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Squamous Cell Papillomatosis in the Setting of Recurrent Respiratory Papillomatosis

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Abstract
A 23 year old male presented to the Otolaryngology clinic with 6 months of hoarseness and poor voice projection without improvement from speech therapy or medical anti-reflux medication. Upon examination he was found to have multiple polypoid lesions emanating from bilateral false vocal folds, left true vocal fold, and the anterior commissure. Biopsy and potassium titanyl phosphate (KTP) laser ablation with bevacizumab injection provided treatment and confirmed the clinical suspicion of squamous cell papilloma. Despite 3 years of treatment, the papillomatosis proved difficult to control, requiring a procedure approximately every 3 months. In an attempt to control the course of the disease the patient received a series of three bevacizumab and three cidofovir injections. Serial biopsies showed mild atypia within the squamous cell papillomas. Two separate biopsies confirmed presence of human papillomavirus (HPV) 6/11 via in situ hybridization with appropriate controls. There is promising research that the quadrivalent HPV (types 6, 11, 16, and 18) vaccine both reduces the disease burden in patients with active disease and reduces the incidence of recurrent respiratory papillomatosis (RRP). Other studies have shown that local immunologic dysregulation may play a role in RRP pathogenesis. Therefore new treatment options, to include PDL-1 blockade, offer hope in treating this benign condition with high morbidity and rare mortality.

Keywords Recurrent respiratory papillomatosis · Squamous cell papilloma · Squamous cell papillomatosis · HPV · Immunologic dysregulation · PDL-1 · RRP

Clinical Features

A 23 year old male presented to the Otolaryngology clinic with the chief complaint of hoarseness and poor voice projection for 6 months. He reported the onset was sudden and began after a weekend of vocal abuse and overuse. Initial laryngoscopic exam showed significant false vocal cord (FVC) and interarytenoid edema with a polypoid mass on left true vocal cord (TVC) near the anterior commissure. At the patient's 3 month follow up appointment he had persistent hoarseness without improvement from speech therapy or medical anti-reflux medication. Examination of the larynx revealed lesions that emanated from bilateral FVCs, left TVC and the anterior commissure. The lesions were exophytic, and polypoid with a pink to red, finely lobulated surface (Fig. 1). The laryngoscopic exam was consistent with recurrent respiratory papillomatosis (RRP). Microscopic examination of the submitted tissue resulted in the diagnosis of multiple squamous cell papillomas (see histologic findings below), confirming the clinical suspicion of RRP. The patient underwent greater than ten procedures to remove and debulk the papillomas. However, the disease continues to persist and is considered aggressive per the criteria described by Omland et al. [1]. The areas that continued to be affected include bilateral FVCs and TVCs, right arytenoid, and the anterior commissure. His treatment included potassium titanyl phosphate (KTP) laser ablation and surgical debulking with a microdebrider. A series of three bevacizumab and three cidofovir injections were also

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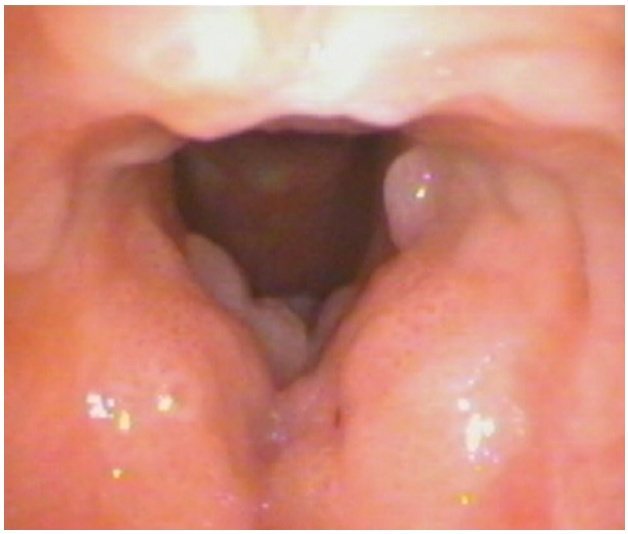

Fig. 1 In-office examination using flexible fiberoptic laryngoscopy

administered. Finally, he was given the Gardasil (quadrivalent human papillomavirus types 6, 11, 16, and 18) vaccine as well as celecoxib in hopes of improvement [2]. The patient continues to suffer with voice weakness and hoarseness. However, since receiving the vaccine and the celecoxib the progression has slowed. He never developed obstructive airway symptoms. Clinically this patient is immunocompetent (without acquired or congenital immunodeficiency) who had normal complete blood count and differential.

