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ORIGINAL RESEARCH

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Impact of human papillomavirus status before cervical conization on the clinical course of patients with cervical intraepithelial neoplasia

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Abstract

Persistent infection with human papillomavirus (HPV) is a key driver in the development of cervical cancer (CC). We aimed to elucidate the relationship between preoperative high-risk HPV status and prognosis of cervical intraepithelial neoplasia (CIN) in patients undergoing cervical conization. We retrospectively analyzed data from 2546 patients with CIN who underwent HPV deoxyribonucleic acid (DNA) testing and cervical conization in two individual years, *i.e.*, 2009 and 2013, at 205 Japanese institutions. Patients were categorized into five groups based on their high-risk HPV status: high-risk HPV negative (Group 1); HPV 16/18 positive (Group 2); positive for HPV types 31, 33, 35, 45, 52 or 58 (Group 3); other high-risk HPV positive (Group 4); and unconfirmed high-risk HPV status (Group 5). Logistic and Cox regression analyses were conducted for statistical assessment. The distribution of participants across Groups 1 to 5 was 8.1%, 26.3%, 20.1%, 3.0% and 42.5%, respectively. Cervical conization identified CC in 3.9% (99 patients) of the cohort. Multivariate analysis revealed that diagnostic conization, preoperative diagnosis of CIN grade 3 and HPV 16/18 positivity were significant risk factors for post-conization CC. Notably, no correlation was found between preoperative HPV status and post-conization recurrence in patients without CC. HPV types 16 and 18 emerged as significant independent risk factors for CC development following conization. The study findings underscore the need for vigilant management of this patient group. However, the presence of high-risk HPV before conization was not correlated with the risk of recurrence.

Keywords

Cervical intraepithelial neoplasia; Conization; Papillomaviridae; Papillomavirus infections; Risk factors

1. Introduction

Cervical cancer (CC) remains a significant concern for women's health globally, with a notable impact in Japan, where recent data indicate an increasing incidence. In 2015, 10,759 new cases of CC were diagnosed and 2813 patients died from CC in Japan. Projections estimate that, by the late 2030s, approximately 10,820 new cases and 3620 deaths will occur annually [1]. Cervical intraepithelial neoplasia (CIN), a key precursor of CC, is characterized by the abnormal proliferation of squamous cells on the cervical surface and is considered a premalignant condition. The primary etiological factor for CIN, and consequently CC, is persistent infection with human papillomavirus (HPV), which is primarily transmitted *via* sexual intercourse [2]. HPV is also implicated in the pathogenesis of other anogenital malignancies and a subset of head and neck cancers [3, 4]. However, only some HPV-infected individuals or infected lesions progress to cancer, and the burden of HPV-associated cancer varies widely depending on the part of the body that is infected. Most cervical HPV infections are transient and resolve within 1–2 years without intervention [4]. Persistent HPV infection is a critical factor in the development of CC. HPV types 16 and 18 are the most significant risk factors for CC and present the highest risk of progression to this malignancy [4, 5]. Additionally, HPV types 31, 33, 35, 45, 52 and 58 were classified as high-risk factors for CC, with a lower risk than that of HPV types 16 and 18 but still a higher risk than that of other HPV types. Evidence from a large cohort study in Japan indicated that CIN grades 1 and 2 (CIN1/2) are less likely to resolve spontaneously and more likely to progress to CIN grade 3 (CIN3) in patients positive for these high-risk HPV types [6]. This finding underscores the importance of differentiating the management of CIN1/2 based on high-risk

HPV status.

In Japan, the specific relationship between preoperative high-risk HPV status in patients with CIN and their subsequent diagnosis and prognosis following cervical conization remains unclear. Therefore, this multicenter retrospective study was designed to investigate these associations with the aim of providing clearer insights into the impact of HPV status on the clinical outcomes of cervical conization in patients with CIN.

