Safety and Immunogenicity of a Nonadjuvant Human Papillomavirus Type 6 Virus-like Particle Vaccine in Recurrent Respiratory Papillomatosis

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Summary: Objectives. To assess the safety and immunogenicity of a nonadjuvant human papillomavirus (HPV) type 6 L1 virus-like particle (VLP) vaccine in recurrent respiratory papillomatosis (RRP) in local Chinese patients.
Methods. Patients with RRP who had undergone surgical treatment before intramuscular administration of an escalating dose of HPV type 6 L1 VLPs (1, 5, and 25 μg at 4 weekly intervals) as part of their treatment were followed up for more than 10 years. Efficacy was assessed by detecting the vaccine-induced type-specific antibody titer, calculating the intersurgical interval, and observing recurrence or remission of papillomas after receiving the vaccine.
Results. Nonadjuvant HPV vaccine was generally well tolerated, with no serious vaccine-related adverse episodes. It induced seroconversion for each vaccine-related HPV type. At week 12 (4 weeks after injecting 25 μg), the vaccine-induced type-specific antibody titer was significantly high. Analysis of all patients found a significant increase in the intersurgical interval and decrease in the scores. One patient (16.7%; female) experienced complete remission. Five patients (83.3%) (two males and three females) experienced partial remission. In total, complete or partial remission was achieved in six (100%) patients.
Conclusions. Administration of nonadjuvant HPV type 6 L1 VLPs vaccine to RRP was generally well tolerated and highly immunogenic.
Key Words: Recurrent respiratory papilloma–HPV vaccine–VLP–Adjuvant therapy–Treatment.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is caused by infection with human papillomavirus types 6 and 11.[1] It affects both children and adults, with a reported incidence of 4.3 and 1.8 cases per 100,000 in children and adults, respectively.[1–3] It is associated with obstruction of the airway, stridor, progressive hoarseness, and respiratory distress.[4,5] Although benign, these lesions commonly recur. The challenge comes from repeated surgical procedures and the disease's unpredictable nature. The condition is extremely difficult to treat and patients usually undergo multiple surgical procedures (such as simple resection, CO_2 laser, or low-temperature plasma) and are given toxic systemic medications to control their symptoms (acyclovir, ribavirin, isotretinoin, indol-3-carbinol, and interferon).[6] However, generation of specific immunity to papillomavirus proteins appears important for clearance, as immunosuppressed individuals with impaired cell-mediated immunity clear papillomas more slowly and more commonly experience recurrence after treatment.

The major papillomavirus protein, L1, self-assembles into virus-like particles (VLPs), which, together with alum-based adjuvants, are the basis of vaccines licensed for use or use for prevention of human papillomavirus (HPV) infection,[7] as Ceravix bivalent vaccine (from GlaxoSmithKline) and the newer 9-valent Gardasil vaccine (from Merck & Co). L1 VLPs, in animal models, can induce strong cell-mediated immune responses including cytotoxic T-cell responses if delivered without adjuvant.[8,9] A phase 1 open label safety trial of nonadjuvant HPV type 6 VLPs (VLP immunotherapy) in patients with treatment for refractory genital warts demonstrated regression of the disease that would not have been expected from previous studies of therapy in similar patients.[10] At the same time, a trial was undertaken to assess the safety and immunogenicity of nonadjuvant HPV type 6 L1 VLP vaccine in RRP.

MATERIALS AND METHODS

Study design

This clinical study was conducted by the Princess Alexandra Hospital and sponsored by the Centre for Immunology and Cancer Research (CICR) at the University of Queensland (Protocol Number: CICR-2002-02). This study was performed at the department of otolaryngology–head and neck surgery, the 2nd Affiliated Hospital and Yuying Stomatology of Wenzhou Medical University in China in compliance with the Australian National Health and Medical Research Council Statement on Human Experimentation, the Declaration of Helsinki, and the International Committee of Harmonization Harmonized Tripartite Guidelines for Good Clinical Practice. The guidelines included provision for the monitoring and the auditing of the study. The protocol was submitted for independent review by the appropriate Institutional Ethics Committees.

