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Distribution of human papilloma virus genotypes and treatment outcomes in definitive radiotherapy for cervical cancer

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ABSTRACT

Most oncogenic human papilloma virus (HPV) genotypes stratify into two species, α-7 HPV and α-9 HPV. There are several studies that evaluate the relationship between HPV species and treatment outcomes and reports that HPV species is prognostic. The HPV genotyping was conducted using biopsy specimens which had been stored in these studies. We conducted the study using the HPV test performed by cytology specimens which is less invasive and more useful in clinical settings. This study enrolled 46 patients who received HPV genotyping before the definitive radiotherapy. The results of the HPV genotyping were classified into HPVα-7, HPVα-9 and negatives. Of the 46 patients, 10 were positive for HPVα-7, 21 positive for HPVα-9 and 15 were negative. The median follow-up period was 38 months (range 4–142). The HPVα-7, HPVα-9 and negative groups showed the 3-year overall survival (OS; 59.3%, 80.4% and 72.2% [$P = 0.25$]); local control (LC; 67.5%, 81% and 80% [$P = 0.78$]); pelvic control (PC) (50%, 81% and 72.7% [$P = 0.032$]); pelvic lymph node (PLN) control (78.7%, 95% and 92.3% [$P = 0.012$]); distant metastasis free (DMF) survival (50%, 75.4% and 42.8% [$P = 0.098$]); and progression free survival (PFS) rate of patients (30%, 66.7% and 38.9% [$P = 0.085$]), respectively. Patients with HPVα-7 showed statistically significant poorer PC than the HPVα-9 group, in multivariate analysis. This result is consistent with previous studies for HPV positive patients. The HPV negativity rate was higher in this study than in other studies and further work on this may be needed for clinical use.

Keywords: cervical cancer; definitive radiotherapy; human papilloma virus (HPV) genotype; prognosis

INTRODUCTION

Cervical cancer is the fourth most common type of malignancy diagnosed and was the fourth leading cause of cancer death in females in 2020 [1]. In Japan, it was estimated that there were about 11 000 new cases in 2018 and about 3000 deaths in 2019 [2].

The human papilloma virus (HPV) is the major cause of cervical cancer, and HPV is classified into different genotype according to their L1 open reading frame. As a result, more than 170 different HPV genotypes have been identified, and about 20 genotypes are known as oncogenic [3]. When two HPV genotypes share 60–70% of the genomic nucleotide they are categorized into the same species. Two species; α-7 (HPV18, 39, 45, 59, 68 and 70) and α-9 (HPV16, 31, 33, 35, 52, 58 and 67) contribute more than 80% of all cervical cancers.

The HPV test is recommended for screening of cervical cancer by the American Cancer Society (ACS) [4], because persistent cervical infection with high risk HPV, representing HPV16 and HPV18 causes cervical cancer.

Several studies evaluated the treatment outcomes of patients treated with primary surgery. Some studies reported that HPV18 positive cases were associated with a poorer prognosis than HPV18 negative cases, and other studies did not show any relationship between HPV genotype and the prognosis [5–7]. Several studies have shown that HPV α-7 positive, primarily HPV18 was associated with a poorer prognosis than HPV α-9 (primarily HPV16) in patients treated with definitive radiotherapy [8–10], and Wang *et al.* reported that local control (LC) of HPVα-7 positive patients was poorer than other genotypes, while Okonogi *et al.* reported that patients with HPVα-7 had poorer disease free survival and distant metastasis free (DMF) survival but not overall survival (OS) and LC [9, 10]. In these studies, the HPV genotyping had been performed by extracting DNA from formalin-fixed paraffin-embedded specimens which had been stored.

Although previous studies using biopsy specimens which had been stored indicate that HPV species has potential as prognostic factor, it is invasive for patients to obtain biopsy specimens. Therefore, it is important to consider less invasive methods, and we planned the study using the results of HPV tests by cytology specimens which has been accepted for primary cervical cancer screening [11]. In this study, we investigated the distribution of HPV genotypes using cytology specimens and evaluated the relationship between HPV species and treatment outcomes in Japanese females treated with definitive radiotherapy. The purpose of this study was to verify the validity of the results which was obtained by the method employed.

