Assessment of Human Papilloma Virus Infection in Adult Laryngeal Papilloma Using a Screening Test

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Summary: Objectives. Human papilloma virus (HPV) infection is involved in both juvenile and adult laryngeal papilloma. We wished to determine which types of adult laryngeal papilloma were clinically related to HPV infection. We hypothesized that multiple-site and recurrent papillomas would have a strong relationship to HPV and conducted the present study to test this hypothesis.

Methods. Thirteen male patients with adult laryngeal papilloma who underwent resection of papilloma between August 2006 and September 2009 were studied. We examined the relationships between whether the tumor was solitary or multiple, presence or absence of recurrence after surgery, and HPV infection. High-risk HPV types (HPV-DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and low-risk HPV types (6, 11, 42, 43, and 44) were tested by a liquid-phase hybridization method. In addition, HPV typing was performed for patients positive for low-risk HPV types. Twenty patients with laryngeal carcinoma or laryngeal leukoplakia were enrolled as the control group.

Results. In the laryngeal papilloma group, all patients tested were negative for high-risk HPV and 69.2% were positive for low-risk HPV. Typing performed for seven of the patients who tested positive for low-risk HPV showed that one patient was positive for HPV-11, whereas the remaining six patients were positive for HPV-6. All patients with recurrent laryngeal papillomatosis (RLP) were positive for low-risk HPV. All patients who were positive for low-risk HPV had RLP. Tumor samples from repeat operations were positive for low-risk HPV in all patients tested. HPV was not detected in the control group.

Conclusions. The relationship between RLP and low-risk HPV was strong, with all cases that were positive for low-risk HPV showing recurrence. Tumor tissue resected at the time of repeat surgery was positive for low-risk HPV in all cases tested.

Key Words: Voice–Liquid-phase hybridization–Recurrent respiratory papillomatosis.

INTRODUCTION

Laryngeal papillomas are classified into juvenile and adult onset. Juvenile-onset papillomas occur in children (<14 years), exhibit multiple infection sites, and often recur. Such infections generally resolve spontaneously around puberty but recur in adulthood. Adult-onset infections may have single or multiple infection sites. Single-site infections can often be cured with a single surgery, whereas most multiple-site infections will recur. Human papilloma virus (HPV) infection is generally regarded as the causal agent in laryngeal papillomatosis. Juvenile-onset laryngeal papillomatosis is thought to be caused by vertical transmission of HPV at birth. Among births to pregnant women with condyloma acuminatum, there were one of 144 cases of juvenile-onset HPV infection, with three of 1000 children developing laryngeal papilloma.[1] Despite the fact that HPV-associated laryngeal papilloma is a benign disease, it is extremely difficult to cure. However, HPV vaccines are now available and already in use in many countries, with the aim of preventing cervical cancer. Cervarix (GlaxoSmithKline, Middlesex, UK)[2,3] contains HPV types 16 and 18 antigens, the major cause of cervical cancer. In contrast, Gardasil (Merck & Co, Whitehouse Station, NJ)[4–6] is a quadrivalent vaccine that also includes HPV type 6 and 11 antigens. Because these latter two HPV types are causally associated with recurrent respiratory papillomatosis (RRP), Gardasil (Registered) would be expected to have a preventative effect on this disease.

It is clear that juvenile laryngeal papillomatosis occurs as a result of HPV infection. However, some cases of adult laryngeal papillomatosis are not related to detectable HPV infection. We therefore wished to address the question, "Which types of papilloma have a clinical relationship with HPV type?" We hypothesized that multiple and recurrent papillomas, that is, recurrent laryngeal papillomatosis (RLP), may have a strong relationship with HPV type. To test this hypothesis, the relationship between adult laryngeal papilloma and HPV infection was investigated using an HPV screening test.

METHODS

Thirteen male patients with laryngeal papilloma who presented and underwent laryngeal microlaser surgery at Nihon University Surugadai Hospital between August 2006 and September 2009 were studied. Their ages ranged from 28 to 86 years with an average age of 50.6 years. Surgery was performed using a holmium-yttrium aluminum garnet laser to completely ablate the laryngeal mucosa at the site of the tumor.

Whether the tumor was solitary or multiple and the presence or absence of recurrence after surgery was examined in relation to HPV infection. HPV infection was determined by an HPV-DNA test using liquid-phase hybridization.[7–10] High-risk types (HPV-DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and low-risk types (6, 11, 42, 43, and 44) were tested. In addition, simultaneous detection of multiple types of HPV-DNA using a consensus primer-directed polymerase chain

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reaction (PCR) system was performed for patients who were positive for low-risk HPV.[11] This test method enables simultaneous detection of nine types of HPV, specifically HPV-6, -11, -16, -18, -31, -33, -42, -52, and -58, with a sensitivity no lower than that of Southern blotting.[11] We were able to perform PCR typing for seven of the nine patients who were positive for low-risk HPV. As a control group, 10 patients with laryngeal carcinoma and 10 with laryngeal leukoplakia were enrolled. The ages of patients in the control group ranged from 34 to 82 years with a mean age of 65 years. An HPV-DNA screening test using liquid-phase hybridization and PCR typing were also conducted in the control group for comparison with the laryngeal papilloma group.