Each subject was given a written description of the procedures, risks, and benefits of participation in the study, in the form of a patient information sheet. They were given sufficient time to consider the information, to ask questions, and to seek advice before being asked whether they wish to participate in the study. Each subject signed a consent form before entering the study. Before the commencement of the study, the sponsor required the completion of a Clinical Trial Agreement between the principal

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investigator and/or co-investigators and the sponsor. The Clinical Trial Agreement included details of financial arrangements and responsibility for appropriate insurances before commencement. The use of CICRVAX6 HPV type 6 L1 VLPs in the study has been approved by the Therapeutic Goods Administration under the conditions of the clinical trial notification scheme.

Study subjects

Between October 2006 and April 2007, six Chinese children with a history of RRP for at least 1 year (two males and four females, median age 9.1 years, range 6.1 to 16.1 years) were invited to take part in a phase 1b, multicenter study of HPV type 6 L1 VLPs as therapy for RRP. All patients underwent direct laryngoscopy with bronchoscopy and ablation of papilloma using the microdebrider more than three times due to recurrence of the disease. Pathology reports indicated HPV types 6/11 infection by in situ hybridization analysis. No immunogenicity tests were performed on the patient. Exclusion criteria included enrollment in studies of other investigational agents, history of allergy to any component of the medicine, history of infection of human immunodeficiency virus, and history of vaccination during the last month. An institutional review board for each institution approved both the protocol and the consent forms. As patients were younger than 18 years of age, parental consent was required. All their parents or legal guardians signed a consent form after review of the protocol procedures.

Procedures

The immunotherapy consisted of 1, 5, or 25 μg of HPV type 6 L1 VLPs suspended in 1 mL of 0.9% NaCl without adjuvant or preservative. VLPs were produced to good laboratory practice standards in insect cells using recombinant baculovirus, purified as previously described,[11] subjected to three Gray of c-irradiation and stored in aliquots at −80°C until use. Batch release qualifications included electron microscopic appearance, immunogenicity, and sterility.

Each participant received three doses of 0.5 mL HPV type 6b VLPs (1, 5, and 25 μg) at day 3, week 4, and week 8. The injection site (ie, left or right deltoid) was alternated for each dose. After vaccination, participants were observed for 2 hours. Temperatures were recorded orally every day for a week after vaccination, and the participant noted adverse events in the vaccination report card for 4 weeks after vaccination. The intensity of nonquantifiable reactions was assessed using a severity scale (mild, moderate, and severe). During the visit, participating clinicians interviewed the patients, transcribed adverse events, and assessed whether adverse events were vaccine related.

Serologic assays

Blood samples were obtained seven times throughout the study. The first sample was tested before enrollment to assess each subject's eligibility and to gather baseline data. During the dosage period and before the subject completing the study, six more blood samples were collected. Three of these samples were collected 4 weeks after each injection for immunologic tests, and three were collected 1 week after each injection for monitoring safety, which included the following: complete blood cell count, platelet count, serum alanine transaminase, and serum creatinine.

IgG-specific HPV type 6 L1 VLP-based enzyme-linked immunosorbent assays (ELISAs) were performed in 96-well plates as described previously.[12] Infection of 293TT cells was monitored by secreted alkaline phosphatase (SEAP) activity in the culture supernatant using a highly sensitive chemiluminescent reporter system. Antibody mediated papillomavirus pseudoviruses neutralization was detected by a reduction in SEAP activity. There are two major steps in the neutralization assay procedure. Initially, 293TT cells were plated in 96-well plates at day 0. After preincubation with sera, pseudoviruses were transferred onto the plated cells for infection. Subsequently, supernatant was collected from the plated cells at day 3, and SEAP was detected using the "Great EscAPe SEAP Chemiluminescence Kit" (Clontech, USA, Ref 631725).

Clinical assays

For patients where there was adequate documentation available, the prevaccine disease course was quantified by measuring the average time between treatments. Destructive therapy, such as surgical excision of lesions, laser therapy, or direct injections of cidofovir or bevacizumab, was defined as an invasive intervention designed to treat or to prevent the disease when the papillomas became so large that the patient had difficulty speaking or breathing. Some dates, such as intersurgical interval (ISI) and the severity scores of lesions, were entered into a matched-pairs comparison with postvaccine time between treatments. The severity scores of lesions were evaluated by electronic laryngoscopes as described previously.[13,14] After vaccination, each participant returned for follow-up visits each year for 10 years, and the same information was collected. We used complete remission, partial remission, and no efficacy to evaluate the clinical efficacy of VLP. Complete remission was defined as the papillomas having disappeared or not recurring after a minimum ISI of 12 months since the last treatment. Partial remission was defined as the papillomas having recurred but the ISI being significantly longer than original measurement. No efficacy was defined as the disease still requiring ongoing surgery.