MATERIAL AND METHODS

Patients

From March 2010 to December 2019, patients with cervical cancer who had received a HPV test before treatment and were treated by definitive radiotherapy at our institution were enrolled in this study, Application of the HPV test was decided by the physician before the treatment.

There were 84 patients who had been treated with definitive radiotherapy during the period. Four patients who were followed for less than 6 months and whose prognoses are unknown were excluded. Forty-six of the remaining 80 patients were subjected to HPV genotyping before the radiotherapy. The patients were clinically staged according to the FIGO (2008) staging criteria. Lymph node metastasis were determined by radiological methods, not surgical staging. This study has been approved by the institutional review board (020-0264).

HPV genotyping

Cervical cells for HPV genotype determination were sampled before starting the treatment. We routinely obtained the cervical epithelium specimens with the Cervex-Brush® and also performed HPV tests using the multiplex polymerase chain reaction (PCR) method (PapiPlex) to specifically detect HPV-6, 11, 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 at the Genetic Lab Co., Ltd. (Sapporo, Japan) [12].

Treatment

All the enrolled patients received external beam radiation therapy (EBRT) and high dose rate intracavitary brachytherapy (HDR-ICBT). One patient was treated with 3D computed tomographic simulation followed by intensity modulated radiotherapy and the remaining were treated with 3D computed tomographic simulation. All patients received whole-pelvic radiotherapy at a dose of 1.8 Gy per fraction, 5 times per week. After 39.6 Gy to the whole pelvis, a-3-cm wide center shield was used and a 10.8 Gy boost was administered to the pelvic sidewall. The total pelvic sidewall dose was 50.4 Gy in 28 fractions. For patients with para-aortic lymph node (PALN) metastasis or with high risk of PALN-node failure, the PALN area was also included.

After adequate tumor shrinkage, HDR-ICBT was delivered with a linear source arrangement, as described elsewhere [13]. The HDR-ICBT dose was 30 Gy in 6 fractions twice a week and prescribed at Point A. No patients were treated with IGBT in this study.

A total of 31 patients received concurrent chemotherapy. A dose of 40 mg/m^2 cisplatin was administrated to these patients once a week during the course of the whole pelvic EBRT. Patient characteristics are shown in Table 1.

Follow up

Patients were given a follow-up evaluation 1 to 1.5 months after the treatment. Then they received follow-up examinations every 1–2 months for the first 2 years, every 3–4 months in years 3–4, every 6 months in year 5 and once a year thereafter.

Statistical analysis

The baseline characteristics among patients with HPVα-7, HPVα-9 and in the HPV negative groups were compared with the Kruskal-Wallis test and Fisher's exact test.

OS was defined as the time from the last day of radiotherapy to the date of death from any cause. LC, pelvic lymph node (PLN) control and pelvic control (PC) were measured from the last day of radiotherapy to the date of the first local recurrence, PLN recurrence, or at any recurrences inside the pelvis, respectively. Local recurrence was defined as the presence of tumor recurrence in the cervix, parametrium. The DMF interval was measured form the last day of radiotherapy to the appearance of tumor outside the pelvis including PALN recurrence. Progression free survival (PFS) was measured from the last day of radiotherapy to the development of any tumor recurrence and/or the date of death.

The OS, LC, PLN, PC, DMF and PFS were calculated using the Kaplan–Meier method. The log-rank test was used to evaluate the

Table 1. Patient characteristics

Characteristics	
Age range (median)	33–89 (61)
FIGO stage (2008)	
1B	5
2A	2
2B	17
3B	19
4A	3
Histology	
Squamous cell carcinoma	37
Others	9
PLN metastasis	
Negative	25
Positive	21
PALN metastasis	
Negative	41
Positive	5
Treatment field	
Whole pelvis	36
Whole pelvis + PALN	10
Concurrent chemotherapy	
Yes	31
No	15

PLN: pelvic lymph node
PALN: para-aortic lymph node

difference between the HPV genotype and the treatment outcomes. The Cox proportional hazards regression analysis was used to identify independent predictors of treatment outcomes. P values of < 0.05 were considered statistically significant. The statistical analyses were performed using JMP Pro14.0.0 (SAS Institute INC., Cary, NC, USA).

RESULTS

HPV genotype distributions

Of the 46 patients, 31 (67.4%) were HPV positive and 15 (32.6%) were HPV negative.

