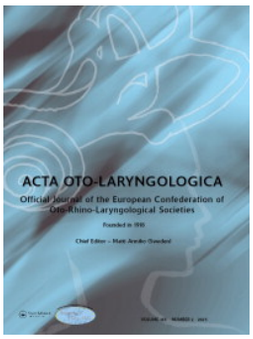

Acta Oto-Laryngologica

ISSN: 0001-6489 (Print) 1651-2251 (Online) Journal homepage: www.tandfonline.com/journals/ioto20

Cross-evaluating Epstein-Barr virus, human papilloma virus, human cytomegalovirus and human adenovirus in nasal polyps and turbinate mucosa

Alexandra Schindele, Anna Holm, Sofie Kraft, Karin Nylander, Annika Allard & Katarina Olofsson

To cite this article: Alexandra Schindele, Anna Holm, Sofie Kraft, Karin Nylander, Annika Allard & Katarina Olofsson (2025) Cross-evaluating Epstein-Barr virus, human papilloma virus, human cytomegalovirus and human adenovirus in nasal polyps and turbinate mucosa, Acta Oto-Laryngologica, 145:2, 164-167, DOI: 10.1080/00016489.2024.2445025

To link to this article: https://doi.org/10.1080/00016489.2024.2445025

ACTA OTO-LARYNGOLOGICA
2025, VOL. 145, NO. 2, 164–167
https://doi.org/10.1080/00016489.2024.2445025

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Cross-evaluating Epstein-Barr virus, human papilloma virus, human cytomegalovirus and human adenovirus in nasal polyps and turbinate mucosa

Alexandra Schindele[a], Anna Holm[a], Sofie Kraft[a], Karin Nylander[b], Annika Allard[c] and Katarina Olofsson[d,e]

[a]Department of Clinical Sciences, Otorhinolaryngology, Umeå University, Östersund, Sweden; [b]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden; [c]Department of Clinical Microbiology, Clinical Virology, Umeå University, Umeå, Sweden; [d]Department of Clinical Sciences, Otorhinolaryngology, Umeå University, Umeå, Sweden; [e]Department of Clinical Sciences, Otorhinolaryngology, Umeå University, Sunderbyn, Sweden

ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common disease in which inflammatory responses to exogenic stressors, such as viral infections, has been recognised. The role of viruses in CRSwNP pathogenesis is unclear.

Aims/objectives: We aimed to characterise Epstein-Barr virus (EBV), human papillomavirus (HPV), human cytomegalovirus (HCMV), and human adenovirus (HAdV) in nasal polyps and adjacent paired healthy turbinate mucosa.

Materials and methods: We used real-time PCR for EBV, HCMV, and HAdV DNA detection, combined PCR/microarrays for HPV detection and genotyping, in samples from 45 patients with CRSwNP. Additionally, we used EBER in situ hybridisation for EBV detection.

Results: EBV detection with EBER-ISH was significantly higher in polyps (36%) versus turbinate mucosa (12%). None of the viral comparisons with PCR between polyps and turbinate mucosa for EBV-, HCMV- or HAdV-DNA showed statistically significant differences. All samples were HPV negative.

Conclusions and significance: We report higher expression of EBV in nasal polyps (36%) than in adjacent healthy turbinate mucosa (12%), using a valid method; EBER-ISH in 45 patients with CRSwNP. EBV might be a possible stressor that can trigger polypoid inflammation.

ARTICLE HISTORY
Received 11 November 2024
Revised 16 December 2024
Accepted 16 December 2024

KEYWORDS
Chronic rhinosinusitis with nasal polyps; Epstein-Barr virus; EBER-ISH; nasal mucosa; HPV; HCMV; HAdV

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common, complex inflammatory disease associated with significant morbidity and burden regarding healthcare consumption and work productivity loss [1]. In the Scandinavian population, the incidence of CRSwNP was 63 cases per 100 000 and year in 2002 [2]. The prevalence was 2.7% in general, and 5% in the elderly ≥ 60 years. Globally, the prevalence of chronic rhinosinusitis (CRS) with or without polyps has been reported to 5–12% of the general population [1]. The definition of CRS is presence of two or more symptoms for ≥ 12 weeks, one of which should be either nasal blockage/obstruction/congestion or nasal discharge, and with or without facial pain/pressure and reduction or loss of smell [1]. Patients with CRSwNP are characterised by a type 2 immune signature and high tissue eosinophil load.