Statistical analysis

The primary hypothesis was addressed using four exact binomial 95% confidence intervals, and a separate test was performed for each HPV dose. The separate test was used to compare the distribution of antibody titers in different doses over time. The Spearman correlation coefficient was used when comparing ELISA results against those obtained by using the neutralization test. Categorized ELISA and neutralization results were compared with the use of the Kappa statistic, and overall agreement percentages were also computed. All statistical tests were two-sided. Statistical significance was set at a P-value lower than 0.05 and all t tests were two-tailed. The difference in ISI was calculated using a paired t test because the data were normally distributed. Statistics were calculated using *GraphPad Prism* version 5.0 for Windows (GraphPad Software, San Diego, CA).

TABLE 1.
Demographic Characteristics of the Study Population

Trial Number	Sex	Inclusion Age (years)	First Treatment Age (years)	Times of Operation	Genital Warts of Parents (Y/N)	Tracheotomy (Y/N) and Times (years)	HPV type	Follow-up Time (months)	ISI (months)		
									Mean	Maximum	Minimum
WRRP01	F	7.0	1.2	17	Y	Y 1.5–5.9	11	122	4.4	13.2	3.0
WRRP02	F	6.8	2.0	3	N	N	6	122	19.0	23.0	15.0
WRRP03	F	6.1	6.7	12	Y	N	6	120	4.0	14.4	1.5
WRRP04	M	16.1	8.2	4	Y	N	6	120	26.8	28.8	18.2
WRRP05	M	11.8	11.0	3	Y	N	6	120	5.1	7.8	2.3
WRRP06	F	6.8	6.3	3	Y	N	6	120	2.9	3.5	2.3

Abbreviations: F, female; M, male; N, no, Y, yes.

RESULTS

Subjects

The trial tested three vaccine doses without adjuvant (1, 5, and 25 μg). Each dose was given at 0, 4, and 8 weeks. The demographic characteristics of the study population are shown in Table 1. Every subject (100%) received all three planned vaccinations, attended all visits, and completed the study up to week 12. The mean age of the subjects who were included in the primary analysis was 9.1 years; with the youngest age of first operation being 5.9 years. In addition, all subjects had been operated on more than three times in the year before vaccination, whereas their mean operation period was less than 11 months.

Safety

In general, HPV type 6 L1 VLP was well tolerated with no reports of any serious vaccine-related adverse effect. The frequency and severity of reactions following each of the three doses were similar. Most of the local and systemic reactions were classified as mild. However, pain was mild to moderate in intensity and resolved spontaneously within 48–72 hours in all subjects. None of the subjects required medication or other clinical intervention. Clinical laboratory results for all subjects were unremarkable.

Immunogenicity

Vaccine-induced immune responses were assessed in the HPV type 6 per-protocol immunogenicity cohort. Before the vaccination, the geometric mean titer (GMT) against HPV type 6 was very low (<0.05). At week 12, the GMT of those subjects injected with 25 μg of the vaccine was substantially higher (0.1290 ± 0.0719 MU/mL) when compared to other doses (Table 2). The higher serum antibody the subject had before the vaccination, the higher the titer after receiving 1 μg of HPV type 6 VLP. Also, GMT in serum after each injection was significantly associated with the GMT before injection, as shown in Figure 1. Apparently, the GMT of serum antibody was significantly associated with the dose of VLP injection.

Clinical results

VLP immunotherapy was effective where destructive therapy alone was insufficient to clear the virus. Before the vaccine treatments, patients' ISI averaged at 10.37 months. Following vaccination, one subject never relapsed while five patients had relapses. Four subjects underwent two further surgeries. One, whose ISI significantly increased to 17.4 months with an associated decrease in morbidity (Table 3), required four additional surgeries. In the first 5 years after vaccination, only one child (16.7%) achieved complete remission and five patients (83.3%) (three females and two males) achieved partial remission. Complete or partial remission was achieved in six (100%) patients. All patients had achieved complete remission when they were followed up for more than 10 years. The patients' condition had improved clinically as they no longer had stridor at rest and a hoarse voice, which was sometimes present before vaccination, and the severity score of lesion improved throughout treatment.

