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Photodynamic Therapy for Cervical Intraepithelial Neoplasia

Satoshi Yamaguchi[a] Hiroshi Tsuda[c] Masayuki Takemori[a] Shinich Nakata[c] Sadako Nishimura[a] Naoki Kawamura[c] Keisuke Hanioka[b] Takeshi Inoue[d] Ryuichiro Nishimura[a]

Departments of [a]Gynecology and [b]Pathology, Hyogo Medical Center for Adults, Akashi, and Departments of [c]Obstetrics and Gynecology, and [d]Pathology, Osaka City General Hospital, Osaka, Japan

Key Words
Human papilloma virus · Excimer dye laser · YAG-OPO laser · Photofrin · Cervical cancer

Abstract
Objectives: Photodynamic therapy (PDT) is a minimally invasive treatment for cervical intraepithelial neoplasia (CIN). We report the effectiveness of PDT in 105 cases of CIN. ***Methods:*** All patients received photofrin (PHE) 2 mg/kg intravenously and, 48–60 h later, phototherapy was performed using the Excimer dye laser or a YAG-OPO laser with an irradiation dose of 100 J/cm^2 using 630 nm wavelength. ***Results:*** Mild photosensitivity occurred in 48% (50/105) of patients. The complete response (CR) rate was 90% (94/105) at 3 months following treatment. In the remaining 11 patients, 5 patients had CIN1, 2 patients had CIN2, and 4 patients had mild cytologic findings. However, in 9 of these 11 patients, CR was achieved 6 months after PDT. In 69 patients, human papilloma virus (HPV) typing was performed before and after PDT therapy. Pre-treatment, 64 of 69 patients (93%), were HPV-positive including 30 cases of high-risk HPV (43%). Testing performed 3, 6 and 12 months following PDT revealed no HPV-DNA in 75% (52/69), 74% (48/65) and 72% (41/57) of patients. At present, the median follow-up period is 636 days (90–2,232 days). In 3 patients, recurrence requiring surgical treatment was identified at 646, 717 and 895 days after PDT. ***Conclusions:*** PDT is an effective and minimally invasive treatment for CIN, which also appears to eradicate HPV infection.

Introduction

Cancer of the uterine cervix is one of the most common malignant neoplasms among women, and remains the leading female malignancy in developing countries [1]. In 1999, about 6,500 women were diagnosed with cervical cancer in Japan [2]. In the USA, approximately 13,000 women developed cervical cancer in the year 2000 [3]. Cervical intraepithelial neoplasm (CIN) is often the precursor to cervical cancer. In 70% of CIN, evidence of the human papilloma virus (HPV) is detected [4]. In cervical carcinogenesis, HPV is thought to inactivate the cell cycle regulators by inhibiting p53 and pRb proteins by E6 and E7 proteins [5–7]. HPV is divided into two types: high-risk types and low-risk types. Only the high-risk HPV can efficiently inactivate p53 and pRb.

The current treatment of CIN is primarily based on the surgical excision using laser, loop electrosurgical procedure or cold knife conization technique. Unfortunately, these treatments often lead to obstetric problems such

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Ryuichiro Nishimura, MD, PhD
Department of Gynecology, Hyogo Medical Center for Adults
13-70, Kitaoji-cho
Akashi 673-8558 (Japan)
Tel. +81 78 929 1151, Fax +81 78 927 9511, E-Mail r_nishim@wk9.so-net.ne.jp

as cervical incompetence in young women who go on to become pregnant [8]. There is a novel alternative for the treatment of neoplasia called photodynamic therapy (PDT). PDT involves the systemic administration of a tumor-localizing photosensitizer, followed by the laser irradiation of the affected area [9–11]. Since PDT is minimally invasive with no surgical excision, it should be a cervix-sparing treatment, which may be particularly attractive to women desiring to preserve fertility. This paper presents a large series of patients with CIN treated with PDT.

In this study we expand on our preliminary report of the therapeutic effect of PDT in 31 CIN cases which suggested that PDT is effective for treating cervical dysplasia, and for the eradication of cervical HPV [12]. We now describe the effectiveness PDT in 105 cases, of CIN with a median follow-up period of 636 days.