2. Materials and methods

A nationwide survey on cervical conization was conducted using the Survey of Cervical Conization in Japan, a subcommittee of the Gynecologic Oncology Committee of the Japan Society of Obstetrics and Gynecology. This survey found that 14,832 women underwent cervical conization in two individual years, *i.e.*, 2009 and 2013, at 205 Japanese institutions. Of these patients, 2546 were preoperatively diagnosed with CIN and underwent HPV deoxyribonucleic acid (DNA) testing (high-risk HPV testing or HPV genotyping) before cervical conization.

Included were 2546 patients preoperatively diagnosed with CIN, and the study was conducted in accordance with the Reporting of Observational Studies in Epidemiology guidelines. Although details of the HPV DNA tests were unavailable, HPV genotyping results were obtained. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 were defined as high risk. The patients were classified as follows based on their high-risk HPV status: high-risk HPV negative (Group 1); HPV 16/18 positive (Group 2); HPV types 31, 33, 35, 45, 52 or 58 positive (Group 3); other high-risk HPV positive (Group 4); and unconfirmed high-risk HPV positive (Group 5). Patients without high-risk HPVs were classified into Group 1. HPV type 16/18-positive patients comprised Group 2, although other high-risk HPV types were also detected. Patients who were negative for HPV types 16 and 18 but positive for one or more HPV types 31, 33, 35, 45, 52 or 58 were classified into Group 3. Patients negative for HPV types 16, 18, 31, 33, 35, 45, 52 and 58 but positive for one or more HPV types 39, 51, 56, 59, 66 and 68 were classified into Group 4. Patients positive for one or more of the high-risk HPV types but with an unknown genotype were classified into Group 5.

Conization was classified as either diagnostic or therapeutic. In therapeutic conization, histological diagnosis and colposcopy suggest a low likelihood of CC, and sufficient resection of the lesion is possible. Diagnostic conization is characterized by a discrepancy between the preoperative diagnosis obtained through biopsy and the diagnosis anticipated by cytology or colposcopy.

The postoperative diagnosis was based on the most significant lesion identified by pathology obtained from preoperative biopsy, cervical conization and additional surgery. Follow-ups were conducted in accordance with the protocols of each institution. Recurrence after conization was defined as detection of low-grade squamous intraepithelial lesions (LSIL) or higher (for cytology) and CIN1 or higher (for histology). The surgical margins of the cone specimens were categorized as positive if precancerous or cancerous lesions were present on the ectocervical or endocervical margins, respectively. Conversely, margins were considered negative in the absence of neoplasia.

The correlation among preoperative high-risk HPV status, postoperative diagnosis and recurrence after cervical conization was examined using a pairwise deletion method to address missing data. Of the 2546 patients, those with a postoperative diagnosis of CC, and those who underwent hysterectomy as additional treatment were excluded from the recurrence analysis. In addition, those with a follow-up of less than 2 years or longer than theoretically possible according to the enrollment criteria were excluded because the former were considered to have insufficient follow-up for analysis, and the latter were considered to have an error. The final analysis included 1460 patients (Fig. 1).