The overall changes in the severity scores of lesions were plotted for all patients (Figure 2). Reduction in the severity of papillomas after therapy was shown for subjects recruited according to vaccine dose. Analysis of all patients showed a mean severity lesion score of 11.1 following the last three episodes of surgery before receiving the vaccine, and a mean sore of 5.3 after receiving the vaccine. A paired *t* test comparing before and after scores showed a significant decrease.

As of the last visit (more than 10 years after the first injection), the clinical score was 0. The patients continue to be followed up in our clinic for symptom exacerbation and possible future interventions. It was surprising that the clinical course did not

TABLE 2.
GMT of Antibodies to Three Doses of HPV Type 6b VLP

	GMT, MU/mL (95% CI)	t	*P*
Before the vaccination	0.0197(0.0045–0.0343)		
1 μg VLP	0.0308(–0.0078 to 0.0694)	1.031	0.350
5 μg VLP	0.0380(0.0126–0.0634)	0.817	0.451
25 μg VLP	0.1290(0.0571–0.02009)	2.937	0.032

The per-protocol immunogenicity population includes all subjects who were not general protocol violators, who received all three vaccinations within acceptable day ranges, and who had day 1, week 4, week 8, and week 12 serum sample collected within acceptable day range.

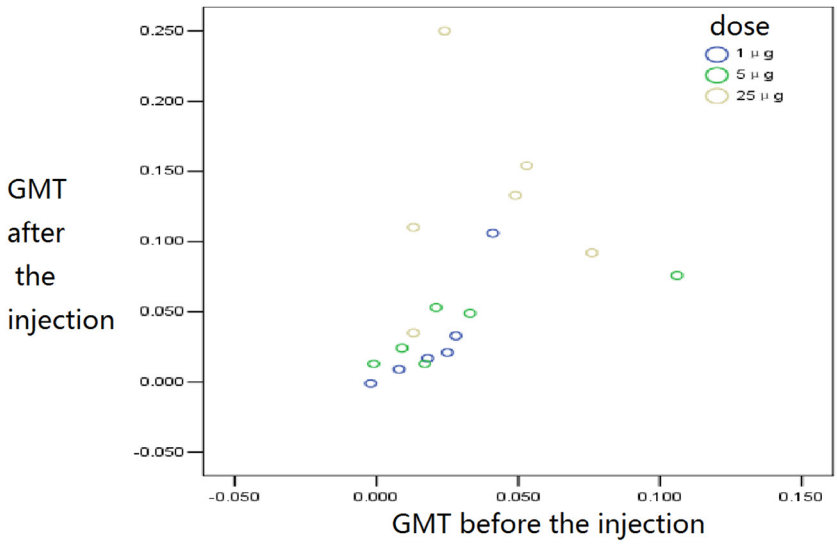

FIGURE 1. The relationship of GMTs between before and after immunotherapy can be seen in the graph. The GMT in serum after each injection of VLP was significantly associated with the GMT before injection, as well as the dose.

follow the natural law while papillomatosis became inactive slowly along with the age growth, but suddenly stopped growing. Moreover, the tumors did not recur after surgical intervention (Figure 3A, B, C). The tumor in one case was growing at the time of follow-up, but it decreased after 8 months without surgical treatment (Figure 4A, B).

DISCUSSION

RRP is a seemingly capricious and potentially fatal disease that is difficult to treat. The patients and their parents perceived a poor quality of life,[15] and they experienced limitations in interactions with their peers. No single type of therapy had been consistently effective in eradicating RRP. When children needed surgical therapy more frequently than four times in 12 months or had evidence of distal spread of RRP outside of the larynx, adjuvant medical therapy was generally considered.

This study demonstrates that administration of three doses of 1 μg or more of a VLP immunotherapy based on the L1 capsid protein of HPV type 6 was associated with a significantly reduced ISI and the severity scores of papillomas after conventional surgical therapy. VLP immunotherapy as a sole therapy was tested in our earlier study with encouraging results.[16] It was suggested that the benefit of VLP immunotherapy was not uniformly observed, but rather was restricted to patients treated with conventional destructive therapy, which on their own were relatively ineffective. Thus, a combined study of immunotherapy and conventional destructive treatment was developed.