Microbial agents are considered important environmental drivers of inflammation in CRS. Epstein-Barr virus (EBV), human papillomavirus (HPV), human cytomegalovirus (HCMV), and human adenovirus (HAdV) are typical upper airway viruses and can establish persistent infections [3–6]. EBV and HPV are also associated with upper airway cancer in humans [7], and inverted papilloma in the sinonasal cavities [8]. Studies have found EBV in nasal polyps in 35%–85% [9,10], and HPV in 13%–40% [9,11].

The inflammatory response to exogenic stressors, such as viral infection, has been postulated as a part of the complex host-environmental interaction [1,9]. Since presence of viruses in CRSwNP is not well described, our aim was to map EBV, HPV, HCMV, HAdV, in nasal polyps and adjacent healthy turbinate mucosa. Our hypothesis was that viruses are expressed in nasal polyps, in contrast to adjacent turbinate mucosa.

CONTACT Alexandra Schindele alexandra.schindele@umu.se Department of Clinical Sciences, Otorhinolaryngology, Umeå University, ÖNH-kliniken, Östersunds Sjukhus, 831 83 Östersund, Sweden

Supplemental data for this article can be accessed online at https://doi.org/10.1080/00016489.2024.2445025.

Materials and methods

Materials

This is a prospective, descriptive, cross-sectional study approved by the Regional Ethical Review Board of Umeå University (2017-277-31 M), and the Biobank North (472-13-008). We are reporting as recommended by the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist. After obtaining informed consent, biopsies from 45 consecutive patients with CRSwNP scheduled for endoscopic sinus surgery were collected during the operation between February 2018 and May 2019. Diagnostic-, and control biopsies were taken from polyp tissue in the nasal cavity and nearby macroscopically healthy mucosa from the inferior turbinate in paired samples. Study participant characteristics are presented in Table S1 (Supplemental Online Material).

Methods

We used real-time PCR for EBV, HCMV and HAdV DNA detection, combined PCR/microarrays (PapilloCheck®, Greiner BioOneGmbH, Frickenhausen, Germany) for HPV detection and genotyping in biopsies. Additionally, we used EBER *in situ* hybridisation (EBER-ISH) for EBV detection.

Each biopsy was cut in five 10-µm sections for DNA preparation, followed by extraction using the QIAamp DNA FFPE Tissue Kit (QIAgen, Hilden, Germany). DNA quality and quantity was measured with spectrophotometry. A real-time PCR assay of the *Bam*HI-W region of the EBV genome was used for EBV detection. HCMV analysis was obtained by real-time PCR towards the polymerase gene of HCMV. Detection of HAdV was performed as described by Hernroth et al. [12], with qualitative real-time PCR. The microarray PapilloCheck® was used for detection and genotyping of 24 HPV-types. It is a multiplex PCR method with fluorescent primers and requires a DNA fragment of 350 bp for successful performance.

EBV was analysed in biopsies by using EBER-ISH, an in-situ hybridisation kit, for mapping of EBER transcripts (Epstein-Barr Encoding Region). From each biopsy, two 5 µm sections were used. The first was incubated with an RNA-positive control probe (800-2846, Ventana), and the second tested for EBER-1 and EBER-2 with the Inform EBER Probe (800-2842, Ventana Medical Systems, Roche Diagnostics GmbH, Mannheim Germany). Visualisation was made by NBT- BCIP Detection System (800-092, Roche Diagnostics GmbH, Mannheim, Germany). For staining, a Ventana BenchMark ULTRA system (Roche Diagnostics AG, Switzerland) was used.

EBER-positive cells were counted in the tissue slides by three of the authors (AS, SK and KN). EBER was graded as positive when there were ≥ 1 positive cell per slide, and negative when there were no positive cells. Consensus was reached after comparison and re-examination of individual results.

Descriptive statistics (IBM SPSS USA, version 26) and cross tabulations were presented. Data were not normally distributed, non-parametric tests (McNemars test and Wilcoxon signed ranks test) were therefore used. A probability value of equal or less than 0.05 (2-sided) was considered statistically significant.

Results

EBV detection with PCR and EBER-ISH

EBV was assessed in two steps. First, we analysed EBV DNA presence or absence with PCR in turbinate mucosa and polyp tissue. In the second step, samples that were PCR positive for EBV DNA underwent EBER-ISH for microscopy of EBER-positive cells and comparison between turbinate mucosa and polyp tissue.

PCR for EBV DNA in turbinate mucosa showed that 32/41 (78%) were EBV positive. Four out of 32 (12%) samples were EBER positive, with one cell each visibly stained in the stroma (Table 1).