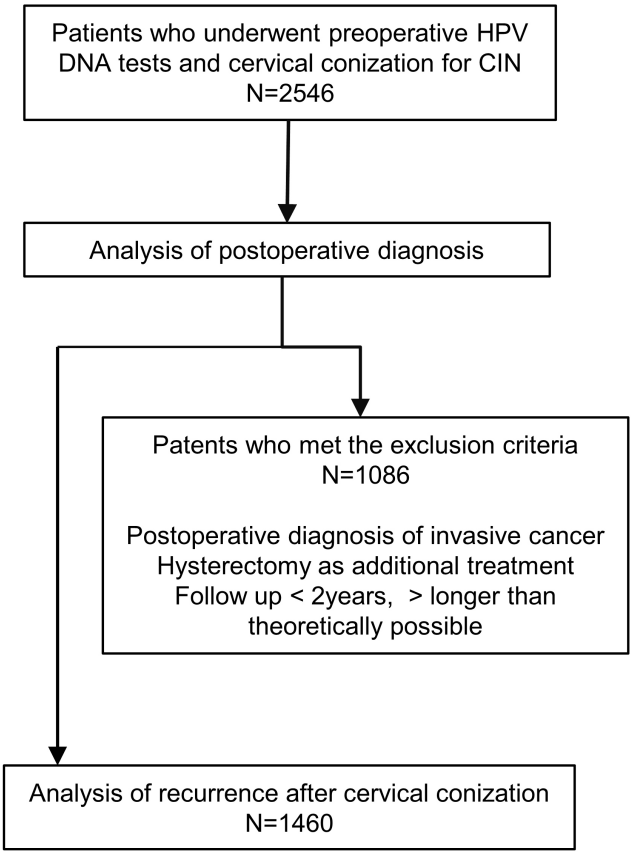

FIGURE 1. Flow chart of the study process. HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia.

Statistical analyses, including logistic regression and Cox regression analyses, were performed using EZR software version 1.36 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/) [7]. Statistical significance was set at $p < 0.05$ was considered significant.

3. Results

The clinical and demographic characteristics of the study participants are summarized in Table 1. The median age of participants was 37 years, with the majority (91.5%, n = 2330) in the premenopausal phase. Regarding preoperative diagnoses, 20.6% (n = 525) of the patients had CIN1/2, while 79.4% (n = 2021), had CIN3. The patient cohort was categorized into

TABLE 1. Pathological and clinical data of 2546 patients who had undergone HPV DNA testing prior to cervical conization.

Parameters	Patients (N = 2546)	
Age (yr)		
Median (quartile)	37	(31, 43)
Under 40 years old	1571	61.7%
40 years old or older	974	38.3%
Unknown	1	0.0%
Para		
Nullipara	973	38.2%
Primipara or multipara	1564	61.4%
Unknown	9	0.4%
Menopause		
Before	2330	91.5%
After	205	8.1%
Unknown	11	0.4%
Preoperative diagnosis		
CIN1/2	525	20.6%
CIN3	2021	79.4%
High-risk HPV status		
Negative	206	8.1%
16/18	669	26.3%
31/33/35/45/52/58	511	20.1%
Other	77	3.0%
Unconfirmed	1083	42.5%

HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia.

five groups according to their distribution as follows: 8.1% (n = 206) in Group 1, 26.3% (n = 669) in Group 2, 20.1% (n = 511) in Group 3, 3.0% (n = 77) in Group 4 and 42.5% (n = 1083) in Group 5. Diagnostic conization was performed in 7.0% (n = 177) of cases, whereas the remaining patients underwent therapeutic conization. The timing of conization was predominantly in non-pregnant women, with 95.4% (n = 2429) of procedures performed when the patient was not pregnant or more than 1 year after delivery. Electrosurgical cauterization was the most common surgical method, used in 28.4% (n = 722) of cases, whereas cold knife conization was the least employed technique, accounting for 4.4% (n = 112) of cases. Most patients had negative surgical margins (85.8%, n = 2184), as shown in Table 2. Postoperative diagnosis revealed that 11.6% (n = 294) of participants had CIN1/2, 83.4% (n = 2123) had CIN3, 0.6% (n = 15) had adenocarcinoma *in situ* and CIN and 3.9% (n = 99) had CC. Of the 1460 patients who were evaluated for recurrence, 100 (6.8%) experienced recurrence (Table 3).

Multivariate logistic regression and univariate analyses identified diagnostic cervical conization (odds ratio (OR): 3.490, 95% confidence interval (CI): 1.940–6.280, $p < 0.001$), a preoperative CIN3 diagnosis (OR: 2.690, 95% CI: 1.360–5.300, $p = 0.004$), and HPV 16/18 (OR: 5.890, 95% CI: 1.810–19.200, $p = 0.003$) as significant risk factors for a postoperative CC diagnosis (Table 4).