Material and Methods

Patients

Between December 1996 and April 2004, 105 nonpregnant women with a histological diagnosis of CIN (CIN1: 4; CIN2: 6; CIN3: 95) were enrolled in this study. All patients hoped to retain their fertility and chose PDT for its potential as a cervix-preserving therapy. The nature and purpose of the study were fully explained to each patient, and all patients gave written informed consent. The study was approved by the institutional review board of Hyogo Medical Center for Adults and Osaka City General Hospital.

PDT

All patients received intravenous PHE, 2 mg/kg to photosensitize the lesions (Photofrin, Japan Wyeth Lederly , Tokyo, Japan). Phototherapy was performed using an Excimer dye laser (EDL) or a YAG-OPO laser (Ishikawajima-Harima, Heavy Industry, Tokyo, Japan) with an irradiation dose of 100 J/cm^2 using a 630-nm wavelength. The laser instruments were mounted on a colposcope with an optical path for the laser. For the endocervix, a specially designed intracervical probe was used. After treatment, all patients were hospitalized in a dark room with protection from light for 3 weeks. For the first week post-treatment, light was limited to 5–20 Lx, for the second week light was limited to 30–50 Lx, and for the third week light was limited to 50–100 Lx. The light was measured using a luxmeter. The patients were examined every 3 months after PDT treatment. The clinical effect was judged using cytology and directed biopsy. The primary responses were determined 3 months later after PDT. When these examinations showed no abnormal findings, the case was considered a complete remission (CR). Minor response (MR) indicates mild histological change indicating low or high grade squamous intraepithelial lesion (LSIL, HSIL) less severe than the primary disease, and partial response (PR) indicates mild cytologic findings indicating LSIL or HSIL without histologic change. Toxicity was determined using NCI-CTC ver2.

Detection of HPV

The cervical smears were collected with a cotton-tipped swab and preserved in the phosphate buffer at –80°C until analyzed. DNA was analyzed following the PCR-based methods previously described by Yoshikawa et al. [13] and Nagano et al.[14]. Briefly, samples were analyzed by L1 consensus primers for amplification and detection of HPV-DNA, and digested with *Rsa*I, *Dde*I, *Hae*III, *Hin*fI, *Xba*I, *Acc*I, *Pst*I and *Kpn*I for HPV typing by restriction fragment length polymorphism (RFLP). HPV-DNA types 16, 18, 31, 33, 51, 52, 56, 58, 61, 70 and 82 were determined with the sensitivity of 0.01–0.1 copy/cell.

Results

Clinical Response

A total of 105 women enrolled in this study. The clinical characteristics of the patients are shown in table 1. Median age is 30 years (range: 19–49). Forty-eight patients were single, 50 patients were married and 7 patients were divorced. Seventy-four patients were nulliparous, and 31 patients were multigravida. Four patients had CIN1, 6 patients had CIN2 and 95 patients had CIN3. Toxicity was predominantly mild cutaneous photosensitivity (grade 1–2 in 49 patients, and grade 3–4 in 1 patient). Grades 1 and 2 cutaneous photosensitivity were cured within 2 weeks without any treatment. One patient suffered from grade 3 photosensitivity, because she worked in the sunshine during the summer season just after being discharged. She was cured with topical steroid treatment. Minimal vaginal discomfort and discharge was also described by some patients. Cervical stenosis occurred in 11 patients. PDT was performed safely for all the patients.

The CR rate was 90% (94/105) 3 months following PDT. In the remaining 11 patients, 5 patients had CIN1, 2 patients had CIN2, and 4 patients had mild cytological findings. However, in 9 of these 11 patients, CR was achieved 6 months following PDT. In contrast, 5 patients had newly detected disease after 6 months including 3 CIN1 lesions and 2 cases of mild cytological findings. For 76 patients, the 1-year follow-up results were as follows: 2 patients had CIN1, 2 patients had mild cytological findings and 72 patients achieved CR (95%: 72/76). In 15 CR cases, cervical cytology and biopsy were performed every 3 days after PDT for 2 weeks, and within 3 days of laser treatment, necrosis of the CIN region occurred and atypical cells disappeared in all cases.

