Prognostic Factors of the Outcome of Cervical Intraepithelial Neoplasia in Association with Human Papillomavirus in Patients with Long-term Follow-up

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ABSTRACT. Objective: To retrospectively analyze the risk factors for the persistence and progression of cervical intraepithelial neoplasia (CIN) in patients with long-term follow-up. **Patients and Methods:** Charts were reviewed to select patients with a diagnosis of CIN, who had been followed up with or without surgical treatments, that is, vaporization or conization. The baseline status of human papilloma virus (HPV) infection was unknown. HPV has been tested along with cytological evaluation for the patient at every visit since then until April 1998. Patients who could be followed up for more than 12 months were analyzed to determine the risk factors for persistent or progressive CIN. **Results:** An analysis of 314 visits by 66 patients was made. The madian overall follow-up duration of the patients was 45 months (range 16-192). The cytological outcome was normalized in significantly more patients with a lower HPV positive rate ($p=0.0276$), and it was significantly better in those patients who had undergone vaporization or conization ($p=0.0286$), particularly in those patients with higher grade CIN. **Conclusion:** Destructive surgical treatments and an HPV positive rate could be prognostic factors for the cytological outcome of CIN.

Key words: Cervical intraepithelial neoplasia — human papilloma virus — long-term follow-up — vaporization — conization

INTRODUCTION

Human papillomavirus (HPV) infection, particularly with type 16 or type 18, is associated with cervical intraepithelial neoplasia (CIN) and the development of cervical carcinoma of uterus.[1,2] Polymerase chain reaction (PCR) technology has made identification of subtype of HPV easier and has made it possible for routine examination of HPV positivity. We began to routinely test the patients with CIN for HPV in 1995. Persistent presence of high-risk subtypes of HPV has been found to be associated with persistent cervical dysplasia or progressive disease.[3-6] Destructive surgical treatments, such as vaporization or conization, may eliminate the presence of HPV. The frequency of positive results of HPV test may be a prognostic factor.

In this study, we retrospectively evaluated the role of surgical treatments and analyzed the relationship between a positive HPV rate and the cytological and HPV outcomes during follow-up.

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PATIENTS AND METHODS

Charts were reviwed to select patients with a diagnosis of CIN made before October 1995 in Kawasaki Medical School Hospital. Since the HPV test was not performed before that time, the baseline HPV status of these patients at their first visit were unknown. However, these patients were tested for HPV every visit there after.

The grade of CIN at the first visit was determined based on the results of a cervical smear and colposcope-guided punch biopsy and a higher degree was applied. Follow-up of the CIN was done only by cytology and colposcopy. A punch biopsy was performed only when progression of the disease was suspected by either cytology or colposcopy. The follow-up interval of patients with cytological findings of class IIIA or IIIB was 3-4 months and that for patients with class I or II was 6-12 months. Patients whose follow-up period was less than 12 months were excluded from this study.

The follow-up until April 1998 was reviewed. Only patients who had been followed up or those who underwent conization or vaporization before October 1995 were evaluated. Conization was performed using either the YAG-laser or the LOOP Electric Excision Procedure (LEEP) system. Vaporization was done using either the YAG-laser or a ball-type tip of the LEEP system.

Detection of HPV DNA and its typing was performed using the PCR, as previously described.[7] All samples for HPV detection were obtained in the same fashion using a cotton swab immediately after obtaining samples for cytological smears. A fresh cotton swab moistened with sterilized saline was used for cytological smears of the exocervix and endocervix. The cervix was scrubbed again twice for the HPV test using the same cotton as used for the cytological smears. The swab was transferred into a test tube and was stored at $-20°C$ or $-80°C$ depending on the time till assayed. The types of HPV tested were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58, which are considered to be high-or intermediate-risk types.

Factors that might influence cytological and HPV outcomes, such as the grade of CIN at the first visit, with or without treatment, and the types oa treatment were analyzed. The positive rate of HPV was also examined in relation to cytological outcomes. The positive rate of HPV was calculated as follows: Positive rate (%) $=100$ x (Number of positive results)/(Number of total visits). The cytological outcome was defined as a result which was worse in the last two visits. The HPV outcome was also determined using the same rule.

Statistical analysis was performed using Fisher's exact test or the Mann-Whitney test depending on the analysis, as indicated in the Results section. Reported P values resulted from the use of two-tailed tests. The actual calculation was done by SPSS for Windows 7.5J, GraphPad Instat version 3.00.

RESULTS

Sixty-six patients met the criteria for this study and a total of 314 visits of these patients were analyzed. Their characteristics are summarized in Table 1.

Table 2 shows the relationship between the degree of CIN and treatments undergone. There was a significant tendency for heavier treatment to be applied as the degree of CIN increased ($p<0.001$). While about half of the

patients with CIN 1 received vaporization or conization, more than 90% with CIN 2 or 3 underwent one of these treatments. Conization was performed on 50% of CIN 2 patients and on 82% of CIN 3 patients.