For patients who underwent a repeat operation, the HPV-DNA screening test was performed again to evaluate whether HPV reinfection was involved in the recurrence.

This study was approved by the Institutional Ethics Committee. The patients were given a full explanation and provided written consent. The Mann-Whitney *U* test was used for comparison between the laryngeal papilloma group and the control group and between solitary and multiple papillomas. A *P* value of 0.05 or lower was considered to represent a significant difference. *StatView* version 4.5J (SAS Institute Japan Ltd, Japan) was used for statistical analyses.

RESULTS

The clinical summaries and HPV-DNA test results of laryngeal papilloma patients are shown in Table 1, and those of control patients are given in Table 2.

As shown in Table 1, the vocal folds accounted for most of the papilloma sites. Tumor tissue from all papilloma patients was negative for high-risk HPV; however, nine of the 13 patients (69.2%) tested positive for low-risk HPV. Six of the seven patients who underwent HPV typing were positive for HPV-6. One patient was positive for HPV-11. Normal laryngeal mucosa was tested for low-risk HPV in five patients with multiple papillomas and was positive in all patients. High- and low-risk HPV were tested in all patients in the control group and results were negative in all cases (Table 2). HPV typing was conducted for 15 of 20 patients in the control group, but all patients tested negative. Low-risk HPV was significantly more common in the laryngeal papilloma group than in the control group ($P < 0.0001$).

The relationships between whether the tumor was solitary or multiple, the presence or absence of recurrence after surgery, and presence of low-risk HPV in laryngeal papilloma patients are shown in Table 3. Tumors were solitary in three patients and multiple in 10. All patients with the solitary tumors were negative for low-risk HPV-DNA. In contrast, nine of 10 patients (90%) with multiple tumors were positive for low-risk HPV. Hence multiple papillomas showed a significantly higher rate of low-risk HPV infection compared with solitary papillomas ($P = 0.0044$).

With the exception of patient 3, all patients have had at least 1 year of postoperative follow-up, and eight patients have had recurrences. All patients who had a recurrence demonstrated multiple papillomas and were positive for low-risk HPV. Recurrence was significantly more common in patients who were positive for low-risk HPV compared with those who were negative ($P = 0.0009$). Patient 11 showed noncontinuous papillomas in the right vocal fold and anterior commissure, but the HPV-DNA test was negative and no recurrence was evident. Patient 3 was excluded from the assessment of recurrence because less than 1 year had passed since surgery, although no recurrence had been seen after 6 months. In other words, all patients who were positive for low-risk HPV showed recurrence. In all eight patients who underwent repeat surgery, the tumor tissue excised at the second operation was positive for low-risk HPV.

DISCUSSION

Adult laryngeal papilloma and HPV-6/11 infection

The types of HPV detected in RRP have been reported in many countries. In reports from Southeast Asia, including

TABLE 1.
Laryngeal Papilloma Patients

Patient Number	Age	Sex	Tumor Site	Tumor Tissue HPV-DNA High Risk	Tumor Tissue HPV-DNA Low Risk	Tumor Tissue HPV Typing	Normal Laryngeal Mucosa HPV-DNA Low Risk
1	28	Male	Bilateral and false vocal folds	Negative	Positive	HPV-6	Positive
2	29	Male	Bilateral and false vocal folds	Negative	Positive	HPV-6	Positive
3	29	Male	Bilateral vocal folds	Negative	Positive	HPV-6	Positive
4	30	Male	Bilateral vocal folds	Negative	Positive	HPV-6	Positive
5	31	Male	Bilateral vocal folds, anterior commissure	Negative	Positive	HPV-11	Not tested
6	41	Male	Bilateral vocal folds, right arytenoid	Negative	Positive	Not Tested	Not tested
7	46	Male	Bilateral and false vocal folds	Negative	Positive	HPV-6	Positive
8	54	Male	Bilateral vocal folds	Negative	Positive	Not tested	Not tested
9	59	Male	Right vocal fold	Negative	Negative	Not tested	Not tested
10	61	Male	Bilateral vocal folds, right arytenoid	Negative	Positive	HPV-6	Not tested
11	67	Male	Right vocal fold, anterior commissure	Negative	Negative	Not tested	Not tested
12	71	Male	Aryepiglottic fold	Negative	Negative	Not tested	Not tested
13	86	Male	Right vocal fold	Negative	Negative	Not tested	Not tested