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ORIGINAL ARTICLE

Association Between Positive Human Papillomavirus Status After Conization and Disease Recurrence in Patients with Cervical Intraepithelial Neoplasia Grade 3

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Abstract

Objective This study aimed to examine the associations of cone margin and human papillomavirus (HPV) status after conization with cytological abnormalities and disease recurrence in patients with cervical intraepithelial neoplasia grade 3 (CIN3).

Methods This is a retrospective study of 366 women with CIN3 who underwent conization at Kagoshima University Hospital between 2004 and 2017. Conization was performed using an ultrasonic scalpel. The polymerase chain reaction for detecting HPV genotypes was performed using fresh cervical cell samples. We examined the associations of margin status and HPV status after conization with cytological abnormalities and recurrence.

Results Among 224 women with CIN3, 193 (86.2%) underwent HPV genotype testing before conization. The HPV-positive rate was 84.9%. The most common HPV genotypes before conization were HPV 16, 31, 58, 52, 18, 35, and 33. In 191 patients, the uterus was preserved after conization. Sixteen patients had pathologically positive margins, 165 had negative margins, and 10 had unclear margins. There was no significant difference in abnormal cytology and recurrence rate after conization between the three groups. Five patients with positive margins and abnormal cytology during follow-ups were HPV16- or HPV58-positive in the preoperative HPV testing. Of the 191 women, 91 (47.6%) underwent pre- and postoperative HPV genotype testing, among whom 14 (15.4%) were HPV-positive after conization. No significant difference in abnormal cytology based on HPV status after conization was found. The recurrence rate tended to be higher in HPV-positive patients than in HPV-negative patients after conization (21.4% vs. 1.3%, $p < 0.05$). Three patients with HPV positivity after conization and recurrence during follow-up were HPV16- or HPV58-positive.

Conclusions HPV positivity after conization for CIN3 was associated with a high recurrence rate, especially in HPV16- and HPV58-positive patients. HPV58 has not received much attention thus far, but abnormalities in cytology and recurrence may be as likely as those associated with HPV16. Thus, a careful follow-up in such patients is recommended.

Keywords CIN · Conization margin · HPV · Recurrence

Introduction

Human papillomavirus (HPV) is one of the leading causes of cervical cancer [1, 2], and more than 35 genotypes are involved in cervical cancer [1, 3]. Cervical intraepithelial neoplasia (CIN) is a precursor to cervical cancer [4], and high-risk HPV is detected in most surgical specimens of CIN lesions [5]. Early detection and treatment of CIN can help in preventing the progression of the disease to cervical cancer. The standard treatment for CIN3 patients is cervical conization [6].

A relapse of CIN is reported to occur in 5–25% of patients after conization [7–9], and a close follow-up by cytology after treatment is recommended. Recently, several studies

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have shown the usefulness of HPV testing for follow-up after conization [6, 10–12]. These studies have shown that HPV testing has a higher sensitivity than cytology and a similar specificity in detecting relapse.

The present study aimed to examine the associations of cone margin and HPV status after conization with cytological abnormalities and recurrence in patients with CIN3 at our institution. We also examined the possible HPV genotypes that caused such cytological abnormalities and recurrence.

Materials and Methods

Study Design and Population

This study is a retrospective analysis of 366 patients who underwent conization at Kagoshima University Hospital between January 2004 and December 2017. Conization was performed in all patients using a harmonic scalpel, one of the hot-knife devices widely used in Japan [9, 13]. Five patients with incomplete records were excluded from the analysis. Of the 361 patients, 224 were pathologically diagnosed with CIN3 from conization specimens and were analyzed further. Patients with invasive cancer, in situ adenocarcinoma, CIN1, CIN2, and other cervical lesions were excluded from the analysis (Fig. 1). The pathological state of the stump lesions in the conization specimens was identified as positive, negative, or unclear.

All procedures were approved by the Ethics Committee of Kagoshima University [K-170369]. Written informed consent for this study was obtained from all participants.

Cytology and HPV Genotype Testing

Cervical samples were obtained for cytology using a Cervex-Brush (Rovers Medical Devices B.V., Lekstraat, The Netherlands). Specimens were then fixed in 95% ethanol, stained using the Papanicolaou method, and classified according to the 2001 Bethesda System.