TABLE 1. Patients' Characteristics

Age	Mean	39
	Range	18-81
CIN Grade	1	39
	2	15
	3	12
Overall Follow-up Duration (months)	Median	45
	Range	16-192
Follow-up during study (months)	Median	28
	Range	13-30
No. of Visits after 1995	Total	314
	Median	5
	Range	2-13

TABLE 2. Degree of CIN and treatment choice

Treatment	CIN Grade			Total
	1	2	3	
No	19	1	1	21
Yes	20	14	11	45
(Vaporization)	17	7	2	26
(Conization)	3	7	9	18
Total	39	15	12	66

The choice of treatment was significantly dependent on the degree of CIN ($p<0.001$*)
*: Tested by x^2 test.

There was no association between the initial grade of CIN and the cytological and HPV outcomes (data not shown). The cytological outcome depended on whether or not one of the treatments (vaporization or conization) was performed (Table 3-1). Although 36.4% of the patients had persistent abnormal cytology in the no treatment group, abnormal cytology was seen in only 6.8% of the patients who received one of the treatments ($p=0.0286$). No significant differences in the cytological outcome were observed with regard to the type of treatment, vaporization or conization. This observation mainly applied to the outcome of patients with CIN 2 or 3 (Table 3-2). Although there was a similar tendency in patients with CIN 1, where the number of patients with CIN 1 in the treated and non-treated groups was well balanced, the number might be too small for statistical significance (Table 3-3).

The HPV outcome was also significantly associated with whether or nottreatment was performed (Table 4) ($p=0.0378$). The association between the cytological outcome and HPV outcome was also statistically significant ($p<0001$, Table 5).

TABLE 3-1. Relationship between treatment and cytological outcome.

Treatment	Cytological Outcome		Total
	Class I + II	Class IIIA + IIIB	
No	14	7	21
Yes	41	4	45
(Vaporization)	25	1	2
(Conization)	16	3	18
Total	55	11	66

The difference in the cytological outcome of patients with or without treatment was significant ($p=0.0286$, calculated by Fisher's Exact test).
The difference in the HPV outcome of the types of treatment (vaporization vs conization) was not significant ($p=0.2954$, calculated by Fisher's Exact test).

TABLE 3-2. Relationship between treatment and cytological outcome in CIN 2 and 3 patients.

Treatment	Cytological Outcome		Total
	Class I + II	Class IIIA + IIIB	
No	0	2	2
Yes	22	3	25
Total	22	5	27

The difference in the cytological outcome of groups with or without treatment was significant ($p=0.0285$, calculated by Fisher's Exact test).

TABLE 3-3. Relationship between treatment and cytological outcome in CIN 1 patients.

Treatment	Cytological Outcome		Total
	Class I + II	Class IIIA + IIIB	
No	14	5	19
Yes	19	1	20
Total	33	6	39

The difference in the cytological outcome of groups with or without treatment was not significant ($p=0.0951$, calculated by Fisher's Exact test).

TABLE 4. Relationship between treatment and HPV outcome.

Treatment	HPV Outcome		Total
	Negative	Positive	
No	12 (54.5%)	10 (45.5%)	22 (100%)
Yes	36 (81.8%)	8 (18.2%)	44 (100%)
(Vaporization)	22	4	26
(Conization)	14	4	18
Total	48	18	66

The difference in the HPV outcome of groups with or without treatment was significant ($p=0.0378$).
The difference in the HPV outcome of the types of treatment (vaporization vs conization) was not significant ($p=0.6971$*).
* : Calculated by Fisher's Exact test.

TABLE 5. Relationship between HPV outcome and cytological outcome.

HPV Outcome	Cytological Outcome		Total
	Class I + II	Class IIIA + IIIB	
Negative	46	2	48
Positive	9	9	18
Total	55	11	66

The association of cytology/HPV status at the last visit was statistically significant ($p<0.0001$, tested by Fisher's Exact test.).

TABLE 6. Relationship between an HPV positive rate parameters which may correlate with an HPV positive rate.

Parameters		HPV Positive rate (%) Median (25%-75% Percentile)	p value*
Initial CIN Grade	1	25.0 (0.0-50.0)	
	2	14.3 (0.0-22.5)	N.S.**
	3	33.9 (0.0-66.7)	
Cytological Outcome	I + II	20.0 (0.0-41.5)	0.0276
	IIIA + IIIB	44.4 (22.5-83.4)	
Treatments	No	30.9 (0.0-50.0)	0.2571
	Yes	17.5 (0.0-42.9)	

*: Calculated by the Mann-Whitney test (two-tailed)
**: The differences between the groups were not statistically significant.

The relationships between an HPV positive rate and the initial grade of CIN, cytological outcome, and whether or not treatment was performed are summarized in Table 6. An HPV positive rate was significantly associated with the cytological outcome. However, it was not related to the initial grade of CIN or to whether or not treatment was performed.