Histologic Findings

Six laryngeal specimens were submitted over the course of 2 years. All specimens were diagnosed as squamous cell papillomas and an increase in atypia was noted over the serial biopsies. Microscopic review of each specimen revealed a proliferation of keratinized stratified squamous epithelium overlying fibrovascular connective tissue cores (Fig. 2). Most tissue samples showed a normal maturation pattern. Lesions submitted later in the 2 year time period showed increasing atypia in the form of basilar hyperplasia, hyperchromatic nuclei, irregularity of the basal cell layer and focal mitotic activity (Fig. 3). Koilocytes, virus-altered cells, were observed throughout the epithelium. A human papillomavirus (HPV) typing panel was performed twice, both via in situ hybridization (ISH) with appropriate controls. The initial specimen showed only rare positive staining for HPV 6/11 and was negative for HPV 16/18 and 31/33 (Fig. 4). A subsequent specimen was also evaluated for HPV with similar results. Both specimens were non-reactive for p16 by immunohistochemistry (IHC). The testing was done

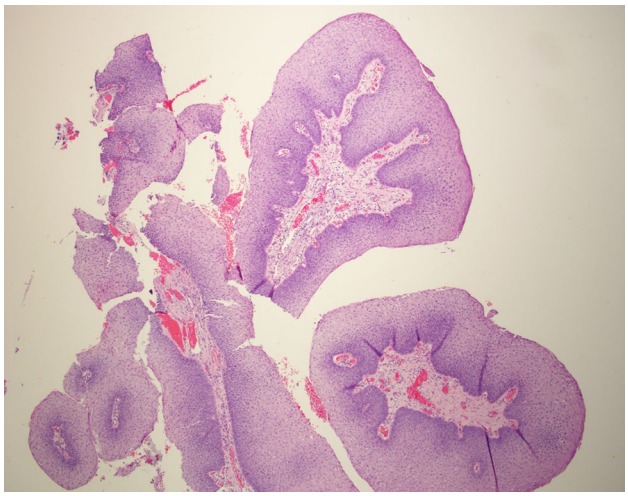

Fig. 2 Histology revealed proliferative well differentiated squamous epithelium overlying fibrovascular cores

for academic interest and is not recommended for routine evaluation of RRP.

Discussion

Squamous cell papillomas of the larynx are the most common benign epithelial tumor of the larynx [3]. Clinically multiple contiguous squamous cell papillomas that are locally recurrent are referred to as recurrent respiratory papillomatosis (RRP). Over 100 HPV types have been identified in humans, but the vast majority (~90%) of RRP and solitary papillomas are associated with HPV genotypes 6 and 11 [4, 5]. Rarely co-infection with HPV 6 and 11 has been identified and even less common are other HPV types

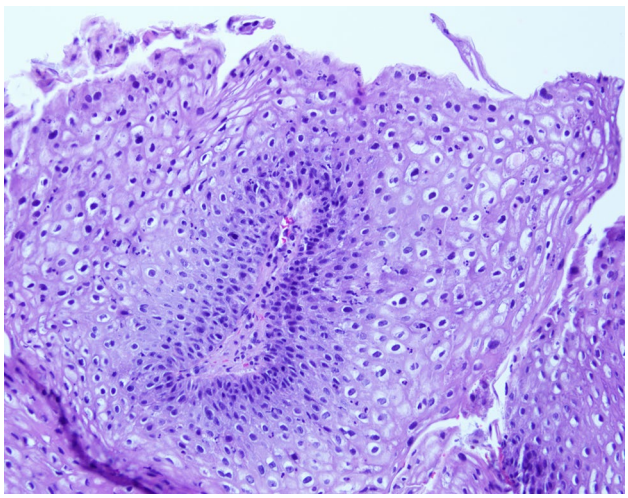

Fig. 3 Koilocytes, virus-altered cells, were observed throughout the epithelium

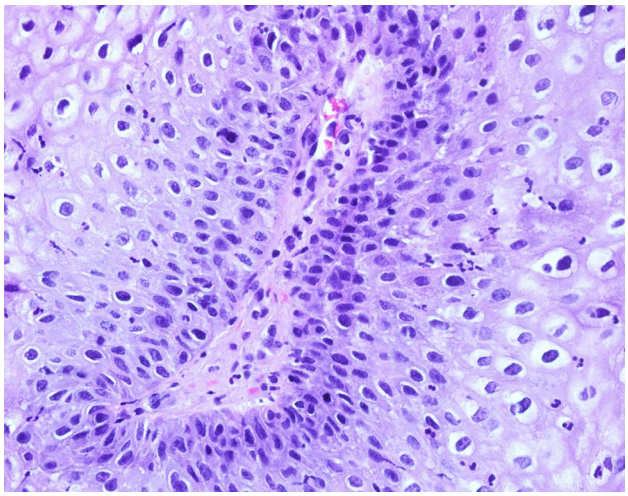

Fig. 4 Atypia seen in the form of basal and parabasal cell hyperplasia, hyperchromatic nuclei, irregularity of the basal cell layer and increased mitotic activity

(16, 31, 33, 35 and 39) [5]. Several studies indicate that HPV 11, regardless the age at diagnosis, has a more aggressive clinical course that may require multiple surgeries [1, 5]. Though RRP of the larynx is a benign disease, it has high morbidity due to issues of vocalization, swallowing and breathing resulting in multiple surgical procedures. Airway obstruction, while rare, is a cause of mortality.