Table 5 presents the CC detection rates according to the cervical conization goal (therapeutic or diagnostic), preoperative diagnosis and high-risk HPV status. Overall, the rates were higher in the diagnostic conization group, which consisted of only a small number of patients, than in the therapeutic group. The CC detection rates in preoperative CIN1/2 patients undergoing therapeutic cervical conization were relatively low (<2%), regardless of high-risk HPV status. In patients with a preoperative CIN3 diagnosis undergoing therapeutic cervical conization, the detection rate in Group 2 was notably high (8.3%), but those in Groups 1 and 3 were relatively low (less than 2%). The detection rate in Group 4 was 5.5%, but only three patients were diagnosed with CC in this group.

Table 6 presents the risk factors for recurrence after cervical conization. Univariate Cox regression analysis revealed that loop electrosurgical excision procedure (LEEP) (hazard ratio (HR), 2.277; 95% confidence interval (CI), 1.066–4.861; $p = 0.033$) and surgical margin positivity (HR, 1.983; 95% CI, 1.235–3.184; $p = 0.005$) were significant risk factors for recurrence. Multivariate analysis revealed LEEP (hazard ratio (HR), 2.358; 95% confidence interval (CI), 1.098–5.064; $p = 0.028$), ultrasonic scalpel use (HR, 2.219; 95% CI, 1.014–4.857; $p = 0.046$) and positive surgical margins (HR, 1.950; 95% CI, 1.203–3.162; $p = 0.007$) as significant risk factors. No association was found between preoperative high-risk HPV status and recurrence, even in patients with HPV 16/18-positive CIN (HR, 0.979; 95% CI, 0.481–1.993, $p = 0.954$).

TABLE 2. Details of cervical conization.

Parameters	Patients (N = 2546)	
Aim of conization		
Therapeutic	2369	93.0%
Diagnostic	177	7.0%
Timing of conization		
Non-pregnant	2429	95.4%
During pregnancy	11	0.4%
Within 1 year after delivery	99	3.9%
Unknown	7	0.3%
Surgical form		
Laser	360	14.1%
Cold knife	112	4.4%
LEEP	458	18.0%
High frequency surgical unit (Shimodaira's method)	292	11.5%
Ultrasonic scalpel	563	22.1%
Electric cautery	722	28.4%
Other or unknown	39	1.5%
Surgical margin		
Negative	2184	85.8%
Positive	348	13.7%
Unknown	14	0.6%

LEEP, loop electrosurgical excision procedure.

TABLE 3. Postoperative diagnosis and prognosis.

Parameters		Patients (N = 2546)	
Postoperative diagnosis			
	CIN1/2	294	11.6%
	CIN3	2123	83.4%
	AIS + CIN	15	0.6%
	CC	99	3.9%
	Other or unknown	15	0.6%
Additional treatment			
	Nothing	2377	93.4%
	Laser vaporization	6	0.2%
	Reconization	26	1.0%
	Hysterectomy	92	3.6%
	Trachelectomy	7	0.3%
	Radiation	1	0.0%
	Other or unknown	37	1.5%
Recurrence[1]			
	No	1360	93.2%
	Yes	100	6.8%
Follow-up period (month)[1]	Median (range)	33	(24–91)

CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma in situ; CC, cervical cancer; [1]Patients to be analyzed for recurrence (1460 cases).

