Prevalence of Dysplasia in Juvenile-Onset Recurrent Respiratory Papillomatosis

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Objectives: To quantify the prevalence of dysplasia and to evaluate the impact of use of cidofovir in recurrent respiratory papilloma biopsy specimens obtained from a pediatric population.


Setting: Children’s Hospital of Wisconsin.

Patients: Patients with a history of operation treated for recurrent respiratory papillomatosis.

Main Outcome Measures: The presence or absence of dysplasia identified in a papilloma specimen and patient characteristics, such as age of initial presentation, number of operations, tobacco exposure, treatment for reflux, and use of cidofovir, were quantified.

Results: Treatment for recurrent respiratory papillomatosis was identified in 21 patients. Age at initial diagnosis ranged from 8 months to 14 years. A total of 123 recurrent respiratory papillomatosis specimens in 20 patients were identified. Dysplasia was seen in less than 1% of samples (1/123), which represents 5% of total patients. Seven patients (35%) received cidofovir treatment and none of them developed dysplasia. These data demonstrate a lack of correlation between cidofovir treatment and dysplasia, with the P value being nonsignificant (Fisher exact test, P=.4).

Conclusion: Dysplasia is a rare event in pediatric recurrent respiratory papillomatosis, and there does not appear to be an association between the use of cidofovir and dysplastic changes.


Juvenile-onset recurrent respiratory papillomatosis (RRP) is generally characterized as an unpredictable, benign, yet debilitating disease of the aerodigestive mucosa caused by the human papillomavirus (HPV). The papillomata cause dysfunction and obstruction of the larynx (Figure 1) and trachea, which leads to significant morbidity that affects approximately 4.3 per 100 000 infants and children in the United States. In addition to surgical debulking, several adjunctive medicines are used to control juvenile-onset RRP. Cidofovir (Vistide; Gilead Sciences, Forest City, California) is an injectable antiviral agent that has gained popularity for use in treatment of juvenile-onset RRP. The medication is a cytosine nucleotide analog indicated for the treatment of cytomegalovirus-associated retinitis in patients with AIDS. Cidofovir is also known to have activity against HPV, varicella, herpes simplex, Epstein-Barr, and hepatitis B viruses. Its mechanism of action is believed to involve inhibition of cytomegalovirus DNA polymerase. However, the mechanism of action that contributes to broader antiviral activity of this drug has not been well elucidated.

Although the literature shows promise for the use of cidofovir in juvenile-onset RRP, questions remain about its potential carcinogenic adverse effects. In a case report by Wemer et al, progressive dysplasia was seen in a 28-year-old with respiratory papillomatosis treated with cidofovir. Histologic test results from this patient showed no dysplasia when only surgical debulking was performed; however, once the administration of cidofovir began, the patient developed severe disease. As highlighted in this report, the question of dysplastic transformation with the introduction of cidofovir remains relevant.

In adults, RRP is believed to become malignant in 2% to 4% of cases; however, malignant transformation in the pediatric population has not been well characterized. The incidence of dysplasia in juvenile-onset RRP has only been recently described, and therefore the nature of premalignant change remains in question. The purpose of this study is to investigate the prevalence of dysplasia in a group of pediatric patients with RRP and investigate any association with the use of cidofovir.
of cidofovir. It is hypothesized that dysplastic and neoplastic transformation of juvenile-onset RRP is low with and without cidofovir treatment.

A retrospective review of medical records and histopathologic test results during the 10-year period from January 1, 1998, through December 31, 2008, was performed. This activity was approved by the institutional review boards of the Medical College of Wisconsin and the Children’s Hospital of Wisconsin. All patients with a diagnosis of RRP who underwent surgical treatment were identified from a clinical database at the Children’s Hospital of Wisconsin. Those who had pathologic specimens available were included in this study. Hospital medical records were reviewed. Demographic and clinical data, such as age at initial presentation, diagnosis, biopsy, and total number of operations, were quantified. Risk factors for dysplasia, such as exposure to tobacco, and symptoms or any treatment for laryngopharyngeal reflux were also recorded. Adjuvant treatment, including the use of cidofovir, was documented.

Histopathologic evaluation was performed on hematoxylin-eosin-stained sections with a microscope (BX51; Olympus Imaging Corporation, Tokyo, Japan) in a masked fashion by a board-certified pathologist. A subset of specimens was additionally reviewed by 2 secondary board-certified pathologists (V.O. and S.S.S.) for internal validity. Features evaluated included extent of mitotic activity, level of mitotic figures in relation to epithelial height, (absence of) abnormal mitotic figures, degree and extent of basal cell hyperplasia, disorderly maturation, presence or absence of maturation on surface, nuclear abnormalities (increased nuclear-cytoplasmic ratio, hyperchromasia, pleomorphism, and altered polarity), extent of classic koilocytic cytoatypia, further features associated with HPV effect (extent of binucleation or multinucleation and dyskeratosis), and features of metaplasia (residual respiratory epithelial features in portions of squamous papilloma).