Because of the bimodal age distribution it has been a matter of clinical practice to further categorize the disease process by age. The juvenile onset recurrent respiratory papillomatosis (JORRP) is diagnosed before age 12 while adult onset recurrent respiratory papillomatosis (AORRP) is diagnosed after age 12, though age cutoff varies among institutions. While the mode of transmission for adults is uncertain, it appears to be associated with sexual contact or reactivation of pediatric infection [6]. Moreover, this age determined classification may be important as younger patients are more likely to have aggressive disease. In children most infections can be traced back to maternal vertical transmission during vaginal delivery. Data has shown that women giving birth with active infection are at over a 200 fold increase risk of having RRP in their child [7]. It has long been established that HPV-11 and diagnosis at a young age (specifically 3 years old or younger) are more likely to develop aggressive disease [5]. Karatayli-Ozgursoy et al. recently looked at which patients are at increased risk for dysplasia transformation. Data suggests, for both the adult and pediatric populations, that the disease onset at a young age is the strongest prediction of dysplasia [8].

Papillomatous lesions occur more often in sites where squamous epithelium abuts columnar or ciliated epithelium, so called "transformation zones." Thus, true and false vocal cords, epiglottis, subglottic area hypopharynx and nasopharynx are preferential sites for these lesions in the respiratory tract, and the larynx is the most common site [3]. When viewed grossly and laryngoscopically, the papillomas are typically pink with a lobulated surface and may appear exophytic with pedunculated or sessile growth, commonly in multiple clusters. Microscopically the lesions display papillary mucosal projections of abundant squamous epithelium overlying a fibrovascular core, often with basal and parabasal cell hyperplasia. A distinguishing feature of HPV infection is koilocytosis most often near the surface of squamous epithelium. This is characterized by dark, large, irregular nuclei surrounded by cleared out cytoplasm. Rarely, specimens may exhibit atypia or dysplasia characterized by increased nuclear:cytoplasmic ratio, nuclear hyperchromasia and increased mitotic activity. RRP is by definition an HPV associated process, therefore HPV testing of routine samples is of little practical use. However, some providers will request such testing and testing is of some academic interest to the pathologist as well. HPV detection in squamous papillomas is accomplished by in situ hybridization (ISH) and polymerase chain reaction (PCR). ISH enables viral DNA detection and even genotyping while preserving tissue morphology as it is carried out on formalin fixed, paraffin embedded tissue. PCR is the most sensitive test but specificity is limited by laboratory contamination or infection with virus particles, cloned HPV plasmids, and PCR products themselves [9].

The standard treatment for RRP involves use of various lasers (CO_2 and KTP are common) for ablative treatment versus microdebrider or cold knife excision. Adjuvant therapy may include bevacizumab, interferon, indol-3-carbinol, and cidofovir [10]. With the advent and use of the quadrivalent HPV vaccine (types 6, 11, 16, and 18) there is evidence for improved long-term outcomes and decreasing incidence of RRP. An Australian study concluded that the quadrivalent HPV vaccine decreased in the incidence of JORRP [11] with an expected similar reduction in AORRP. Villa et al. have shown that after the Gardasil vaccination those with baseline antibodies to the HPV virus had a 12 to 26 fold increase of antibodies than those observed in baseline-naïve group [12]. Two other studies followed a total of 21 men with HPV positive RRP who received combination therapy of resection and the Gardasil vaccine series. At six and seven months follow up after the initial vaccine there was a dramatic increase of HPV antibodies [13, 14]. The authors believe that as the pre-vaccine antibody levels were mostly undetectable therefore the natural antibody production capacity was too low to affect the course of the disease immunologically [13]. Clinically there was significantly less recurrences and a decrease in surgical rates after receiving the vaccine [14]. Gi et al. concluded that the quadrivalent HPV vaccine could help in the treatment of RRP as patients showed a robust

immunological response regardless of age, age of onset or HPV type [14].

There is mounting evidence that an immunosuppressive microenvironment, generated by persistent HPV infection, imparts some of the long term recurrence and the lack of improvement in RRP patients. Several studies of RRP patients found that the peripheral blood mononuclear cells behaved normally (compared to controls) but within the milieu of the HPV infection there was immune dysregulation, namely enrichment of regulatory T-cells, histiocytic defects, natural killer T-cell dysfunction as well as skewing of the T helper cells to the T_H2 phenotype [15, 16]. Lui et al. completed a study of six JORRP patients and found in a subset of patients showed increased expression of PD-L1 in tumor cells and inflammatory cells as well as increased CD8+ infiltrating lymphocytes. This highlights the potential benefit of tumor immunotherapy [17]. Similarly, Ahn et al. found that RRP specimens compared to normal controls had significantly increased PD-L1 staining with 68% of the epithelium and 76% of the infiltrating immune cells. They concluded that this checkpoint pathway could be contributing to the local immunosuppression and also saw a possible role for the use of PD-blocking treatment [16]. These new possibilities of medical treatment via immune therapy and vaccines bring hope to improve outcomes in this benign disease with high morbidity and rare mortality.

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