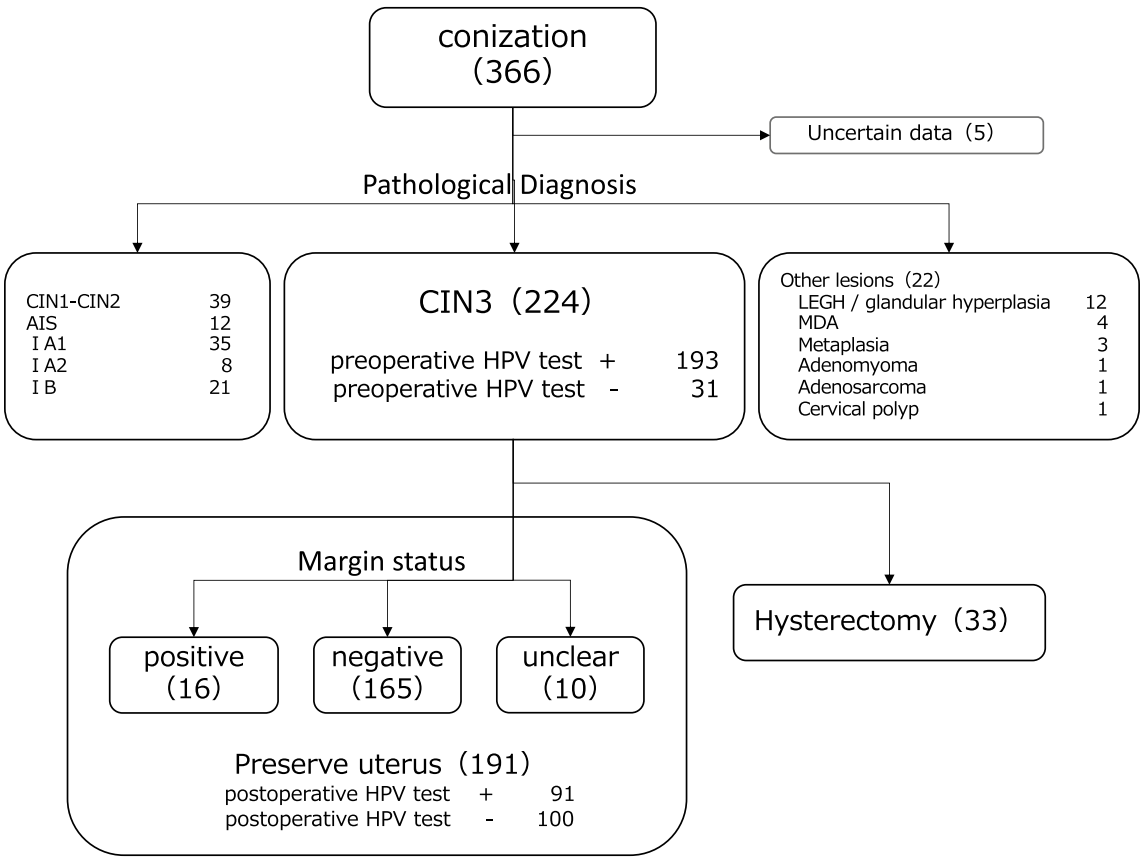

Fig. 1 Composition of the study population. Of the 366 women who underwent conization at Kagoshima University Hospital, 224 were diagnosed with CIN3. Among them, 193 had HPV genotype testing before conization. Thirty-three patients with CIN3 had hysterectomy immediately after conization. In 191 patients, the uterus was preserved, and the group was categorized into three according to cone margin status. Among the 191 patients, 91 underwent HPV genotype testing after conization. *CIN3* grade 3 cervical intraepithelial neoplasia, *HPV* human papillomavirus

The PCR for detecting an HPV DNA genotype was performed using fresh cell samples from the cervix, and high-risk HPV-positive samples were defined as HPV-positive.

The detection and typing of genital high-risk HPV DNAs in cervical scrapes were carried out using the E6/E7-specific consensus PCR [14]. This test can identify 11 high-risk HPV genotypes, namely 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58.

HPV genotype testing was performed immediately before and after conization. The postoperative HPV genotype testing was performed several times between 1 and 12 months after conization. The preoperative testing was performed only once, and it was determined whether the test was positive. The postoperative testing was determined to be positive if it was positive at least once.