At present, the median follow-up period is 636 days (range: 90–2,232 days). In 3 patients, recurrence occurred at 646, 717 and 895 days after treatment. Two of these

Table 1. Clinical course in all cases

Patient	Age	MA	PRG	DEL	HIS	PS	HPV PRE	HPV 3M	HPV 6M	HPV 12M	HPV 24M	Effect 3M	Final prognosis	Duration
1	27	1	0	0	CIN3	G1	NEG	NEG	NEG			CR	NED	1,253
2	35	1	4	1	CIN3	G1	16+OT	NEG	52	52+OT	NEG	CR	NED	1,361
3	29	1	0	0	CIN3	G1	16	NEG	OT	OT	OT	CR	NED	1,409
4	33	1	4	0	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,244
5	37	1	2	1	CIN3	G0	16	NEG	NEG	NEG	NEG	CR	NED	1,395
6	28	1	4	1	CIN3	G1	16	NEG	NEG	NEG		CR	NED	567
7	29	1	1	1	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,255
8	26	1	1	1	CIN3	G1	16	OT	61	51	NEG	CR	NED	1,293
9	20	0	1	0	CIN3	G0	51	NEG	NEG	NEG	NEG	CR	NED	721
10	32	0	0	0	CIN2	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,099
11	28	0	4	2	CIN3	G1	18	NEG	NEG	NEG	NEG	CR	NED	1,104
12	29	1	1	0	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,103
13	30	1	0	0	CIN3	G0	61	NEG	NEG	NEG	NEG	CR	NED	1,140
14	32	1	0	0	CIN3	G1						CR	NED	1,140
15	25	0	4	0	CIN3	G0	53+OT	NEG	NEG	NEG	NEG	CR	NED	1,116
16	33	1	6	2	CIN3	G0	52	NEG	NEG		NEG	CR	NED	1,011
17	38	2	1	0	CIN3	G1	58	NEG	NEG	NEG	NEG	CR	NED	1,060
18	30	1	2	1	CIN3	G1	58	NEG	NEG	NEG	OT	CR	NED	810
19	36	0	0	0	CIN3	G0	35	NEG	16	OT	NEG	CR	NED	1,024
20	30	1	1	1	CIN3	G0	52	NEG	NEG	NEG	NEG	CR	NED	917
21	30	1	1	1	CIN3	G1	16	NEG	NEG	NEG		CR	RE	717
22	25	0	0	0	CIN3	G1	16	NEG	OT	NEG		CR	NED	372
23	33	0	0	0	CIN3	G0	52+OT	OT	NEG		52	CR	NED	919
24	19	0	1	0	CIN3	G0	70+OT	70	NEG	NEG	OT	MR	NED	970
25	33	1	0	0	CIN3	G0	31	31	OT	NEG	OT	PR	RE	895
26	40	1	0	0	CIN3	G1	16+58	OT	OT	OT	OT	CR	NED	913
27	35	2	3	1	CIN3	G1	16	51	51	NEG	NEG	CR	NED	902
28	37	1	4	0	CIN3	G1	NEG	NEG	NEG	NEG	NEG	CR	NED	902
29	35	2	3	1	CIN3	G0	52+OT	NEG	NEG	NEG	58	MR	NED	810
30	42	0	1	0	CIN3	G0	82	16	NEG	16	16	CR	NED	893
31	29	1	2	1	CIN2	G1	52	NEG	NEG	NEG		CR	NED	803
32	28	0	0	0	CIN3	G1	OT	NEG	NEG	NEG	NEG	CR	NED	886
33	28	1	0	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	733
34	39	1	1	0	CIN3	G0	16	OT	NEG	NEG	OT	CR	NED	756
35	29	1	1	1	CIN3	G0	OT	NEG	NEG	NEG	OT	MR	NED	620
36	30	1	1	1	CIN3	G0	82	NEG	NEG	NEG		CR	NED	727
37	31	1	0	0	CIN3	G0	51	NEG	NEG	NEG		MR	NED	557
38	33	1	3	2	CIN3	G0	51	NEG	NEG	51		CR	NED	594
39	26	1	1	0	CIN3	G0	NEG	NEG	OT	NEG		CR	NED	371
40	23	0	0	0	CIN3	G0	51	NEG	NEG		16+53	CR	NED	636
41	36	1	0	0	CIN3	G1	16+35	NEG	NEG	NEG		CR	NED	698
42	26	0	3	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	558
43	23	0	0	0	CIN2	G0	OT	52	52+OT	54		CR	NED	547
44	33	0	0	0	CIN3	G0	51	NEG	NEG	NEG		CR	NED	568
45	25	0	0	0	CIN3	G0	16	52	52	52		CR	NED	529
46	28	0	0	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	529
47	30	2	2	1	CIN3	G1	OT	34	NEG	16		CR	NED	529
48	29	0	4	0	CIN3	G1	31	NEG	OT	NEG		CR	NED	374
49	27	0	0	0	CIN3	G0	16	NEG	NEG	OT		CR	NED	286
50	34	0	0	0	CIN2	G1	16	NEG		68		CR	NED	395
51	37	1	1	0	CIN3	G1	16	NEG	NEG	NEG		CR	NED	381
52	31	0	3	0	CIN3	G0	18	18	18	18		CR	NED	381
53	37	1	1	1	CIN3	G1	16	NEG	NEG	NEG		CR	NED	371
54	32	1	2	0	CIN3	G0	16	NEG	NEG	NEG		CR	NED	371
55	30	0	0	0	CIN3	G0	52	OT	51	68+OT		CR	NED	359