Among the HPV positive patients, HPV16 was the most frequently detected, followed by HPV18, HPV52, HPV31 and HPV45. One patient was positive for multiple HPV types (HPV 16, 52 and 58), the others were positive for a single type; 10 patients were categorized into HPV-α7 and 21 were categorized into HPVα-9. Details of the distribution of the HPV genotype are shown in Table 2.

Table 3 shows the characteristics of the patients stratified by HPV groups. There is a significant difference in the histologic distribution among the three groups. There were no significant differences in age among the HPV-α7, HPV-α9 and negative groups, the FIGO stage, PLN metastasis, PALN metastasis and administration of concurrent chemotherapy.

Treatment outcomes

The median follow-up was 38 months (range 4–142). By the end of the study, 29 patients were alive, 17 patients had died; 24 patients had experienced treatment failure and 15 patients had died of cervical cancer. The 3-year OS in the HPVα-7, HPVα-9 and negative groups were 59.3%, 80.4% and 72.2% ($P = 0.25$); for LC it was 67.5%, 81% and 80% ($P = 0.78$); for PC it was 50%, 81% and 72.7% ($P = 0.032$); for PLN it was 78.7%, 95% and 92.3% ($P = 0.012$); for DMF it was 50%, 75.4% and 42.8% ($P = 0.098$); and for PFS it was 30%, 66.7% and 38.9% ($P = 0.085$) (Fig. 1). For the FIGO stage, histology, PALN and HPV were analyzed with multivariate analysis. No factor was shown to be an independent predictor of PC nor PLN when HPV was divided into the three groups (HPVα-7, HPVα-9 and HPV negative) (Table 4). However, HPV was an independent predictor factor of PC (hazard ratio [HR] 6.29 95% confidence interval [CI] 1.47-27.0) $P = 0.016$) when the HPV negative group was excluded (Table 4). The HPV status remained significant in predicting PC when a multivariate analysis was performed using two factors, the HPV status and another factor (FIGO stage, histology, PALN) as the variable (Supplementary Table 1).

DISCUSSION

This study reports the distribution of HPV genotypes and species using the results of HPV tests of cytology specimens and evaluated the relationship between HPV species and treatment outcomes.

In this study, HPV16 was the most common genotype followed by HPV18, HPV52 and HPV31. This result is consistent with the previous worldwide study reporting HPV genotype attribution in invasive cervical cancer [14]. HPV16 and HPV18 were the most and the second most common genotypes worldwide. Several studies reported that the third to eighth most common HPV genotypes were HPV31, 33, 35, 45, 52 and 58, with variation in the ranking order of individual genotypes by region. The incidence of HPV52 and HPV58 was higher in Asia (especially in East Asia) than in other regions [15].

In this study, 32.6% of patients were HPV negative, and there is variation in the HPV-negativity rate of patients treated a by definitive radiotherapy. Wang *et al.*, Hall *et al.* and Okonogi *et al.* reported 18 of 1010 patients (1.8%), 22 of 202 patients (10.9%) and 14 of 83 patients (16.9%) with HPV negative tumors, respectively [8–10]. The HPV-negativity of this study is higher than these previous studies. Among the HPV negative cases in this study, false negative cases could be expected to be present. Arezzo *et al.* pointed out several reasons which cause false negatives [16]. First, sampling errors such as low cellularity would cause false negative. Hall and Okonogi carried out HPV genotype tests that used paraffin-embedded specimens which had been obtained before treatment and only patients with specimens containing some amount of tumors were eligible for inclusion in those studies [8, 10]. In this study, the specimen used for the genotyping was obtained for HPV tests, and there was no restriction that the specimen had to contain a specified amount of tumor, this suggests that inappropriate specimens could be a cause of the high HPV negativity. Second, the sensitivity of HPV genotyping we used would affect the results. In this study, we used commercially available HPV genotyping methods, Papipulex [12]. Papipulex has been validated with cytology specimens but not with biopsy specimens, and the relationship between the results of Papipulex and cytological groups such as normal, ASCUS, LSIL and HSIL has been investigated, but not with invasive cancer. The low sensitivity of the primer (Papipulex) would also result in an increase in negativity. Finally, we used cytology specimens for the HPV