The nature of the cellular immune response required for clearance of papillomas in humans is unknown, although studies of clearance of cervical cancer associated persisting HPV infections suggest that helper T-cell responses to viral nonstructural proteins correlate with virus clearance. In addition, the VLP immunotherapy intervention used in this study induced significant delayed type hypersensitivity to L1 protein,[17] which is a measure of induced CD4 T-cell mediated response to antigen. Natural resolution of dog and rabbit papillomavirus-associated warts is also associated with CD4 mediated delayed-type hypersensitivity responses to papillomavirus antigens.[18] The major capsid protein, L1, is the only protein to which measurable humoral immune responses are commonly found during the course of papillomas infections in humans.[19] We also observed weak delayed-type hypersensitivity responses to L1 in patients before VLP

TABLE 3.
The Overall Change in ISI for All Patients Before and After Receiving the Vaccine

Trial Number	Times of Operation Before	Times of Operation After	Mean ISI Before	Mean ISI After	Mean Scores Before*	Mean Scores After†	Times of No Recurrence (Months)
WRRP01	17	4	4.4	17.4	8	5.7	56
WRRP02	3	0	24	NA	4.7	0	125
WRRP03	12	2	4	7.2	15	6.3	119
WRRP04	4	2	26.8	22.8	10	6	92
WRRP05	3	2	5.1	5.7	10.3	8.7	115
WRRP06	3	2	2.9	2.7	16.7	7	117

* Mean severity score of lesion along the last three times surgeries before.
† Mean severity score of lesion along the next three times surgeries or visits.
Abbreviations: NA, not applicable.

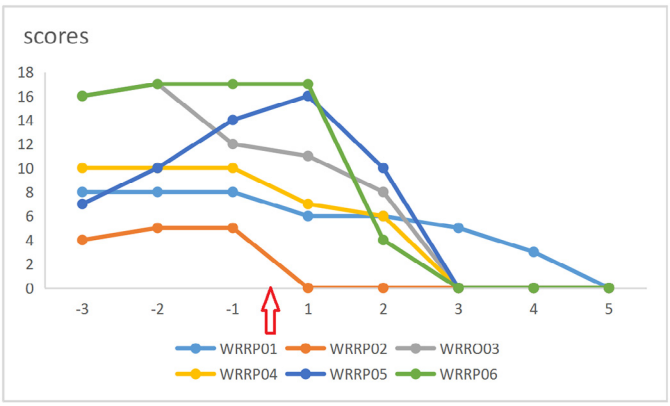

FIGURE 2. The *arrow* indicated where immunotherapy started. −1, −2, −3 were the scores of the last three times of surgeries before immunotherapy; 1, 2, 3, 4, 5 were the scores of morbidity after immunotherapy, including the visits if the patient did not accept surgery; and 5 was defined as last visit on May 2017.

immunotherapy. It has also been suggested that either disease or destructive treatment induces some cell-mediated immunity, albeit insufficient for disease resolution. Children with RRP had been shown to have lower anti-HPV antibody titers than those who were exposed to HPV but no active disease. It was thought that there was an immune dysfunction that allowed for the proliferation of HPV-induced papillomas.[20]

L1 protein is predominantly expressed in the superficial layers of the epidermis during the course of natural infection, where it would not be thought susceptible to cell-mediated immune responses of the sort induced by VLP immunotherapy.[20] However, sufficient L1 protein for susceptibility to cell-mediated immunity is presented by immature cells, and protein from immature[21,22] epithelial cells can be cross-presented from skin by vascular endothelial cells to disable epithelial rejection.[23] As local inflammation has been shown important for local function of immune effector cells induced by vaccination,[24] the current study was designed to use VLPs as immunotherapy following conventional surgical therapy, which was expected to induce the unnecessary local inflammation.

HPV type 6 L1 VLPs were expressed in baculovirus-infected Sf9 insect cells (Novavax, Rockville, MD). Production of clinical lots of recombinant HPV type 6 L1 VLP vaccine was performed in accordance with good manufacturing practices guidelines as previously reported.[25] This stimulated T-cell immunity and caused an increase in anti-HPV antibody titers.[26] The antibodies then neutralized the virus to prevent entrance into cells and to promote phagocytosis. This study showed that all patients had significantly higher anti-HPV antibody titers after