PCR for EBV DNA in polyp tissue revealed 34/42 (81%) EBV positive samples. The remaining 8 samples (19%) were EBV negative and excluded from EBER-ISH analysis. In addition, one sample was excluded for EBER count due to mucus only. Thus, 33 samples had preserved RNA and successful EBER-ISH. In 12/33 (36%) of the samples, one or more positive EBER cell was visible in the stroma at microscopy. There were <10 positive cells/sample, except for two cases with 20 and 90 cells, respectively (Table 1).

The number of EBER-positive cells was significantly higher in polyps versus turbinate mucosa ($p = 0.022$), which was not the case with analysis for EBV-DNA with PCR ($p = 1.000$).

HPV-, HCMV- and HAdV-DNA detection with PCR

HPV, HCMV and HAdV were analysed with PCR for DNA, and thereafter compared in turbinate mucosa and polyp tissue.

All patients were HPV-DNA negative in turbinate (41) and polyp tissue (42). HCMV-DNA was present in turbinate mucosa in 6/41 (15%) and in polyp tissue in 1/42 (2%) of samples ($p = 0.146$). HAdV-DNA was present in turbinate mucosa in 1/41 (2%) and in polyp tissue in 4/42 (10%) of samples ($p = 1.000$) (Figure 1).

Table 1. EBER-stained cells in nasal inferior turbinate mucosa and nasal polyp tissue samples frequencies.

No of EBER-stained cells	Nasal inferior turbinate mucosa (%)	Nasal polyp tissue (%)
0	28 (88)	21 (64)
1	4 (12)	7 (21)
2	0	1 (3)
6	0	1 (3)
7	0	1 (3)
20	0	1 (3)
90	0	1 (3)
Total	32 (100)	33 (100)
Median	0	0
Mean	0.13	4.09
Interquartile range	0–0	0–1

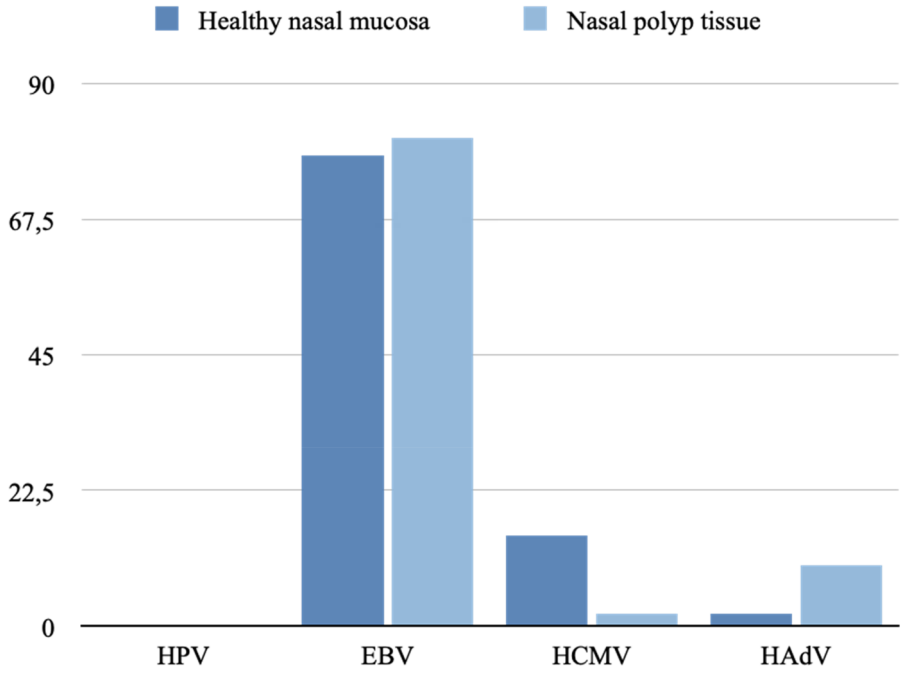

Figure 1. HPV-, EBV-, HCMV- and HAdV-DNA detection with PCR. Frequencies in nasal turbinate mucosa versus polyp tissue.

Discussion

The method EBER-ISH, in contrast to PCR, reveals EBV expression in a significantly higher degree (36%) in nasal polyps versus adjacent healthy turbinate mucosa (12%) in paired samples. No differences in expression of HCMV or HAdV were detected by PCR. HPV was not expressed in analysed samples.