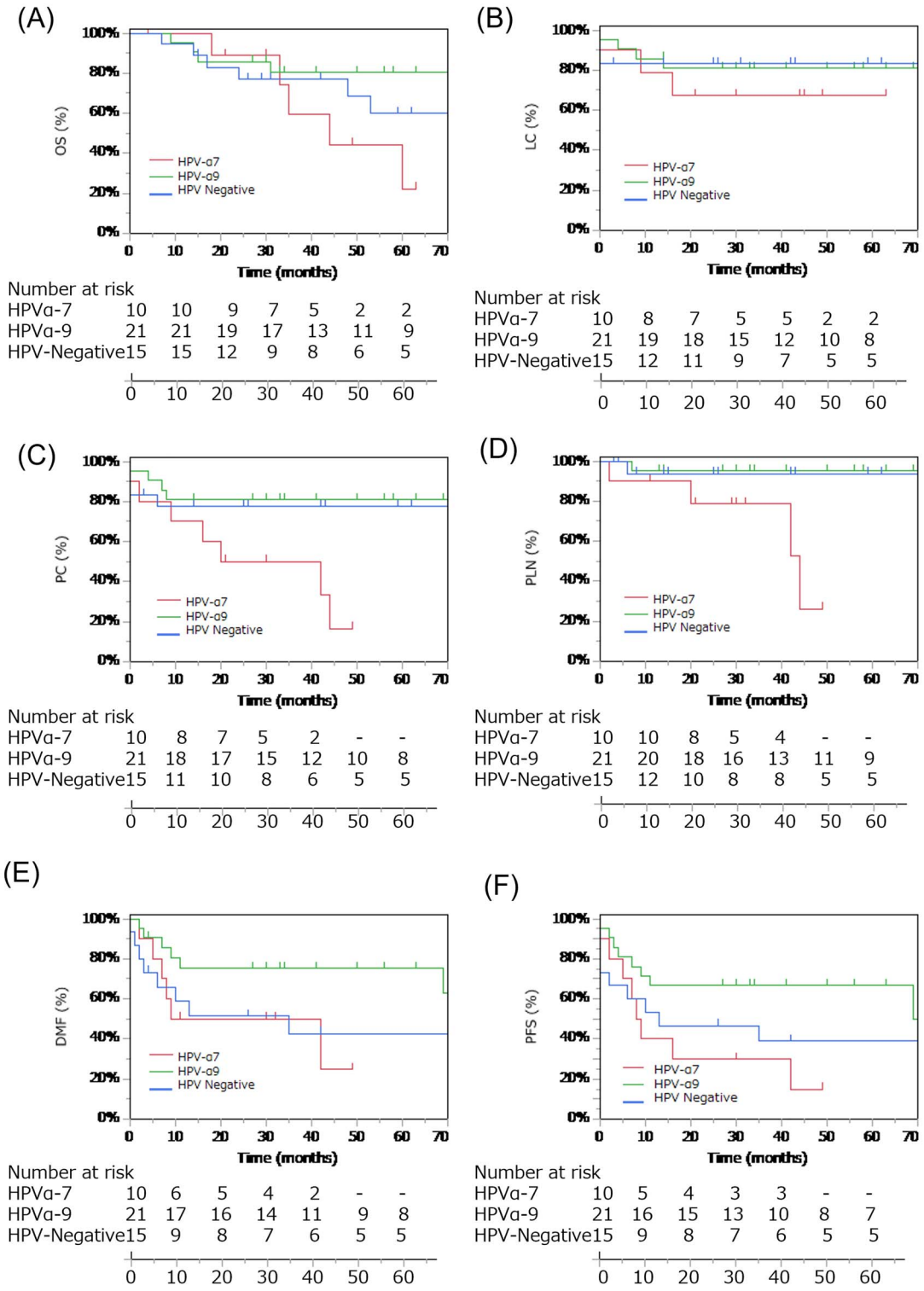

Fig. 1. Kaplan–Meier curve of (A) OS, (B) LC, (C) PC, (D) PLN, (E) DMF, (F) PFS according to HPV Status. OS: overall survival, LC: local control, PC: pelvic control, PLN: pelvic lymph node control, DMF: distant metastasis free, PFS: progression free survival.