There were two CIN 2 patients who underwent hysterectomy during this study period because progression of the disease was observed. One of them had had follow up only and the other patient had undergone vaporization before the study began.

DISCUSSION

In this study, probable factors relating to the outcome of CIN were examined. One factor which may have a significant impact on the outcome of CIN could be destructive treatments of the cervix including vaporization or conization. In fact, significant improvement in cytological results and the HPV outcome was observed in patients who underwent one of these treatments (Tables 3 and 4). This was particularly true in patients with CIN 2 and 3. Therefore, it is assumed that these destructive treatments could eliminate HPV.

As previously reported,[10] it is believed that many cases of CIN 1 regress spontaneously. However, in our study, although it was not statistically significant, the cytological outcome also tended to be better in patients with CIN 1 who underwent destructive treatments. This result suggests that there

could be a subpopulation of CIN 1 patients for whom these treatments are beneficial. Factors which determine the prognosis of CIN 1 patients, therefore, should be further clarified.

In spite of the positive impact of these destructive treatments on the improvement of cytological and HPV outcomes, the HPV positive rate was not associated with whether or not the treatments were performed (Table 6). There is no clear explanation for these conflicting results, although an increase in the statistical power may resolve this question.

We found that a patient with a lower HPV positive rate was more likely to achieve improvement in CIN and HPV outcome (Table 6). Therefore, the HPV positive rate could be a prognostic factor for the persistence and/or progression of CIN during a long-term follow-up.

One of the most important limitations of this study was the fact that the patients' HPV status at the first visit was not known because of the nature of the nature of this retrospective analysis. As Ho *et al*[8] and Moscicki *et al*[9] demonstrated, a positive HPV test will become negative within 6-24 months, particularly in young women, and only women who have persistence of HPV are at high risk for abnormal cytology. These studies suggested that a baseline HPV status may not play an important role in the follow-up of CIN patients. However, since the patients in our study were a selected population who had shown abnormal cytology in the first place, a simple comparison with these two important studies is impossible. Therefore, the importance of the initial HPV status as a prognostic factor should be compared with the HPV positive rate in a future prospective study.

Another important issue to be addressed is cost-effectivenss. A relatively expensive HPV test is needed with each visit of a patient. As Kaufman *et al*[11] suggested that the HPV profile test is not a cost-effective triage for patients with atypical squamous cells of undetermined significance in low-grade squamous lesions, it is possible that determination of the HPV positive rate is not cont-effective. A similar conclusion may be reached in a future study. Careful examination is required.

At this moment, a prospective study is in progress to clarify which of the following is the most significant prognostic factor in CIN ; initial HPV status, an HPV positive rate, or whether or not treatment was performed.

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REFERENCES

1) Mitchell MF, Tortolero LG, Wright T, Sarkar A, Richards KR, Hong WK, Schottenfeld D : Cervical human papillomavirus infection and intraepithelial neoplasia : a review. J Natl Cancer Inst Monogr, 17-25, 1996
2) Koutsky LA, Holmes KK, Critchlow CW, Stevens CE, Paavonen J, Beckmann AM, DeRouen TA, Galloway DA, Vernon D, Kiviat NB : A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med **327** : 1272-1278, 1992

3) Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, Basu J, Tachezy R, Lewis R, Romney S : Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia [see comments]. J Natl Cancer Inst **87** :1365-1371, 1995
4) Remmink AJ, Walboomers JM, Helmerhorst TJ, Voorhorst FJ, Rozendaal L, Risse EK, Meijer CJ, Kenemans P : The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease : natural history up to 36 months. Int J Cancer **61** : 306-311, 1995
5) Katase K, Teshima H, Hirai Y, Hasumi K : Natural history of cervical human papillomavirus lesions. Intervirology **38** : 192-194, 1995
6) Konno R, Sato S, Yajima A : Progression of squamous cell carcinoma of the uterine cervix from cervical intraepithelial neoplasia infected with human papillomavirus : a retrospective follow-up study by in situ hybridization and polymerase chain reaction. Int J Gynecol Pathol **11** : 105-112, 1992
7) Yoshikawa H, Kawana T, Kitagawa K, Mizuno M, Yoshikura H, Iwamoto A : Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. Jpn J Cancer Res **82** : 524-531, 1991
8) Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD : Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med **338** : 423-428, 1998
9) Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N, Darragh TM, Brescia R, Kanowitz S, Miller SB, Stone J, Hanson E, Palefsky J : The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr **132** : 277-284, 1998
10) Ostor AG : Natural history of cervical intraepithelial neoplasia : a critical review. Int J Gynecol Pathol **12** : 186-192, 1993
11) Kaufman RH, Adam E, Icenogle J, Reeves WC : Human papillomavirus testing as triage for atypical squamous cells of undedetemined significance and low-grade squamous intraepithelial lesions : sensitivity specificity, and cost-effectiveness. Am J Obstet **177** : 930 -936, 1997