Management After Conization

Patients who had no wish for future pregnancy consented to undergo a subsequent hysterectomy. Those who did not undergo a subsequent hysterectomy were followed up every 3–4 months during the first year, every 6 months during the second year, and yearly after that. Cytology was performed at every visit. A finding of atypical squamous cells of undetermined significance (ASC-US) or worse was defined as abnormal cytology. Colposcopy was performed only when a cytological abnormality was detected. Recurrence was determined when a pathological CIN was detected through a punch biopsy.

Statistical Analysis

Statistical analyses were performed using R version 3.4 (The R Foundation for Statistical Computing), and Fisher's exact test was used to compare variables.

Results

Among the 366 women who underwent conization, 224 were diagnosed with CIN3. The median age was 36 years (range 23–78). Among the 224 patients, 193 (86.2%) had HPV genotype testing immediately before conization. The most frequently detected HPV genotypes were HPV16 (77), HPV31 (35), HPV58 (33), HPV52 (30), HPV18 (18), HPV35 (3), HPV33 (3), and HPV39 (1) (Fig. 2a). A total of 135 patients were positive for a single high-risk HPV genotype, but 29 were positive for multiple high-risk HPV genotypes. High-risk HPV genotypes were not detected in 29 patients (15.0%), and HPV genotype testing was not performed in 31 patients (16.1%). Thirty-three women (14.7%) had hysterectomy immediately after conization, while 191 women had a preserved uterus. The follow-up period was at least 2 years for each case. During follow-up, 30 patients (15.7%) presented with cytological abnormalities worse than ASC-US, and 6 (3.1%) were diagnosed with a pathological recurrence of CIN1. There was no recurrent case worse than CIN2. The average age of patients with cytological abnormalities was 36 years (range 25–70), and that of patients with recurrence was 43.5 years (range 29–70).

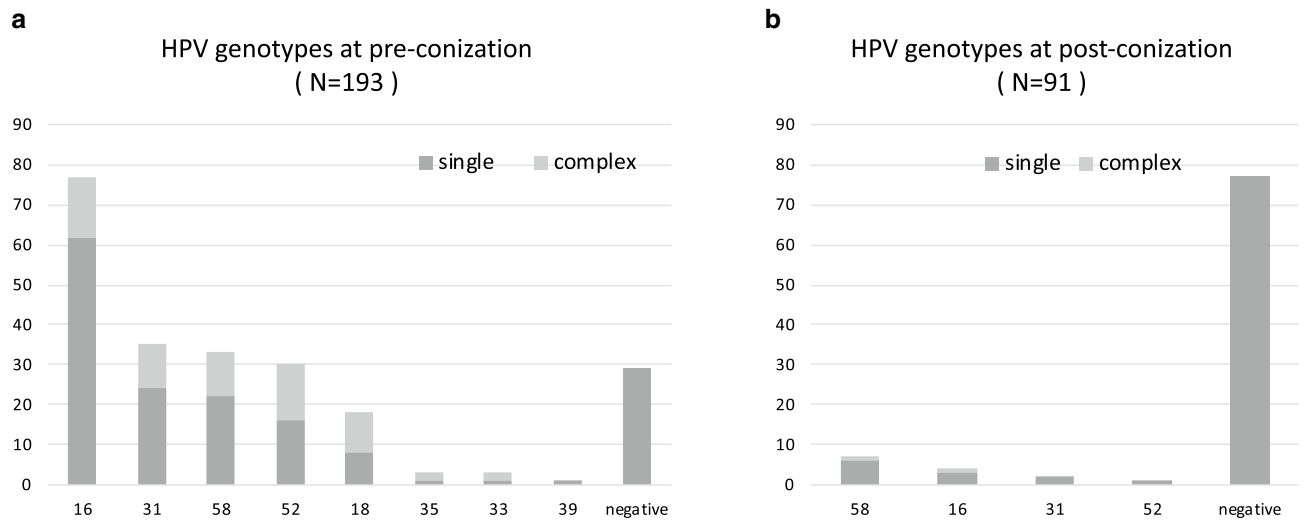

Fig. 2 High-risk HPV genotypes detected before and after conization. **a** In the preoperative HPV genotype testing, 135 patients were positive for single high-risk HPV infections, while 29 were positive for multiple high-risk HPV infections. High-risk HPV infections were not detected in 29 patients, and the HPV test was not performed in 31 patients. **b** Postoperative HPV genotype testing was performed in 91 patients whose uteri were preserved after conization. Twelve patients tested positive for single high-risk HPV infections, while two patients were positive for multiple high-risk HPV infections. *HPV* human papillomavirus