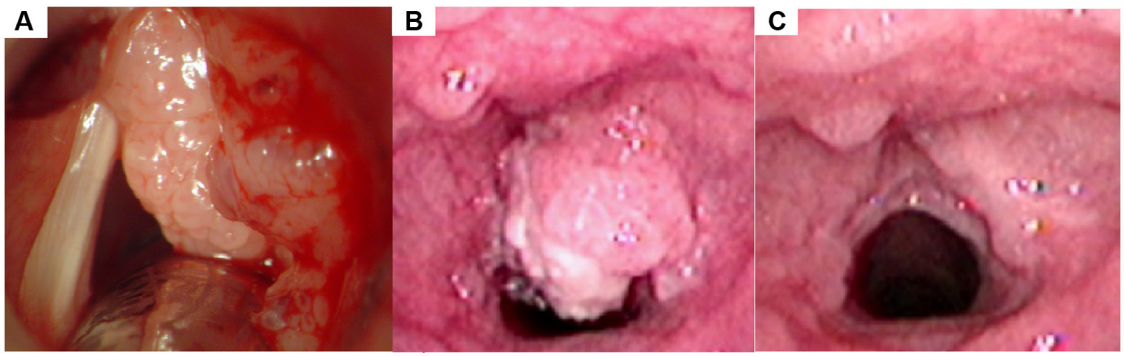

FIGURE 3. (**A**) Some tumors in the right vocal cord can be seen on the first operation of the child. (**B**) Then the bilateral vocal cords filled with tumors were visible through laryngoscope before vaccination, and the tumor grew more and the range became wider. However, the tumors did not recur after vaccination which can be seen on (**C**).

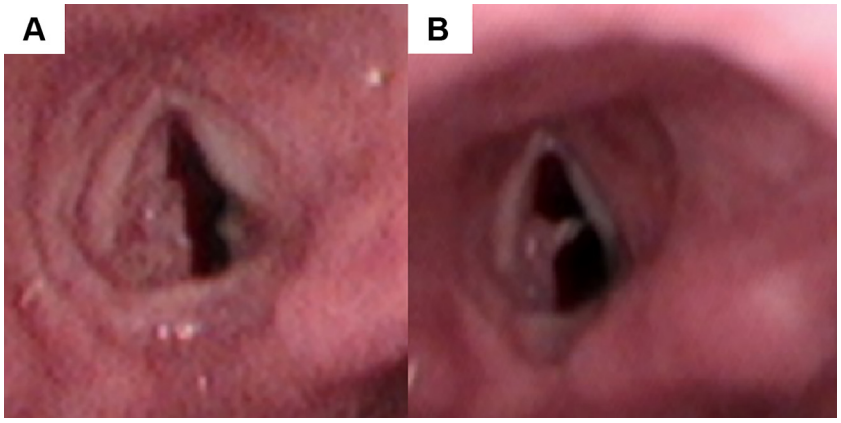

FIGURE 4. (**A**) Some tumors in the left vocal cords were visible after vaccination, but the range became smaller after 8 months without surgical treatment, which can be seen on (**B**).

vaccination. In addition, the titers were associated with the former serum antibody as well as the dose of VLP injection. Three doses were well tolerated and the 25 μg of HPV type 6b VLP was the most effective dose that stimulated the most anti-HPV antibody titers.

While it was understood that cell-mediated responses are necessary to destroy previously infected squamous cells, it was theorized that the increased humoral response would slow the course of disease, lengthen the ISI, and decrease morbidity. Patients were treated with the HPV vaccine to hopefully induce an immunologic response against the HPV infection to prevent future papillomas from forming. In our sample of patients, the addition of HPV type 6 L1 VLPs were associated with a significant increase in the ISI and all cases of complete sustained remission after 10 years.

The RRP was characterized by spontaneous remissions and recurrences and it was therefore difficult to isolate the effects of treatment from the natural course of the disease. Patients affected by RRP present with different clinical histories, including variations in the number of surgeries required for the control of relapses, time between surgeries and recurrences, extent of airway involvement, and the age of onset of RRP. Even a single patient may experience fluctuations in the intervals between operations over time, whether or not adjuvant treatments are used.[27]