PCR versus EBER-ISH for Epstein-Barr virus

Evidence of latent EBV infection with PCR has been presented in CRS mucosa in 42% of cases compared to controls [13]. Ioannidis et al. used quantitative real-time PCR and found EBV in nasal polyps (26.4%) compared to controls (10.5%) [14]. Zaravinos et al. detected EBV by PCR in 35% of nasal polyps, but not in control tissue from turbinates [9]. EBV prevalence in nasal mucosa and polyp tissue varies due to geographical sites, sampling and detection methods. We found a higher EBV expression in polyp versus turbinate mucosa using EBER-ISH but not when using real-time PCR, reflecting the sensitivity differences in methodology. EBER-ISH is considered the most reliable reference method for histopathological analysis of EBV in tissue biopsies [15]. The polyp samples had few EBER-stained cells (<10) each, except for two cases with 20 and 90 cells. Therefore, the results need to be interpreted with caution. However, EBV might be a possible stressor in the context of polypoid inflammation, due to the detection of latent EBV in polyp stroma.

Human cytomegalovirus (HCMV) and human adenovirus (HAdV)

HCMV and HAdV are usually not pathogenic in immunocompetent subjects. Data showed low expression of HCMV and HAdV in both polyp and turbinate mucosa.

Low prevalence of HCMV has been presented in previous studies, comparing nasal polyp to control tissue from turbinates in immunocompetent patients [9,14]. However, in immunocompromised patients, uncommon pathogens such as HCMV can manifest as rhinitis and sinusitis [5], and in HIV/AIDS patients, cases are reported with nasal polyps infected by HCMV in CRS [16].

HAdV and EBV was detected by DNA hybridisation technique in a pilot study in 9 nasal polyp patients compared to 6 controls, with negative results in both groups [17]. A Chinese study of nasal microbiota of antrochoanal polyps in paediatric patients versus healthy controls revealed a significantly higher HAdV and HCMV positivity in the control group than in the antrochoanal polyp group [18].

Absence of HPV

The prevalence of HPV in healthy nasal mucosa and polyps varies in earlier reports. HPV-DNA was absent in this cohort. Knör et al. reported HPV-DNA in 5.8% of nasal turbinate mucosa and 15.1% in nasal polyps, where a subgroup of antrochoanal polyps had a higher prevalence of 53.8%, predominantly HPV16 [19]. Oton-Gonzales et al. detected HPV16 in the antral part of the samples, but not in the nasal part. Viral DNA was presented at a low load and in episomal or integrated form [20]. The divergency in HPV outcome may be due to various detection methods. We used PapilloCheck®, considered a sensitive, robust and readily available method, routinely used in the clinical setting for detection of both high- and low risk HPV genotypes. However, it requires a DNA fragment of 350 bp for successful performance, missing out shorter or fragmented DNA copies, with the risk of false negative results.

Methodological considerations/limitations

It is likely that the distribution of viral particles and genetic material is heterogenous in polyps and variable based on sampling bias and region of tissue labelling. Therefore, it is possible that we could have missed certain rich regions of virus material in nasal polyps, which may explain the data being contradictory in EBER-stained tissue.

We analysed the samples that tested positive for EBV in PCR with EBER-ISH and is it possible that the tissue in which the majority of the EBV expression occurred was used for the PCR and not necessarily reflected in what was left for the EBER-ISH. Also, we did not try EBER-ISH in tissues which tested negative for EBV by PCR to confirm that the PCR was accurate in identifying EBV positive specimens. In future studies, it would be of interest to perform the PCR for EBV-DNA and EBER-ISH on all samples and compare the results, for better understanding EBV's role in CRSwNP.

We present data on viral presence analysed with PCR in a descriptive format, though a larger sample cohort is needed to have more confidence in identifying differences between groups.

We consider the prospective nature, the variety of viruses examined and the cross-evaluation of viruses in nasal polyps and turbinate mucosa tissue in the same patient at the same time, as positive factors and strengths of this study.

Conclusion

Data revealed a significantly higher expression of EBV in nasal polyps (36%) than in adjacent healthy nasal turbinate mucosa (12%), using EBER-ISH. Choice of method is important in future analysis if EBV might be a possible stressor that can trigger the polypoid inflammation, resulting in adult-onset of CRSwNP.

Acknowledgements

We would like to thank Anna Lindam, PhD, for statistical advice, Irene Eriksson for laboratory work and Professor Michael Haney for text revision.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This article was supported by Cancer Research Foundation in Northern Sweden; Region Jämtland Härjedalen; and Västerbotten Läns Landsting.