TABLE 4. Risk factors for postoperative diagnosis of cervical cancer.

	n	CC	(%)	Univariate analysis[1]				Multivariate analysis[1]			
				OR	95% CI		*p*-value	OR	95% CI		*p*-value
Age											
Under 40 years old	1567	62	4.0%	1.000				1.000			
40 years old or older	970	37	3.8%	0.963	0.635	1.460	0.857	1.280	0.812	2.010	0.289
Para											
Nullipara	969	44	4.5%	1.000				1.000			
Primipara or multipara	1562	55	3.5%	0.767	0.512	1.150	0.200	0.838	0.547	1.280	0.416
Menopause											
Before	2323	94	4.0%	1.000				1.000			
After	204	5	2.5%	0.596	0.240	1.480	0.265	0.554	0.207	1.480	0.239
Aim of conization											
Therapeutic	2361	83	3.5%	1.000				1.000			
Diagnostic	177	16	9.0%	2.730	1.560	4.770	<0.001	3.490	1.940	6.280	<0.001
Preoperative diagnosis											
CIN1/2	523	10	1.9%	1.000				1.000			
CIN3	2015	89	4.4%	2.370	1.220	4.590	0.011	2.690	1.360	5.300	0.004
High-risk HPV status											
Negative	204	3	1.5%	1.000				1.000			
16/18	669	50	7.5%	5.410	1.670	17.500	0.005	5.890	1.810	19.200	0.003
31/33/35/45/52/58	510	8	1.6%	1.070	0.281	4.060	0.923	1.190	0.312	4.560	0.797
Other	77	3	3.9%	2.720	0.536	13.800	0.227	2.950	0.577	15.100	0.194
Unconfirmed	1078	35	3.2%	2.250	0.685	7.380	0.181	2.390	0.724	7.880	0.153

OR, odds ratio; CI, confidence interval; HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; CC, cervical cancer; [1]*Statistical analysis was performed by logistic regression analysis.*

TABLE 5. Detection rates of cervical cancer.

Aim of conization	Preoperative diagnosis	High-risk HPV status	n	CC	
				n	(%)
Therapeutic					
	CIN1/2	Negative	23	0	0.0%
	CIN1/2	16/18	120	1	0.8%
	CIN1/2	31/33/35/45/52/58	118	2	1.7%
	CIN1/2	Other	16	0	0.0%
	CIN1/2	Unconfirmed	177	2	1.1%
	CIN3	Negative	166	3	1.8%
	CIN3	16/18	506	42	8.3%
	CIN3	31/33/35/45/52/58	364	4	1.1%
	CIN3	Other	55	3	5.5%
	CIN3	Unconfirmed	816	26	3.2%
Diagnostic					
	CIN1/2	Negative	5	0	0.0%
	CIN1/2	16/18	13	1	7.7%
	CIN1/2	31/33/35/45/52/58	13	1	7.7%
	CIN1/2	Other	3	0	0.0%
	CIN1/2	Unconfirmed	35	3	8.6%
	CIN3	Negative	10	0	0.0%
	CIN3	16/18	30	6	20.0%
	CIN3	31/33/35/45/52/58	15	1	6.7%
	CIN3	Other	3	0	0.0%
	CIN3	Unconfirmed	50	4	8.0%

HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; CC, cervical cancer.

TABLE 6. Risk factor for recurrence.

	n	Recurrence	Univariate analysis[1]				Multivariate analysis[1]			
			HR	95% CI		*p*-value	HR	95% CI		*p*-value
Age										
Under 40 years old	886	53	1.000							
40 years old or older	573	47	1.394	0.941	2.066	0.098				
Para										
Nullipara	555	40	1.000							
Primipara or multipara	900	60	0.943	0.632	1.408	0.775				
Menopause										
Before	1336	88	1.000							
After	121	12	1.499	0.819	2.742	0.189				
High-risk HPV status										
Negative	132	11	1.000				1.000			
16/18	340	25	0.979	0.481	1.993	0.954	0.940	0.459	1.924	0.865
31/33/35/45/52/58	304	19	0.813	0.387	1.710	0.586	0.828	0.389	1.761	0.624
Other	43	5	1.433	0.497	4.126	0.505	1.544	0.533	4.471	0.424
Unconfirmed	641	40	0.793	0.407	1.546	0.496	0.801	0.407	1.577	0.520
Surgical form										
Laser	221	9	1.000				1.000			
Cold knife	66	4	1.418	0.436	4.612	0.561	1.416	0.431	4.652	0.566
LEEP	276	26	2.277	1.066	4.861	0.033	2.358	1.098	5.064	0.028
High frequency surgical unit (Shimodaira's method)	181	12	1.695	0.714	4.027	0.232	1.778	0.742	4.257	0.197
Ultrasonic scalpel	295	23	2.066	0.954	4.470	0.066	2.219	1.014	4.857	0.046
Electric cautery	408	26	1.525	0.714	3.256	0.275	1.708	0.791	3.689	0.173
Surgical margin										
Negative	1262	78	1.000				1.000			
Positive	190	22	1.983	1.235	3.184	0.005	1.950	1.203	3.162	0.007
Postoperative diagnosis										
CIN1/2	162	10	1.000							
CIN3/AIS	1298	90	1.055	0.548	2.029	0.873				