Table 1 (continued)

Patient	Age	MA	PRG	DEL	HIS	PS	HPV PRE	HPV 3M	HPV 6M	HPV 12M	HPV 24M	Effect 3M	Final prognosis	Duration
56	24	1	4	1	CIN3	G1	NEG	NEG	NEG			CR	NED	366
57	35	0	0	0	CIN3	G1	16+70	16+70	NEG	NEG		CR	NED	356
58	33	1	1	1	CIN3	G0	16	NEG	NEG			CR	NED	480
59	31	1	4	2	CIN3	G1	16	NEG	NEG	NEG		CR	NED	276
60	21	1	3	2	CIN3	G1	59	59	NEG			CR	NED	194
61	35	2	5	4	CIN3	G1	OT	NEG	NEG	OT		CR	NED	283
62	22	0	0	0	CIN1	G0	52	OT	52+OT			CR	NED	276
63	27	0	0	0	CIN3	G0	59+OT	OT	33+OT	OT		MR	NED	324
64	29	1	0	0	CIN1	G0	58	NEG	NEG			CR	NED	352
65	26	0	0	0	CIN3	G0	NEG	NEG	NEG			CR	NED	269
66	21	0	1	0	CIN3	G1	16	NEG	OT			CR	NED	273
67	33	1	0	0	CIN3	G0	16	NEG	NEG			CR	NED	269
68	24	0	0	0	CIN3	G1						CR	MC	269
69	29	0	3	1	CIN3	G1						MR	NED	269
70	21	0	0	0	CIN3	G0						CR	NED	269
71	31	1	2	1	CIN3	G1						CR	NED	266
72	41	2	0	0	CIN3	G0						CR	NED	276
73	24	0	3	0	CIN3	G1						CR	NED	273
74	34	0	4	0	CIN3	G1						MR	MC	153
75	40	0	0	0	CIN3	G1						CR	NED	184
76	27	0	1	0	CIN3	G0						CR	NED	90
77	38	0	4	0	CIN3	G1						CR	NED	118
78	32	0	0	0	CIN1	G1						CR	NED	94
79	33	1	1	1	CIN3	G0	16	NEG		NEG		PR	RE	646
80	25	0	0	0	CIN3	G1						CR	NED	648
81	36	0	0	0	CIN3	G1						CR	NED	101
82	35	2	1	1	CIN3	G3						CR	NED	643
83	21	0	0	0	CIN3	G0						CR	NED	616
84	32	1	0	0	CIN3	G0						CR	NED	901
85	22	0	0	0	CIN3	G0						CR	NED	749
86	34	0	1	0	CIN3	G0						CR	NED	845
87	24	1	0	0	CIN3	G1						CR	NED	1,015
88	29	0	0	0	CIN3	G0						CR	NED	1,114
89	20	0	0	0	CIN3	G0						CR	NED	1,298
90	20	0	0	0	CIN3	G1						CR	NED	1,112
91	27	1	1	0	CIN3	G1						CR	NED	1,850
92	49	0	0	0	CIN3	G0						CR	NED	2,116
93	25	1	1	1	CIN3	G1						CR	NED	1,410
94	26	1	0	0	CIN3	G0						CR	NED	2,232
95	29	0	0	0	CIN3	G0	58	NEG		NEG	NEG	PR	NED	2,065
96	28	1	4	1	CIN2	G0	16	NEG		NEG	NEG	CR	NED	2,035
97	25	1	0	0	CIN3	G0						CR	NED	1,653
98	34	0	1	0	CIN2	G0						CR	NED	820
99	40	1	2	2	CIN3	G1						PR	NED	481
100	35	1	2	1	CIN3	G0						CR	NED	365
101	35	1	0	0	CIN3	G1						CR	NED	360
102	39	0	0	0	CIN3	G0						CR	NED	354
103	33	1	0	0	CIN3	G0						CR	NED	180
104	30	0	0	0	CIN1	G1						CR	NED	95
105	32	1	0	0	CIN3	G0						CR	NED	93