Dysplasia was defined as an absence of maturation above the basal cell layer. It is present when the cells above the basal cell layer continue to have a high nuclear-cytoplasmic ratio and lack nuclear polarization. The presence of mitoses outside the basal cell epithelium was also considered a feature of dysplasia and ascertained. When these changes are present within the lower one-third of the epithelium, dysplasia was rated as low grade. Moderate-grade dysplasia is present when these changes are present within two-thirds of the epithelium. High-grade dysplasia was defined as an absence of maturation within the entire thickness of the epithelium.

Our hypotheses were tested with a $\chi^2$ or Fisher exact test. $P<.05$ was considered statistically significant to reject the null hypothesis.

Treatment for RRP was identified in 21 patients. Age at initial diagnosis ranged from 8 months to 14 years. A total of 362 operations were performed in this cohort; however, many of these did not have a pathologic specimen submitted. One patient did not have any pathologic specimens and therefore was left out of further data evaluation. The mean (SD) age at presentation was 3.5 (3.6) years. The mean (SD) age at biopsy was 3.6 (3.7) years, with a median of 2 and a mode of 1, which demonstrates a sample set skewed toward infancy. A total of 123 specimens from patients with RRP were identified. The mean (SD) number of specimens per child was 6.2 (8.8); however, the median was 2 per child because 4 of our patients had 10 or more samples during the study period. The distribution of patient age at the time of the last biopsy and those with more than 10 specimens is displayed in Figure 2.

Dysplasia was seen in less than 1% of samples (1/123), which represents 5% of the total patients. Figure 3 and Figure 4 are examples of papilloma histopathologic findings. The first masked re-review of specimens showed complete correlation to the original pathology report. The second pathologic review for internal validity showed complete agreement as well.

The 1 patient with dysplasia had a total of 10 specimens during the study period. Only 1 of these demonstrated dysplasia, which was graded as focal moderate. The biopsy specimen with dysplasia was identified when the patient was 18 years old. Two subsequent biopsy specimens obtained on this patient within 2 years showed no evidence of significant dysplasia.

No specimens showed severe dysplasia or carcinoma in situ. Multiple specimens from 1 patient exhibited features of metaplasia, which included occasional residual goblet cells in squamous epithelium typically in a back-
ground of reactive epithelial features; only a few additional specimens from other patients showed similar features focally. Five specimens were either small or partly limited by laser cautery artifact, but all showed at least koilocytic changes.

In patients with multiple biopsy specimens, which typically clustered at young ages, the constellation of histologic features tended to remain similar over time, often with initial high mitotic activity, typically restricted to the basal third of the epithelium. Rare mitoses were seen up to half of the epithelial height in 23% of specimens but without associated worrisome features. Although in some patients koilocytic features were well developed and extensive in early biopsy specimens, in others these became accentuated at later times. Subtle variations in the degree and/or extent of individual evaluated parameters, within the realm expected for HPV effect, were not infrequent in successive biopsy specimens. However, no consistent pattern could be deciphered.

Seven patients (33%) received cidofovir treatment during the study. Dosage ranged from 0.2 to 3 mL of cidofovir solution injected percutaneously and intralesionally at the time of operative management. The concentration of the solution ranged from 5 to 10 mg/mL, with most patients receiving a solution of 7.5 mg/mL. One patient received 55 injections during the study period. Patients who received cidofovir tended to have greater disease burden (as defined by the volume of papilloma and frequency of return to the operating room) than those who did not. Those who received cidofovir had an operative frequency of approximately 3 to 15 times per year, whereas those who did not receive cidofovir had frequencies of approximately 1 to 5 times per year. No patient who had been treated with cidofovir developed dysplasia. These data demonstrate a lack of correlation between cidofovir treatment and dysplasia, with the $P$ value being non-significant (Fisher exact test, $P=.4$).

With respect to other adjuvant therapy, 2 patients (10%) received the mumps vaccine and 1 (5%) received photodynamic therapy. The single dysplastic specimen came from the patient who had undergone photodynamic therapy.

Three patients (15%) were treated for laryngopharyngeal reflux with either a histamine2-blocker or a proton pump inhibitor. Two patients (10%) had secondhand tobacco exposure and none of these had dysplasia. Neither reflux nor tobacco exposure was associated with dysplasia ($\chi^2$ test, $P=.025$); however, the power of this statistic is admittedly weak.
Tissue infection with HPV is well known to be associated with epithelial proliferation, atypia, dysplasia, and neoplastic transformation. This has been most classically characterized in dysplastic transformation of the uterine cervix. Both HPV-16 and HPV-18 are the primary high-risk subtypes for malignant transformation, and HPV-6 and HPV-11 are the low-risk HPV subtypes. Most laryngeal papilloma cases occur because of infection with subtypes 6 and 11, and therefore these cases are typically considered low risk for malignancy potential. Our study demonstrates that dysplasia in juvenile-onset RRP, a known HPV infection, is rare and confirms data published recently by Lindsay et al. The authors had evaluated 95 specimens from 17 pediatric patients, 7 of whom had received cidofovir adjuvant treatment and showed no dysplasia in their specimens. Our group recently reported a review of adult-onset RRP biopsy specimens. Across 2 institutions, we found dysplasia in 13% of specimens and 22% of patients. We found no correlate to dysplasia and exposure to potential mutagenic agents, including cigarette smoking and cidofovir, in this adult study. In another review of RRP and dysplasia, Johnson et al noted a dysplasia rate of 55%. These studies imply that dysplasia is a much more frequent finding in adult patients. Why then is juvenile-onset RRP different?