ORCID

Alexandra Schindele http://orcid.org/0000-0003-0007-8716
Anna Holm http://orcid.org/0000-0003-3522-1842
Karin Nylander http://orcid.org/0000-0002-4831-4100
Annika Allard http://orcid.org/0000-0001-6949-1213
Katarina Olofsson http://orcid.org/0000-0002-0649-3825

References

[1] Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on rhinosinusitis and nasal polyps 2020. Rhinology. 2020;58(1):1–1. doi: 10.4193/Rhin20.401.
[2] Larsen K, Tos M. The estimated incidence of symptomatic nasal polyps. Acta Otolaryngol. 2002;122(2):179–182. doi: 10.1080/00016480252814199.
[3] Taylor GS, Long HM, Brooks JM, et al. The immunology of Epstein-Barr virus-induced disease. Annu Rev Immunol. 2015;33(1):787–821. doi: 10.1146/annurev-immunol-032414-112326.
[4] Doorbar J. Latent papillomavirus infections and their regulation. Curr Opin Virol. 2013;3(4):416–421. doi: 10.1016/j.coviro.2013.06.003.
[5] Rombaux P, Bertrand B, Eloy P. Sinusitis in the immunocompromised host. Acta Otorhinolaryngol Belg. 1997;51(4):305–313.
[6] Usman N, Suarez M, Adenoviruses. StatPearls.Treasure Island (FL): StatPearls Publishing; 2020. Copyright © 2020, StatPearls Publishing LLC.; 2020.
[7] Guidry JT, Scott RS. The interaction between human papillomavirus and other viruses. Virus Res. 2017;231:139–147. doi: 10.1016/j.virusres.2016.11.002.
[8] Sham CL, To KF, Chan PK, et al. Prevalence of human papillomavirus, Epstein-Barr virus, p21, and p53 expression in sinonasal inverted papilloma, nasal polyp, and hypertrophied turbinate in Hong Kong patients. Head Neck. 2012;34(4):520–533. doi: 10.1002/hed.21772.
[9] Zaravinos A, Bizakis J, Spandidos DA. Prevalence of human papilloma virus and human herpes virus types 1-7 in human nasal polyposis. J Med Virol. 2009;81(9):1613–1619. doi: 10.1002/jmv.21534.
[10] Tao Q, Srivastava G, Dickens P, et al. Detection of Epstein-Barr virus-infected mucosal lymphocytes in nasal polyps. Am J Pathol. 1996;149(4):1111–1118.
[11] Pei F, Chen XP, Zhang Y, et al. Human papillomavirus infection in nasal polyps in a Chinese population. J Gen Virol. 2011;92(Pt 8):1795–1799. doi: 10.1099/vir.0.031955-0.
[12] Hernroth BE, Conden-Hansson AC, Rehnstam-Holm AS, et al. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the first Scandinavian report. Appl Environ Microbiol. 2002;68(9):4523–4533. doi: 10.1128/AEM.68.9.4523-4533.2002.
[13] Wood AJ, Antoszewska H, Fraser J, et al. Is chronic rhinosinusitis caused by persistent respiratory virus infection? Int Forum Allergy Rhinol. 2011;1(2):95–100. doi: 10.1002/alr.20030.
[14] Ioannidis D, Lachanas VA, Florou Z, et al. Herpes viruses and human papilloma virus in nasal polyposis and controls. Braz J Otorhinolaryngol. 2015;81(6):658–662. doi: 10.1016/j.bjorl.2015.08.010.
[15] AbuSalah MAH, Gan SH, Al-Hatamleh MAI, et al. Recent advances in diagnostic approaches for Epstein-Barr virus. Pathogens. 2020;9(3):226. doi: 10.3390/pathogens9030226.
[16] Yoskovitch A, Cantrell H. Cytomegalovirus infection presenting as chronic sinusitis and nasal polyposis: a case report. Ear Nose Throat J. 1998;77(1):35–38. doi: 10.1177/014556139807700108.
[17] Kozak FK, Mahony JB, Chernesky MA, et al. Nasal polyposis: in search of a viral etiology using DNA hybridization. J Otolaryngol. 1991;20(6):404–407.
[18] Song B, Zheng H, Han S, et al. Detection of nasal microbiota in pediatric patients with antrochoanal polyps by TLDA. Int J Pediatr Otorhinolaryngol. 2020;130:109811. doi: 10.1016/j.ijporl.2019.109811.
[19] Knör M, Tziridis K, Agaimy A, et al. Human papillomavirus (HPV) prevalence in nasal and antrochoanal polyps and association with clinical data. PLoS One. 2015;10(10):e0141722. doi: 10.1371/journal.pone.0141722.
[20] Oton-Gonzalez L, Rotondo JC, Cerritelli L, et al. Association between oncogenic human papillomavirus type 16 and Killian polyp. Infect Agent Cancer. 2021;16(1):3.