Table 2. Distribution of HPV genotypes

HPV species	N(%)	HPV Genotype	n(%)
HPVα-7	10(21.7)	HPV18	7(15.2)
		HPV39	0(0)
		HPV45	2(4.4)
		HPV59	1(2.2)
HPVα-9	21(45.7)	HPV16	11(23.9)
		HPV31	3(6.5)
		HPV33	0(0)
		HPV52	5(10.9)
		HPV58	1(2.2)
		HPV16.52.58	1(2.2)
Negative	15(32.6)		

HPV: human papilloma virus

Table 3. Patient characteristics according to HPV species

Characteristics	Species			*P*-value
	HPVα-7 *n* = 10	HPVα-9 *n* = 21	Negative *n* = 15	
Age range (median)	36–73(61.5)	33–89 (54)	46–77(64)	0.17
FIGO Stage (2008)				
I,II	7	9	8	0.36
III,IV	3	12	7	
Histology				
Squamous cell carcinoma	7	21	9	0.0024
Others	3	0	6	
PLN metastasis				
Negative	6	10	9	0.74
Positive	4	11	6	
PALN metastasis				
Negative	8	21	12	
Positive	2	0	3	0.057
Concurrent chemotherapy				
No	2	6	7	0.37
Yes	8	15	8	

PLN: pelvic lymph node
PALN: para-aortic lymph node

genotyping in this study. The cytology-based HPV test has been accepted for primary cervical cancer screening [11]. For invasive cervical cancer, Smith *et al.* summarized the results of 130 studies about the HPV prevalence among 14 595 patients with invasive cervical cancer [17]. Cytology-based HPV tests were used in 29 of the 130 studies and the percentage of HPV positive case ranged from 34.7% to 100% while it ranged from 37% to 100% in biopsy-based tests. Barreto *et al.* [18] reported that 96 of 183 patients (52%) receiving curative treatment for cervical cancer were HPV negative. They used biopsy specimens embedded in paraffin blocks. They concluded that the cause of the high prevalence of HPV-negative cases would be attributes of the quality of the materials. As various rates of HPV negative cases were reported regardless of method used to obtain the specimens, the quality of the materials used and the sensitivity of the HPV genotyping would be the main cause of the higher HPV negativity. Re-examination of HPV genotyping using other specimens could reduce sampling errors and result in reductions in the false-negative case ratio. When multiple tests are assumed, it is desirable that obtaining specimens be minimally invasive and the use of cytology specimen seems to be applicable.

Although the majority of cervical cancers are associated with HPV infection, a small portion of cervical cancers are not. Kaliff *et al.* reported that HPV-negativity was associated with high patient age, longer storage time and adenocarcinoma histology [19]. The median age of HPV-positive patients and HPV-negative patients were 56 years and 64 years, respectively in this study. Six of the 15 HPV-negative cases were adenocarcinoma or adenosquamous cell carcinomas, showing the

Table 4. Multivariate analysis of PC and PLN control

	PC		PLN	
	HR (95% CI)	*P*	HR (95% CI)	p
Whole ($n = 46$)				
HPV species		0.093		0.33
α-7	4.60 (1.12–18.96)		2.11 (0.11–39.76)	
α-9(reference)	1		1	
Negative	1.72 (0.39–7.46)		0.47 (0.014–14.16)	
FIGO Stage		0.45		0.19
1,2 (reference)	1		1	
3,4	1.51 (0.524–4.32)		0.25 (0.026–2.39)	
Histology	1	0.9	1	0.17
SCC (reference)				
Others	1.08 (0.31–3.81)		4.24 (0.52–34.69)	
PALN		0.99		0.13
Negative (reference)	1		1	
Positive	1.0 (0.25–4.01)		4.99 (0.60–41.09)	
HPV positive ($n = 31$)				
HPV species		0.016		0.38
α-7	6.29 (1.47–27.0)		5.90 (0.35–100.26)	
α-9 (reference)	1		1	
FIGO Stage		0.37		0.61
1.2 (reference)	1		1	
3, 4	1.78 (0.50–6.26)		0.56 (0.058–5.41)	
Histology		0.57		0.61
SCC (reference)	1		1	
Others	1.67 (0.27–10.15)		1.80 (0.19–16.8)	
PALN		0.96		0.55
Negative (reference)	1		1	
Positive	0.95 (0.15–6.0)		2.07 (0.20–21.2)	

PC: pelvic control
PLN: pelvic lymph node control
PALN: para-aortic lymph node

HPV-negative cases in this study to have characteristics similar to the HPV-negative cases in the previous studies.