MA = Marriage (0: single, 1: married, 2: divorced); PRG: pregnancy times; DEL = delivery times; HIS = histology; PS = photosensitivity; HPV PRE: HPV type before treatmenrt; HPV 3M = HPV type 3 months after PDT; NEG = negative; OT = Other; Effect 3M = effect of PDT after 3 months; PR = partial response; MR = minor response; NED = No evidence of disease; MC = minor change; RE = recurrence; Duration = duration after treatment (days).

patients had CIN3, and 1 patient (case 25) developed stage IB1 cervical cancer. All 3 patients required surgical intervention. Two additional patients had mild cytological changes. Of all 105 patients, 14 patients have become pregnant following PDT including 6 women who have delivered term babies without complications. The outcomes of the other 8 pregnancies include: 1 preterm delivery, 1 spontaneous miscarriage, 2 therapeutic abortions, 1 molar pregnancy, and 3 ongoing pregnancies. All clinical histories are summarized in table 1.

HPV

HPV typing was performed before and after PDT therapy for 69 patients. Before treatment, HPV was detected in 64 of 69 patients (93%), including 30 patients with high-risk HPV (16, 18). Three months after PDT, HPV-DNA could not be detected in 47 of 64 patients (73%) who showed HPV-DNA positive cervical smears before treatment. Seventeen patients still had HPV-DNA positive cervical smears, and in 13 of these 17 cases, HPV typing changed. Six months after PDT, 17 of 65 (26%) examined patients still had HPV-DNA positive cervical smears; however, these 16 patients had no abnormal cytological or histological findings. Additionally, in 15 of these 16 HPV-DNA positive cases, HPV typing changed compared to pre-PDT testing. One year after PDT, 16 of 57 (28%) examined patients still had HPV-DNA in cervical smears. Of these 16 patients, only 1 patient had mild abnormal cytological findings indicating LSIL. Additionally, in 13 of 16 HPV-DNA-positive patients, HPV typing changed compared to pre-PDT testing. Two years after PDT, 11 of 31 (35%) examined patients still had HPV-DNA in cervical smears. In 10 of 11 HPV-DNA positive patients, HPV typing changed compared to pre-PDT testing. Finally, 3 patients had recurrence (2 cases: CIN3; 1 case: invasive cancer); however, these 3 patients had negative HPV-DNA in cervical smears 1 year after PDT treatment.