Sixteen patients had pathologically positive margins, 165 had negative margins, and 10 had unclear pathological margins for CIN. In the 16 patients with positive margins, 5 patients had endocervical margins, 4 patients had ectocervical margins, and 7 patients had both endocervical and ectocervical margins. Abnormal cytology was observed in 5 of 16 cases with positive margins, 24 of 165 cases with negative margins, and 1 of 10 cases with unclear margins. There was no significant difference in abnormal cytology in the follow-up period between these three groups (Table 1). All five patients with positive margins who presented with abnormal cytology during the follow-up were HPV16-positive preoperatively, and one patient was infected with both HPV16 and HPV58. CIN recurrence was observed in 2 of 16 cases with positive margins and 4 of 165 cases with negative margins. No recurrence was found in the group with unclear margins. There was no significant difference in the recurrence rate between the three groups (Table 1).

Of the 191 women with a preserved uterus, 91 (47.6%) underwent pre- and postoperative HPV genotype testing. Among them, 14 women were HPV-positive and 77 were HPV-negative. The most frequently detected HPV genotypes in the postoperative examination were HPV58 (7), HPV16 (5), HPV31 (2), and HPV52 (1) (Fig. 2b). In 12 of 14 cases, the same HPV genotype was detected as before conization. In one patient who was positive only for HPV16 before conization, HPV16 and HPV58 were detected after conization. In another patient with both preoperative HPV16 and HPV58 infection, only HPV58 was detected after conization. However, there was no significant difference in abnormal cytology based on the HPV genotype after conization (Table 2). CIN recurrence was observed in 3 of 14 HPV-positive cases and 1 of 77 HPV-negative cases. The recurrence rate was significantly higher in the HPV-positive group than in the HPV-negative group after conization (21.4% vs. 1.3%, $p < 0.05$) (Table 2). The three HPV-positive cases with recurrence after conization consisted of an HPV16-positive infection, an HPV58-positive infection, and a double infection with HPV16 and HPV58. Of the 14 patients who were HPV-positive after conization, only 1 had pathologically positive margins and 13 had negative margins. The positive margin case was observed in a 70-year-old woman, and she had both endocervical and ectocervical positive margins. In addition, this case involved abnormal cytology and recurrence of CIN2 after conization.

Table 2 Abnormal cytology and recurrence after conization according to the HPV status

	HPV-positive 14	HPV-negative 77	
Abnormal cytology	5 (35.7%)	16 (20.1%)	$p = 0.30$
Recurrence of CIN	3 (21.4%)	1 (1.3%)	$p < 0.05$

Postoperative abnormal cytology and recurrence were examined according to the high-risk HPV status after conization. No significant difference in abnormal cytology was found, but the recurrence of CIN was significantly higher in the HPV-positive group than in the HPV-negative group

CIN cervical intraepithelial neoplasia, *HPV* human papillomavirus

Discussion

Conization is widely performed as a fertility-sparing treatment for CIN and early cervical cancer. However, treated women are at an increased risk for subsequent invasive cervical cancer compared with the general population for at least 10 years after the procedure [15]. Several variables, such as older age, multiparity, high CIN grade, posttreatment HPV-positive status, short cone length, positive margin, and lesion size, have been associated with the risk of recurrent disease. As most CIN3 cases do not recur after treatment, it is important to determine which cases should be carefully monitored. Cytology-based follow-up is commonly performed after CIN treatment [7, 11, 16]. In CIN3, recurrences occur in 5–25% of cases following conization [10, 17], and many studies showed that patients with a positive cone margin and positive HPV status after conization are at a high risk of disease recurrence [10–12]. In the present study, we retrospectively investigated the margin status and HPV infection after conization in patients with CIN3. However, we did not assess the diameter of the tumor and the height of the conization specimens. During follow-ups, 30 patients (15.7%) presented with cytological abnormalities

Table 1 Abnormal cytology and recurrence after conization according to the margin status

	Margin positive 16	Margin negative 165	Margin unclear 10	
Abnormal cytology	5 (31.3%)	24 (14.5%)	1 (10.0%)	$p = 0.147$
Recurrence of CIN	2 (12.5%)	4 (2.4%)	0 (0%)	$p = 0.089$

Postoperative abnormal cytology and recurrence were examined in the three groups according to the cone margin status. No significant difference in abnormal cytology and recurrence of CIN during follow-up was found between the three groups

CIN cervical intraepithelial neoplasia

and 6 (3.1%) were diagnosed with a pathological recurrence of CIN. These findings are consistent with those of another study, which found a cytological abnormality rate of approximately 18% [6]. The recurrence rate in our study is lower than the overall average of 6.6% found in a recent meta-analysis [10]; in long-term cohort studies, the recurrence rate is 11.5–18.3% [7, 18]. The reason for the low recurrence rate is unknown. As long-term follow-up has been shown to be associated with a high recurrence rate, the follow-up period in our study might be a problem.