Adult-onset RRP is thought to develop either after reactivation of a latent infection or after a newly acquired infection. Juvenile-onset RRP is considered to occur after HPV exposure at birth, with maternal-to-fetal transmission that occurs from direct contact of the infant to the birth canal in mothers with condylomata. This concept of vertical transmission via direct contact during the birth process, however, has been debated in the literature because there have also been cases of juvenile-onset RRP occurring in those who have been delivered by cesarean section. It has been hypothesized that inoculation alone with HPV may not be enough to cause the clinical disease of RRP. An underlying immunodeficiency has long been postulated. In many children, the disease subsides with maturity, and many adults with the disease have no prior history of childhood illness. Does this gradual maturity of the immune system clear the virus? Unfortunately, no long-term studies of biopsy evaluation have been performed in those in whom clinical disease has cleared, and it remains unknown whether the virus is eradicated or whether some type of immune tolerance develops. A long-term study of patients with juvenile-onset RRP with tissue analysis after clinical resolution could provide answers to these questions, which may be especially important as we enter an era of the quadrivalent HPV vaccine, of which use in the treatment or prevention of RRP is being considered.

Figure 4. Focal moderate dysplasia, with disorderly maturation extending into the upper half of the epithelium and occasional dysplastic nuclei, still with maturation at the surface. A and B, Hematoxylin-eosin stains (original magnification, ×400). The double arrows indicate the basal layer, and there are rare mitoses close to the surface. A, The single arrow points to a mitotic figure relatively close to the surface. B, The asterisk indicates nucleomegaly close to the surface.
Another difference between pediatric and adult populations is their exposure and longer time of exposure to potential carcinogens known to be associated with head and neck squamous cell carcinoma and dysplasia. The patients in this current study had a median age of 2 years, which precludes any prolonged exposures. In addition, only 2 patients in this cohort had any documentation of exposure to secondhand smoke. In the previously reported assessment of adult patients, there existed a higher incidence of tobacco exposure for longer periods; however, this cohort also did not exhibit a correlation between tobacco exposure or advanced age and prevalence of dysplasia.

Cidofovir has shown promise in the treatment of juvenile-onset RRP and is part of the arsenal of adjuvant therapy for this disease. There has been ongoing concern since the initial use of this medicine with respect to its own carcinogenic potential. Cidofovir was used in approximately one-third of the patients, and none of them developed dysplasia during the study period. There is a potential error in the conclusion of the articles by Wemer et al and others that suggests a causative effect between the cidofovir treatments and subsequently observed dysplastic or neoplastic changes. Although this concept is important to highlight to the readership and practicing community, conclusions about cause and effect can be difficult to make on the basis of case reports. Furthermore, data from this and other recent studies show that dysplastic transformation is rare in RRP. A simpler conclusion may be that papilloma followed its natural history over time and developed these changes independently of external factors. Surgically, it remains a challenge to remove 100% of the affected laryngeal tissue, and in many cases, it simply may not even be the goal of the operation (ie, vocal vs airway restoration). If all grossly identified papillomas are removed during direct laryngoscopy, the assumption is that lesions recur at the periphery of the infected but grossly uninvolved mucosa or in grossly flat or nonpapillomatous but affected mucosa. If debulking is incomplete and a portion of the lesion is allowed to mature over years, it may seemingly progress rapidly and become dysplastic.

The large number of masked reviewed pathologic specimens is a major strength of this article; however, it suffers from the typical deficiencies afforded to retrospective reviews, such as that of weakened statistical extrapolation toward the sampled population. No consistent data were obtained about HPV subtyping, and therefore any link between HPV subtype and dysplasia in juvenile-onset RRP cannot be made. In our pediatric practice, pathologic specimens are obtained at initial presentation and then sporadically, such as with a change in clinical presentation or papilloma burden. Only approximately 40% of the cases across the study period had any pathologic results to review, which possibly highlights a missed chance to obtain more data. In addition, our study population was skewed toward a young group, with a median age of 2 years. Perhaps if we continue to follow up some of these patients into adulthood, some would develop dysplasia. We anticipate a future study to evaluate these phenomena. The identification of factors associated with premalignant transformation in RRP warrants further investigation.

In conclusion, juvenile-onset RRP is rarely associated with laryngeal epithelial dysplasia. Use of cidofovir does not seem to induce dysplastic changes in the HPV-infected laryngeal epithelium.

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REFERENCES