C, Attamante L, et al. CO_2 laser vaporization for the treatment of vaginal intraepithelial neoplasia: effectiveness and predictive factors for recurrence. *Eur J Gynaecol Oncol* 2015;36:383–8.
6. Buck HW, Guth KJ. Treatment of vaginal intraepithelial neoplasia (primarily low grade) with imiquimod 5% cream. *J Low Genit Tract Dis* 2003;7:290–3.
7. Fiascone S, Vitonis AF, Feldman S. Topical 5-fluorouracil for women with high-grade vaginal intraepithelial neoplasia. *Obstet Gynecol* 2017;130: 1237–43.
8. Caglar H, Hertzog RW, Hreshchyshyn MM. Topical 5-fluorouracil treatment of vaginal intraepithelial neoplasia. *Obstet Gynecol* 1981;58: 580–3.
9. Rhodes HE, Chenevert L, Munsell M. Vaginal intraepithelial neoplasia (VaIN 2/3): comparing clinical outcomes of treatment with intravaginal estrogen. *J Low Genit Tract Dis* 2014;18:115–21.
10. Benedet JL, Wilson PS, Matisic JP. Epidermal thickness measurements in vaginal intraepithelial neoplasia. A basis for optimal CO_2 laser vaporization. *J Reprod Med* 1992;37:809–12.
11. Gunderson CC, Nugent EK, Elfrink SH, et al. A contemporary analysis of epidemiology and management of vaginal intraepithelial neoplasia. *Am J Obstet Gynecol* 2013;208:410.e1–6.
12. Hodeib M, Cohen JG, Mehta S, et al. Recurrence and risk of progression to lower genital tract malignancy in women with high grade VAIN. *Gynecol Oncol* 2016;141:507–10.
13. Jentschke M, Hoffmeister V, Soergel P, et al. Clinical presentation, treatment and outcome of vaginal intraepithelial neoplasia. *Arch Gynecol Obstet* 2016;293:415–9.
14. Rountis A, Pergialiotis V, Tsetsa P, et al. Management options for vaginal intraepithelial neoplasia. *Int J Clin Pract* 2020;74:e13598.
15. De Waal J. Skin tumour specimen shrinkage with excision and formalin fixation—how much and why: a prospective study and discussion of the literature. *ANZ J Surg* 2021;91:2744–9.
16. Naumovska M, Sheikh R, Engelsberg K, et al. Temporal artery biopsies contract upon surgical excision, but do not shrink further during formalin fixation. *Scand J Rheumatol* 2020;49:84–6.
17. Fang S, Jiandong W, Shiqian Z, et al. Expert consensus on the diagnosis and treatment of vaginal intraepithelial neoplasia (2020). *Chin J Pract Gynecol Obstet* 2020;36:722–8.
18. Song YB, Hwang K, Kim DJ, et al. Innervation of vagina: microdissection and immunohistochemical study. *J Sex Marital Ther* 2009;35:144–53.