The treatment outcomes of patients with HPVα-7 tended to poorer than HPVα-9. The HPVα-7 cases showed significantly poorer PC than the HPVα-9 cases in the multivariate analysis. The PLN outcomes showed significant differences for the HPVα-7, HPVα-9 and HPV-negative cases in the log-lank test, but not in the multivariate analysis. The DMF and PFS outcomes did not show statistically significant differences for the HPVα-7, HPVα-9 and HPV-negative cases, the 3-year DMF and PFS with HPVα-7 were more than 20% lower than the results of HPVα-9. The small number of patients and relatively short surveillance period may result in the lack of significant difference of DMF and PFS.

Previous studies reported that HPVα-7 cases had poorer treatment outcomes than others. Wang *et al.* reported that only patients with HPVα-9 had better outcomes for LC and disease-specific-survival than others, whereas Okonogi *et al.* demonstrated that patients with HPVα-7 had poorer DFS and DMFS but not OS and LC [9, 10]. Kang *et al.* reported that the 5-year PFS rate for HPV18 positive patients was lower than for HPV18 negative patients in primary surgery [5]. Hall *et al.* investigated the intrinsic radiosensitivity of HPV-α7 and HPVα-9 using clonogenic assays and showed that there was no difference between the two kinds of HPV species [8]. Taken together, it seems that the reason the prognosis of HPVα-7 positive patients is poorer than others cannot be attributed to differences in radio sensitivity.

HPV is a cause of both cervical cancer as well as head and neck cancers. Especially, HPV-related oropharyngeal cancer has been increasing in the USA for several decades [20]. HPV positive patients show significantly better OS and PFS than HPV negatives in oropharyngeal cancer [21]. This has led to the eighth edition of the TNM staging classification to separate HPV positive oropharyngeal cancer from negative cases [22]. Kreimer *et al.* investigated the genotypes of HPV in oropharyngeal cancer. They reported that HPV16 accounted for 86.7% of HPV positive oropharyngeal cancer and HPV18 accounted for only 2% [23]. Better prognosis in HPV positives for oropharyngeal cancer may be attributed to the rarity of HPV18.

The mechanisms that result in the prognostic difference between HPV16 and HPV18 are not fully understood. *In vitro* studies have

demonstrated the differences between HPV16 and HPV18. Arends *et al.* [24] showed that HPV18 was associated with significantly less apoptosis than HPV16. Villa *et al.* [25] reported that the transformation activity of HPV18 was much higher than that of HPV16. Previous studies showed that high-risk HPVE6 proteins have interactions with cellular PDZ domain-containing proteins and promote cell invasion and the epithelial-to-mesenchymal transition (EMT) [26, 27]. There are significant differences between HPV16 and HPV18 regarding the interaction with PDZ domain-containing proteins [26, 28]. These differences would result in the biological differences between HPV16 and HPV18.

As the HPV genotype does not have any effect on the treatment strategy, HPV genotyping is not performed routinely in clinical settings at present [29, 30]. However, this would change if the HPV species were confirmed as prognostic. In this study, the distribution of HPV genotypes and the relationship between treatment outcomes and HPV species were consistent with previous studies for HPV positive cases [8, 10]. This study is thought to be meaningful because the HPV genotyping using cytology specimens is less invasive than genotyping by biopsy.

There are several limitations in this study. First, this study included small number of patients. Second, as the study design was retrospective, the methods of obtaining specimens and the treatment methods also varied.

In conclusion, this study investigated the distribution of HPV genotypes using cytology specimens and evaluated the relationship between HPV species and treatment outcomes in Japanese females treated with definitive radiotherapy and found that the distribution of the HPV genotype and relationship between treatment outcomes and HPV species were consistent with previous studies for HPV positive cases. HPV-negativity in this study was higher than in previous studies. In order to reduce the HPV-negative cases, it is necessary to consider a re-examination of HPV-tests by using clinically available specimens.

SUPPLEMENTARY DATA

Supplementary data is available at *RADRES Journal* online.

CONFLICT OF INTEREST

There are no conflicts of interest to report related to this study.

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PRESENTATION AT A CONFERENCE

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DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material.

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