In this study, 16 (8.4%) patients had pathologically positive margins, 165 (86.4%) had negative margins, and 10 (5.2%) had unclear margins. According to a systematic review, the positive margin rate for the treatment of precancerous lesions is 23.1% [10]. In our data, even if unclear cases were included, the positive margin rate was only 14%. This finding might be attributed to the surgical method and equipment used, but the exact cause cannot be determined. Abnormal cytology was observed in 30 patients (15.7%), but there was no significant difference in the follow-up period between the three groups (Table 1). All five patients with positive margins, who presented with abnormal cytology during follow-ups, were HPV16-positive preoperatively, and one patient had a mixed infection of HPV16 and HPV58. CIN recurrence was observed in six patients (3.1%). Numerous studies have confirmed that patients with a positive cone margin for CIN are at a high risk of disease recurrence [10–12, 18, 19]. However, there was no significant difference in the recurrence rate in our data.

Several systematic reviews have provided consistent evidence that high-risk HPV testing is an accurate method to predict residual or recurrent CIN after the treatment of cervical precancer [10, 11]. High-risk HPV infection post-treatment predicts treatment failure more accurately than the margin status [10]. Recently, Bruhn et al. reported that with HPV testing, cytology can be omitted from the posttreatment management without lowering the negative predictive value [12]. Our data also support that a positive HPV status after conization predicts recurrence more accurately than the margin status.

The HPV genotypes detected before conization are shown in Fig. 2a. A total of 135 patients were positive with single high-risk HPV infections, while 29 were positive with multiple high-risk HPV infections. As in other studies [20–22], HPV16 was the most commonly detected genotype in our study. The second most common type was HPV31, followed by the so-called Asian types HPV58 and HPV52, and the fifth most common type was HPV18. This trend in HPV infection is almost consistent with that in other reports from Asia [23–25].

Of the 191 women whose uteri were preserved after conization, 91 received pre- and postoperative HPV genotype testing. Among them, 14 (15.4%) were HPV-positive and 77 (84.6%) were HPV-negative after conization. In other studies, over half of the HPV types detected in the postoperative genotype testing were different from those detected in the preoperative testing [24, 26]. However, no study has clearly shown why HPV genotypes change before and after treatment. In our data, the postoperative HPV genotypes were almost the same as the preoperative ones. Even if the postoperative HPV genotype testing result was positive, in most cases, it subsequently became negative. The most commonly detected HPV genotype in the postoperative examination was HPV58, followed by HPV16, HPV31, and HPV52 (Fig. 2b). Nam et al. reported that HPV16 is an important independent factor for HPV persistence based on logistic regression analysis [27]. In other reports, HPV58 is the most common [24], second most common [21], or third most common HPV genotype [22] after conization. Although HPV58 is not the most common HPV genotype found in preoperative examinations, it is less likely to be cleared by conization to the same extent as HPV16. In one study, HPV was detected after a loop electrosurgical excision procedure after 3 months (45.6%), and the rate gradually decreased after 6 months (14.3%), 9 months (6.3%), 12 months (2.2%), 18 months (1.5%), and 24 months (1.1%) [28]. However, the study did not investigate HPV genotypes. In another study that calculated the HPV detection rate by dividing it by the postoperative period, 9.4% of all HPV infections continued for 4–6 months after surgery, but the rate decreased to 2.2% after 8–12 months [20]. In that study, HPV58 was higher than average, at 12.5%, 4–6 months after surgery but was rather low, at 0%, after 8–12 months. Such a difference in clearance could be due to the HPV genotype, and the clearance of HPV58 may be as poor as that of HPV16.

In conclusion, this study showed that a positive HPV status after conization in patients with CIN3 was associated with a high recurrence rate, especially in patients who were positive for HPV16 and HPV58 genotypes. Therefore, a careful follow-up is recommended in these patients.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The data in this manuscript is approved by the Ethics Committee of Kagoshima University.

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