Human Papillomavirus in Recurrent Respiratory Papilloma, Sinonasal Inverted Papilloma and Non-Malignant Tonsils

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Akademisk avhandling

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Avhandlingen kommer att förvaras på engelska.

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Abstract

Background: Human papillomavirus (HPV) is known to cause recurrent respiratory papilloma (RRP) and certain types of oropharyngeal cancer. HPV has also been associated with sinonasal inverted papilloma (SIP). HPV transmission routes are under investigation and the conviction is that the infection occurs sexually at an adult stage, however, vertical transmission at birth with a dormant viral condition until disease eruption/co-activation has been stated as a possibility.

Purpose: The purpose of this work was to contribute to the understanding of HPV related chronic diseases in the airway. Specific aims were: 1. To increase understanding regarding changes in the immune system as well as of the glycosaminoglycan hyaluronan in patients with RRP. 2. To evaluate prevalence of HPV and its surrogate marker p16 in SIP as well as HPV, p16 and Epstein-Barr virus (EBV) in benign tonsillar disease. HPV and EBV in non-malignant tonsillar disease were studied due to the fact that incidence of HPV positive tonsillar cancer is increasing and the time of viral infection is unknown.

Methods: A phenotypic characterization of peripheral blood from 16 RRP patients and 12 age-matched controls, using immunoflow cytometry, and monoclonal antibodies against differentiation and activation markers, was performed. The cytokine mRNA profile of monocytes, T helper-, T cytotoxic-, and NK cells was assessed using RT-qPCR. 54 SIP samples were studied of which 53 were available for analyzation with PCR. Genotype screening for 18 high risk and six low risk HPV types was performed using the PapilloCheck® HPV-screening test (a PCR method). 54 samples were immunohistochemically (IHC) stained for p16. Biopsies from vocal folds (VFs) and false vocal folds (FVFs) were collected from 24 patients with RRP, 12 were randomly selected to histochemistry for Hyaluronan (HA) and IHC staining for CD44 in the epithelium, stroma and RRP lesions. The remaining 12 patients were analyzed for HA molecular mass distribution with a gas-phase electrophoretic molecular mobility analyzer (GEMMA). Eight VF samples and four FVF samples were successfully analyzed. Biopsies from 40 non-malignant tonsils were analyzed using Papillocheck® for HPV, IHC for p16 and EBER analysis for EBV.

Results: We found a dominance of cytotoxic T cells, activated NK cells, and high numbers of stressed MIC A/B (MHC class 1 chain-related molecule A/B) expressing lymphocytes. The HPV analysis was successful for 38 SIP samples and two (5%) were positive for HPV 11. Notably, p16 was present in the epithelia of all samples and in the papilloma portions in 37 of 38 samples. We found extensive HA staining in the stroma of both VFs and FVFs. CD44 was expressed throughout the epithelium, stroma, and RRP lesions in both FVFs and VFs, it did however, not concur with the expression of HA. Very high mass HA was found in both VFs and FVFs, though more variation regarding amounts of HA was seen in the VFs compared to FVFs. No HPV was found in non-malignant tonsils, the p16 levels were low and the counted EBER positive cells showed great variation in numbers.

Conclusions: Our findings demonstrate an immune dysregulation with inverted CD4+/CD8+ ratio and aberrant cytokine mRNA production in RRP patients, compared to healthy controls. We concluded that p16 cannot be used as a surrogate marker for high-risk HPV-infection in SIP and that HPV incidence was low (5%). CD44 does not seem to bind to HA, which might explain the non-inflammatory response previously described in RRP. Very high mass HA possibly crosslinked was seen in both VFs and FVFs. A possibility to counteract inflammatory crosslinking of HA may be found for medical treatment options in RRP.

Keywords

Human papillomavirus, recurrent respiratory papillomatosis, sinonasal inverted papilloma, non-malignant tonsillar disease, Epstein-Barr virus, immune system, p16, Hyaluronan