The following variabilities in clinical manifestations were also observed among patients in the present study: the ages at the onset of RRP ranged from 1.2 to 11 years; the total number of previous surgeries was between 3 and 17 (Table 1); the mean ISI before were between 2.9 and 26.8 months; the mean ISI after were between 2.7 and 22.8 months and then no recurrence; the mean severity scores of lesion for the last three surgeries were between 4.7 and 16.7, the s-mean scores for the next three surgeries or visits were between 0 and 8.7 (Table 2); and the times of no recurrence after the last surgical treatments were between 57 and 125 months. In an attempt to minimize fluctuations in the clinical manifestations of the disease, the persistent postvaccination observation periods were restricted to 10 years. It has been shown that in most cases, disease severity improves over time.[28] In our sample of patients, one patient still decreased the time between surgeries and recurrences after receiving 1.5 μg of HPV type 6b VLP, while two patients (33.3%) showed no change in the frequency of surgery. Similar results were observed in the severity scores. One patient continued to suffer more severe disease, while four patients (66.7%) showed a little improvement in the severity scores. Some months after the last dose of 25 μg of HPV type 6b VLP, however, was remarkable that the tumor suddenly stopped growing and did not recur after surgical intervention. In addition, the size of papillomatosis in one case became smaller without surgical treatment at the time of follow-up. Thus, lengthening the postvaccination observation period could increase the likelihood of observing reductions in the severity of the disease that might not be attributable to the vaccination.

Throughout the study, care was taken not to change the routine procedures with regard to surgical indications and the surgical techniques used. In our hospital, the same surgical technique is used on all of the patients and just the recurrence of a lesion is not a surgical indication criterion. A need for surgery is indicated only when there is dyspnea or dysphonia that interferes with life activities. Changes in the surgical indications or in the surgical techniques employed could have interfered with the results, especially for the intervals between surgeries and the number of surgeries, which could be erroneously interpreted as a treatment effect. To exclude other influences that might have interfered with the outcome of the treatment, patients using adjuvant medicine were not included in the study.

The HPV type may be one factor that was associated with a poor prognosis. RRP was almost universally caused by HPV types 6 and 11; there were cross antigenic determinants because of the similar L1 protein amino acid sequence. Most studies had shown that type 11 is associated with poor disease prognoses.[29] Only one patient in the study was infected with HPV type 11 (Patient 1). This patient had the highest number of previous surgeries, a lower age of disease onset, higher severity scores, and shorter ISI compared with the other patients. Patient 1 was also the only patient to present with tracheotomy. In addition, this patient needed more time to complete remission. Complete remission was defined as complete absence of disease, whereas partial remission was defined as a period greater than 12 months where no appreciable growth of papilloma was noted on laryngeal examination. Patient 1 still underwent surgery 5 years after vaccination within 13 months of ISI with no recurrence for 56 months. Thus, lengthening the postvaccination observation period may help determine the likelihood of complete remission and partial remission.

LIMITATIONS

The limitations of our study are similar to those found with any retrospective chart review. These include possible selection bias, small sample number, lack of blinding, and absence of randomization to account for outliers. It should be stressed that our findings do not prove causality between the HPV vaccine and remission of RRP; they merely suggest a possible association that would benefit further investigation.

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REFERENCES

1. Derkay CS, Wiatrak B. Recurrent respiratory papillomatosis: a review. *Laryngoscope*. 2008;118:1236–1247.
2. Omland T, Akre H, Vardal M, et al. Epidemiological aspects of recurrent respiratory papillomatosis: a population-based study. *Laryngoscope*. 2012;122:1595–1599.
3. Derkay CS. Task force on recurrent respiratory papillomas. A preliminary report. *Arch Otolaryngol Head Neck Surg*. 1995;121:1386–1391.
4. Venkatesan NN, Pine HS, Underbrink MP. Recurrent respiratory papillomatosis. *Otolaryngol Clin North Am*. 2012;45:671–694, viii-ix.
5. Siegel B, Smith LP. Management of complex glottic stenosis in children with recurrent respiratory papillomatosis. *Int J Pediatr Otorhinolaryngol*. 2013;77:1729–1733.