HR, hazard ratio; CI, confidence interval; HPV, human papilloma virus; LEEP, loop electrosurgical excision procedure; CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma in situ; [1]*Statistical analysis was performed by Cox regression analysis.*

4. Discussion

The following results were obtained from a nationwide survey of cervical conization in Japan. Mikami *et al*. [8] recommended continuous observation and selective cervical conization to managing CIN1 and CIN2, which are currently standard practices in Japan. However, postmenopausal status (or age) has been reported as a risk factor for pathological upgrading in conization specimens, positive surgical margins, additional treatment requirements and recurrence [9–11]. Surgical methods are also associated with positive surgical margins after therapeutic conization [11]. We examined data from this survey to explore the correlation between preoperative high-risk HPV status and the prognosis of cervical conization. Multivariate analyses indicated that diagnostic cervical conization, preoperative CIN3 diagnosis, and the presence of HPV 16/18 were significant risk factors for postoperative diagnosis of CC. However, no relationship was observed between preoperative high-risk HPV status and recurrence after cervical conization in patients without CC, even in Group 2. In patients with CIN, HPV types 16 and 18 were significant independent risk factors for the development of CC after cervical conization, whereas high-risk HPV before cervical conization was not a risk factor for recurrence.

In this study, CC was identified after cervical conization in approximately 4% of the patients initially diagnosed with CIN. Ueda *et al*. [12] reported that CC was detected in 151 of 2107 patients (7.2%) based on conization specimens, and CC was significantly higher in patients with preoperative CIN3 than in those with CIN1/2. This finding is in agreement with our