Discussion

In this study, we examined the effect of PDT on CIN and HPV in over 100 women. We found that over 90% of patients achieved CR after 3 months. Only three patients ultimately developed recurrent disease. There are several studies reporting disappointing results using PDT for the treatment of CIN [15–20]; however, these investigators used 5-aminolevulinic acid for a sensitization agent which we believe is inferior to the agent used in our study. For example, Hillemanns et al. [16] performed PDT using 5-aminolevulinic (5-ALA) for sensitization and an argon-ion-pumped dye laser in 7 women with high grade CIN. However, PDT did not appear to be effective in all patients. Keefe et al. [18] performed PDT using 5-ALA and argon-pumped dye laser in 40 CIN2 or 3 patients, and reported success rates at 4, 8 and 12 months were 51, 46 and 31%. Barnett et al. [19] reported that the response rate of PDT using 5-ALA was 33% in CIN1/2 patients. These reports suggest that PDT using 5-ALA is not effective for CIN. We achieved much better response when treating CIN with PDT using PHE for photo sensitization, and an ELD or YAG-OPO laser. In addition, PHE is reported to be more effective for cascular endothelial cell than 5-ALA [21, 22]. Muroya et al. [23] performed PDT using PHE and EDL for 56 patients (39 CIS and 17 dysplasia), and achieved high complete response rate comparable to our current study (96.4%, 54/56). However, in the Muroya report, follow up duration was short and HPV typing was not evaluated. Di Saia and Creasman [24] reported that the surgical treatment including cold-knife excision, electrocautery, cryosurgery and laser ablation achieved high success rates between 90 and 98%. Recurrence rate of conization for CIN was reported to be 0.6% [25–27]. From these findings, PDT may be somewhat inferior to surgical treatment for CIN. A comparative study is needed to solve this problem.

It is well known that HPV is the most prevalent etiologic agent in neoplastic transformation of squamous epithelial cells. Cervical carcinogenesis is related to specific high risk types of HPV, most commonly HPV 16 and 18. In our series, HPV was detected in 64 out of 69 patients (93%) and high-risk HPV (16, 18) detected in 30 (43%) patients before treatment. Finally, HPV-DNA was not detected in 75, 74 and 72% at 3, 6 and 12 months after PDT. These data are consistent with Wierrani et al.'s [17] report of 19 patients undergoing PDT. One year after PDT, 16 of 57 (28%) patients in our study still had HPV-DNA in cervical smears. Of these 16 patients, only 1 patient had mild abnormal cytological findings. Additionally, in 13 of 16 HPV-DNA-positive patients, HPV typing changed compared with testing before PDT. This suggests that the 13 patients might have been re-infected with other types of HPV since treatment. Furthermore, the 3 patients with recurrent pre-invasive or invasive disease had no HPV-DNA detected in cervical smears 1 year after PDT. Persistent HPV infection did not predict the recurrence of CIN. In fact, 16 patients with HPV persistence or re-infection had no recurrence of CIN during the follow-up period.

The follow-up period in our study is too short to determine the long term effectiveness of PDT for CIN treatment. Ylitalo et al. [28] reported that among HPV 16-positive women, the median incubation period from infection to carcinoma in situ was 7–12 years. In our study, 3 patients experienced recurrence despite negative HPV testing 1 year after PDT. Possibly, our HPV detection system may have lacked sensitivity in these cases. Alternatively, not only HPV infection, but also the status of the immune system, abnormality of cell cycle regulators, and p53 polymorphisms may contribute to the development cervical neoplasia [4, 29–31]. However, given the known lengthy incubation period of neoplasia, we speculate that these recurrent cases might be due to small undetectable lesions of CIN that persisted following PDT. Further study is needed to better understand the cases that are not cured by PDT.

In this study, all patients were hospitalized to ensure light deprivation for 3 weeks. With this rigorous light-deprivation protocol, 50 of 105 patients (48%) developed mild cutaneous photosensitivity (grades 1 and 2). Only one patient suffered from grade 3 cutaneous photosensitivity. It may be because she worked in the sunshine during the summer season just after being discharged. In other reports of PDT using 5-ALA, cutaneous photosensitivity was not reported despite light exposure [15–20]. However, we believe the superior therapeutic profile of PHE justifies its use despite the increased phototoxicity. We recognize that our protocol may be prohibitively expensive and inconvenient in many settings. Since toxicity was minimal, more liberal protocols of light deprivation may be appropriate. It was reported that lower doses of PHE such as 1 mg/kg was effective for cutaneous cancer [32–33]. Decreasing the PHE dose may reduces cutaneous photosensitivity. Establishing an outpatient protocol is one of our goals for future study.

In conclusion, PDT is an effective treatment for CIN, and for HPV infection. PDT may be an attractive alternative for women desiring to preserve cervical function for pregnancy. Furthermore, in our study, the persistence of HPV following treatment did not correlate well with CIN recurrence.

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S. Yamaguchi and H. Tsuda have contributed equally to this study.

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