6. Goon P, Sonnex C, Jani P, et al. Recurrent respiratory papillomatosis: an overview of current thinking and treatment. *Eur Arch Otorhinolaryngol*. 2008;265:147–151.
7. Dion GR, Teng S, Boyd LR, et al. Adjuvant human papillomavirus vaccination for secondary prevention: a systematic review. *JAMA Otolaryngol Head Neck Surg*. 2017;143:614–622.
8. Peng S, Frazer IH, Fernando GJ, et al. Papillomavirus virus-like particles can deliver defined CTL epitopes to the MHC class I pathway. *Virology*. 1998;240:147–157.
9. Kaufmann AM, Nieland JD, Jochmus I, et al. Vaccination trial with HPV16 L1E7 chimeric virus-like particles in women suffering from high grade cervical intraepithelial neoplasia (CIN 2/3). *Int J Cancer*. 2007;121:2794–2800.
10. Jardine D, Lu J, Pang J, et al. A randomized trial of immunotherapy for persistent genital warts. *Hum Vaccin Immunother*. 2014;8:623–629.
11. Zhang LF, Zhou J, Chen S, et al. HPV6b virus like particles are potent immunogens without adjuvant in man. *Vaccine*. 2000;18:1051–1058.
12. Schiller JT, Lowy DR. Prospects for cervical cancer prevention by human papillomavirus vaccination. *Cancer Res*. 2006;66:10229–10232.
13. Pasquale K, Wiatrak B, Woolley A, et al. Microdebrider versus CO2 laser removal of recurrent respiratory papillomas: a prospective analysis. *Laryngoscope*. 2003;113:139–143.
14. Lei W, Su Z, Wen W, et al. [Quantitative evaluation of the focus range of juvenile onset recurrent respiratory papillomatosis]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi*. 2006;20:970–972.
15. Montano-Velazquez BB, Nolasco-Renero J, Parada-Banuelos JE, et al. Quality of life of young patients with recurrent respiratory papillomatosis. *J Laryngol Otol*. 2017;131:425–428.
16. Hermann JS, Weckx LY, Monteiro Nürmberger J, et al. Effectiveness of the human papillomavirus (types 6, 11, 16, and 18) vaccine in the treatment of children with recurrent respiratory papillomatosis. *Int J Pediatr Otorhinolaryngol*. 2016;83:94–98.
17. van der Burg SH, de Jong A, Welters MJP, et al. The status of HPV16-specific T-cell reactivity in health and disease as a guide to HPV vaccine development. *Virus Res*. 2002;89:275–284.
18. Jain S, Moore RA, Anderson DM, et al. Cell-mediated immune responses to COPV early proteins. *Virology*. 2006;356:23–34.
19. Wikstrom A, Eklund C, von Krogh G, et al. Antibodies against human papillomavirus type 6 capsids are elevated in men with previous condylomas. *APMIS*. 1997;105:884–888.
20. Young DL, Moore MM, Halstead LA. The use of the quadrivalent human papillomavirus vaccine (gardasil) as adjuvant therapy in the treatment of recurrent respiratory papilloma. *J Voice*. 2015;29:223–229.
21. Block SL, Nolan T, Sattler C, et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16 , and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics*. 2006;118:2135–2145.
22. Bellone S, El-Sahwi K, Cocco E, et al. Human papillomavirus type 16 (HPV-16) virus-like particle L1-specific CD8+ cytotoxic T lymphocytes (CTLs) are equally effective as E7-specific CD8+ CTLs in killing autologous HPV-16-positive tumor cells in cervical cancer patients: implications for L1 dendritic cell-based therapeutic vaccines. *J Virol*. 2009;83:6779–6789.
23. Valujskikh A, Lantz O, Celli S, et al. Cross-primed CD8(+) T cells mediate graft rejection via a distinct effector pathway. *Nat Immunol*. 2002;3:844–851.
24. Zhong J, Hadis U, De Kluyver R, et al. TLR7 stimulation augments T effector-mediated rejection of skin expressing neo-self antigen in keratinocytes. *Eur J Immunol*. 2008;38:73–81.
25. Qi YM, Peng SW, Hengst K, et al. Epithelial cells display separate receptors for papillomavirus VLPs and for soluble L1 capsid protein. *Virology*. 1996;216:35–45.
26. Stanley MA. Immune responses to human papilloma viruses. *Indian J Med Res*. 2009;130:266–276.
27. Ongkasuwan J, Friedman EM. Juvenile recurrent respiratory papilloma: variable intersurgical intervals. *Laryngoscope*. 2012;122:2844–2849.
28. Silverberg MJ, Thorsen P, Lindeberg H, et al. Clinical course of recurrent respiratory papillomatosis in Danish children. *Arch Otolaryngol Head Neck Surg*. 2004;130:711–716.
29. Wiatrak BJ, Wiatrak DW, Broker TR, et al. Recurrent respiratory papillomatosis: a longitudinal study comparing severity associated with human papilloma viral types 6 and 11 and other risk factors in a large pediatric population. *Laryngoscope*. 2004;114(11 suppl 104 pt 2):1–23.