results, indicating that CC was significantly higher in preoperative CIN3 (4.4%) than in CIN1/2 (1.9%). Patients with HPV 16/18 are known to have the highest risk of developing CC [13, 14]. Indeed, the presence of HPV 16/18 before cervical conization was a risk factor for postoperative diagnosis of CC in our study. Furthermore, a prospective cohort study conducted in Japan concluded that CIN1/2 patients with HPV 16, 18, 31, 33, 35, 52 or 58 exhibited a higher progression rate to CIN3 than did those with low-risk or no HPV [6]. Another large prospective cohort study performed in Denmark estimated that the probabilities of patients with HPV types 16, 18, 31 and 33 and other high-risk types developing CIN3 or worse lesions within 12 years of follow-up were 26.7%, 19.1%, 14.3%, 14.9% and 6.0%, respectively [15]. Globally, HPV 45 is recognized as one of the most prevalent high-risk HPV types in women with CC, followed by HPV types 16 and 18 [14]. In Japanese patients with CC, the prevalence of HPV 45 is notably low, with only 0.3% testing positive for this type [13]. In Japan, HPV types 16, 18, 31, 33, 35, 52 and 58 are associated with a high risk of progression to CC. The World Health Organization's histological classification of tumors of the female reproductive organs has adopted the squamous intraepithelial lesion (SIL) classification, including LSIL and high-grade SIL, with revisions made in 2014 [16]. However, in Japan, patient management is still based on CIN classification. The Japan Society of Gynecologic Oncology guidelines recommend treating CIN3 as a precancerous cervical squamous cancer lesion [17]. In general, conservative management is applied to CIN1/2, while CIN2 in the presence of HPV types 16, 18, 31, 33, 35, 45, 52 and 58 is regarded as a treatable condition. Our study identified the HPV 16/18 status as an independent risk factor for CC. These types were detected in 8.3% of patients who underwent therapeutic cervical conization with a preoperative CIN3 diagnosis. We could not analyze patients with CIN1 and CIN2 separately because CIN1 and CIN2 were grouped as CIN1/2 in the case report form used in this study. Thus, the independent detection rates of CC in CIN1 and CIN2 were not calculated. However, the detection rate in the CIN1/2 group must be almost the same as that in the CIN2 group, particularly in the therapeutic conization group, because therapeutic conization is rarely performed in patients with CIN1 regardless of high-risk HPV status. In patients with CIN1/2 without suspicion of CC based on cytology and colposcopy, conization is not always required to test for cancer owing to the low CC detection rates, irrespective of the HPV type. However, from the perspective of CC prevention, treating HPV 16/18-positive CIN2 seems appropriate owing to the high incidence of CC in patients with HPV 16/18-positive CIN3. Otherwise, close follow-up with conservative management is recommended. A previous Japanese study suggested that HPV types 31, 33, 35, 52 and 58 are high-risk types and are considered risk factors for the progression of cervical precursor lesions [6]. Our study could not differentiate the significance of detecting occult CC between Groups 1 and 3, 4 or 5. Furthermore, the detection rate of occult CC was only 1.1% in Group 3 patients diagnosed with CIN3 undergoing therapeutic conization. Therefore, we cannot definitively recommend treatment for Group 3 patients diagnosed with CIN2. This difference may be due to variations in the study design. The previous study was a prospective cohort study describing the association between the progression of CIN1/2 to CIN3 and HPV genotypes [6].

Various treatments are available for CIN, including cervical conization, ablation (laser and cryotherapy) and photodynamic therapy [18, 19]. The choice of treatment primarily depends on the requirements of the diagnostic specimen and future reproductive risks. In patients with CIN3 and HPV 16/18, an excisional procedure, such as cervical conization, should be chosen to obtain a diagnostic specimen because of the heightened risk of detecting occult CC.

A relationship between high-risk HPV status before cervical conization and recurrence was not identified in this study, even in patients with HPV 16/18. A previous study reported that HPV 16 positivity was a risk factor for recurrence after therapy [20]. However, the recurrence rate in the high-risk HPV-positive group of the present study did not differ from that in the high-risk HPV-negative group, even when analyzing only HPV 16-positive patients (data not shown). There are two possible explanations for this discrepancy. First, surgical margin positivity emerged as an independent risk factor for recurrence in the multivariate analysis of this study; however, the previous study used univariate analysis and did not investigate other factors for recurrence, including surgical margin status. Second, the definitions of recurrence differed. In a previous study [20], recurrence was defined as the detection of CIN3, whereas our study defined recurrence as detection of LSIL or more severe lesions on cytology, and CIN1 or more severe lesions on histology. Although predicting recurrence based on the pretreatment high-risk HPV status remains controversial, HPV DNA testing after treatment may be useful. However, there is currently no consensus on the optimal approach for post-treatment HPV DNA testing (*e.g.*, testing interval, follow-up time, number of post-treatment tests and assays used) [21]. Guidelines from the American Society of Colposcopy and Cervical Pathology recommend that post-treatment management of histological high-grade SIL should differ according to the results of HPV DNA testing at six months [22]. These results suggest that management after conization depends on the postoperative high-risk HPV status but not on the preoperative status.

Compared with laser conization, cervical conization with LEEP and ultrasonic scalpel were independent risk factors for recurrence. There are two plausible explanations for this phenomenon. First, specimens obtained by LEEP may not be suitable for determining the status of the surgical margins. Thermal tissue degeneration occurred at the margins during conization except when a cold knife was used. In addition, the cervix is often excised from multiple LEEP sections, further complicating the pathological diagnosis of the surgical margin status. The rate of false-negative surgical margin assessments may be particularly high in patients undergoing conization with LEEP. Secondly, vaporization can be performed at the surgical margins of the cervix after laser conization. Ikeda *et al*. [11] observed a lower recurrence rate with therapeutic conization using a laser than with other methods in patients with a postoperative diagnosis of CIN3 and positive margins. Furthermore, the authors proposed that vaporization of the surgical margins of the cervix after cone excision could destroy and

remove residual lesions, thereby reducing the recurrence rate [11]. Recurrence rates of conization using an ultrasonic scalpel compared with those using other instruments have rarely been reported [23, 24]. Notably, thermal tissue degeneration at the margin was significantly lower with conization using the ultrasonic scalpel than with LEEP or laser. Although this may contribute to a more accurate determination of surgical margin status, it may potentially increase the recurrence rate. In addition, Ikeda *et al*. [11] indicated that the ultrasonic scalpel does not have the ability to vaporize as well as LEEP.

This large-scale, multicenter study provides insights into the correlation between high-risk HPV status and CIN prognosis in Japan. However, follow-up methods vary among hospitals. A more serious problem is that detailed information on HPV DNA testing is unavailable. The CC screening program in Japan will eventually shift to a process based on HPV DNA testing, but it is currently based on cervical cytology. Women with abnormal cytology findings are recommended to undergo colposcopy and biopsies. As an exception, women with a Pap result of "atypical squamous cells of undetermined significance" (ASC-US) may undergo high-risk HPV testing for triage management. Therefore, it is expected that the women enrolled in this study were those who underwent high-risk HPV testing for an ASC-US Pap result and HPV genotyping for management triage of CIN or research purposes. Therefore, these results may have been affected by inconsistencies in HPV DNA testing. A study in which all patients underwent HPV genotyping is warranted to examine the relationship between preoperative high-risk HPV status and prognosis in patients with CIN more accurately. By contrast, high-risk HPV-negative CIN is rare in countries where high-risk HPV testing is the primary method of CC screening. This is because only women who test positive for HPV are referred for further evaluation. Sigurdsson *et al*. [25] reported that in all patients with CIN2, CIN3 and CC screened by cytology, high-risk HPV-negative occurred in 8%, 4% and 8%, respectively. In this study, high-risk HPV-negative CIN accounted for 8% of all CIN cases, and three developed CC after conization, comparable to the results of Sigurdsson *et al*. [25]. This suggests that HPVs, other than high-risk HPV, can induce CIN2, CIN3 and CC and are valuable data that will not be collected when the screening method changes from cytology to high-risk HPV testing in many countries.

5. Conclusions

In conclusion, HPV 16/18 is a significant independent risk factor for postoperative CC. Therefore, examining the HPV 16/18 status may be useful in the management of CIN. However, the other HPV types did not increase the rate of CC diagnosis after conization. Additionally, no relationship was observed between the preoperative high-risk HPV status and recurrence after conization.

ABBREVIATIONS

CI, confidence interval; CIN, intraepithelial neoplasia; HPV, human papillomavirus; HR, hazard ratio; CC, cervical cancer; OR, odds ratio.

AVAILABILITY OF DATA AND MATERIALS

Data presented in this study are available upon request from the corresponding author.

AUTHOR CONTRIBUTIONS

MI, MM, TE, YK, SN, MY and HK—designed and conducted the study and supervised and reviewed the drafts of the manuscript. KY and AK—analyzed and interpreted the data, prepared the manuscript for publication, and reviewed the draft of the manuscript. All authors have contributed to the editorial changes in the manuscript. All the authors have read and approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committees of Tokai University (Institutional Review Board registration number 15R-095) and Niigata Cancer Center Hospital. The requirement for informed consent was waived due to the retrospective nature of the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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