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Prevalence of Epstein–Barr virus in tonsils and adenoids of United Arab Emirates nationals

Suhail Al-Salam [a,*], Shamma Al Dhaheri [b], Aktham Awwad [b], Sayel Daoud [b], Ahmed Shams [b], Mouied Al Ashari [b]

[a] Pathology Department, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates
[b] Department of Laboratory Medicine, Tawam Hospital, Al Ain, United Arab Emirates

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ABSTRACT

Objective: Given that Epstein–Barr virus (EBV) often inhabits human tonsils and adenoids, it remains to be distinctively determined its prevalence and in which cell and microenvironment the virus is present.
Methods: To determine the prevalence of EBV in the tonsils and adenoids of the United Arab Emirates (UAE) nationals and to provide a basis for understanding the origin and biology of EBV-infected cells, the immunophenotype of all EBV-infected cells in 46 tonsils and 46 adenoids was determined by EBER in situ hybridization and immunohistochemistry with monoclonal antibodies to T cells (CD3), B cells (CD20), and epithelial cells (cytokeratin AE1/AE3), as well as immunostaining with antibodies to EBV latent membrane protein-1 (LMP-1).
Results: EBV was found in 43% of tonsillectomy specimens and 15% of adenoidectomy specimens. All EBV-infected cells were found to be B lymphocytes. About 90% of the infected B cells are found in the interfollicular regions of tonsils and adenoids and the remaining 10% are found within the follicles. There is no significant association between EBV infection, age ($P = 0.324$) and gender ($P = 0.442$).
Conclusion: EBV is associated with tonsillar hypertrophy and is prevalent in 43% of our cases. EBV is only detected in B lymphocytes and we believe that B lymphocytes are sites of primary infection and latency. In situ hybridization is the gold standard for the detection of EBV in tissue.

1. Introduction

Tonsils and adenoids, the common sites for recurrent inflammation in the pediatric population, are continually exposed to antigens, hence hyperplasia of their lymphoid component accounts for the increase in their size [1]. Some authors have demonstrated a strong relation between viral infection [2–4] and recurrent pharyngotonsillitis. There are many viruses involved in the pathogenesis of pharyngotonsillitis including adenoviruses, parainfluenza viruses, rhinoviruses, herpes simplex viruses, respiratory syncytial viruses, Epstein–Barr viruses (EBV), influenza viruses, coxsackie A viruses, corona viruses, and cytomegaloviruses [2,3,5,6]. In spite of high prevalence of viral infection in adenotonsillar tissue, the methods to detect viruses make this approach difficult in routine practice [2,3,5,6].

Commonly causing infectious mononucleosis, EBV is a member of γ1-herpesvirus and has a genome comprised approximately 172 nucleotide base pairs [7]. EBV has a linear genome which is characterized by a distinctive sequence reiteration. At the termini there are 20 copies of a 500 bp repeated sequence that is complementary and therefore permits circularization of the linear genome to form the EBV episome. EBV episome is the molecular basis for latent infection. It is a circular intracellular form of genome that maintains a persistent relationship within the cell, like an autonomous piece of DNA situated in the chromatin [7,8]. EBV is B-lymphotropic and has the ability to transform memory B-cells into blast cells, with permanent proliferation, leading to tonsillar enlargement [9]. EBV is associated not only with infectious mononucleosis but also with benign diseases, such as oral hairy leukoplakia, and malignancies, such as Hodgkin's lymphoma, non-Hodgkin's lymphomas, nasopharyngeal carcinoma, gastric carcinoma and breast carcinoma. It is recently being associated with autoimmune diseases, such as lupus erythematosus and multiple sclerosis [7,10,11]. For these reasons, our study is designed to identify the magnitude of EBV infection and types and pattern of distribution of EBV-infected cells in tonsils and adenoids among UAE population which will be of great help in future planning to prevent this infection.

* Corresponding author. Tel.: +971 37672000x464; fax: +971 37671966.
E-mail address: suhaila@uaeu.ac.ae (S. Al-Salam).

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2. Materials and methods

2.1. Review of cases

In total, 46 cases of tonsillectomy with adenoidectomy, which were performed due to tonsillar and adenoidal enlargement, were randomly selected from surgical pathology archive in the department of pathology at Tawam hospital in Al Ain city for the period June 2004 through May 2005. Forty-six paraffin blocks of tonsillectomy specimens and another 46 paraffin blocks of adenoidectomy specimens were available for this study. The available Hematoxylin and eosin (H&E) and immunohistochemical stained sections were reviewed. The age, sex, and clinical presentation were obtained by reviewing all the histopathologic reports and request forms of all cases. For the detection of EBV, two methods were used to increase the specificity and sensitivity of detection, the streptavidin-biotin immunohistochemical immunoperoxidase method to detect Epstein–Barr virus latent membrane antigen type I (EBV-LMP-1) and in situ hybridization (ISH) for EBV encoded RNA (EBER).

2.2. Immunohistochemistry (IHC)

Immunohistochemical (IHC) staining was performed by standard streptavidin–biotin immunoperoxidase technique [12] using the following mouse antihuman monoclonal antibodies (DAKO Cytomation, Glostrup, Denmark); EBV-latent membrane protein-1(LMP1)(clone CS 1–4), CD3 (clone PC3/188A), CD20 (clone L26), cytokeratin (clone AE1/AE3), all diluted to 1:100, and visualized by a commercially available detection kit (DAKO EnVision Plus-HRP, DAKO, Glostrup, Denmark) and 3-3′-diaminobenzidine (DAKO, Glostrup, Denmark) as a chromogen substrate to obtain a brown end-product. Lymph node sections were used as positive controls for CD20, CD3. Skin epidermis was used as a positive control for cytokeratin AE1/AE3. Hodgkin Lymphoma (HL) LMP-1-positive sections were used as positive controls for LMP-1. For negative controls, primary antibody was replaced with normal mouse serum.

2.3. In situ hybridization (ISH)

In situ hybridization (ISH) was performed by standard techniques using a specific oligonucleotide probe (Novocastra–LEBV-K, UK) which hybridizes to EBV encoded RNA (EBER) transcripts concentrated in the nuclei of latently infected cells. With each batch of cases studied, positive and negative control slides were also run. The positive control slide was a known case of EBV positive HL to which a specific EBER oligonucleotide probe was added. The negative control slide was another section of the same case of known EBV positive HL to which a random probe consisting of fluorescein labeled oligonucleotide cocktail was added. In addition, for each case studied two sections were used; the EBER oligonucleotide probe was added to one section, and the random probe was added to the other. Using this random probe as a negative background control alongside the EBV probe contributes to the validation of staining obtained by the EBV probe. If this negative control slide showed significant background staining in a particular case, the slide having the EBER probe was considered non-interpretable and the test was repeated for that particular case.

2.4. Triple staining technique

Five-micrometer sections were stained for EBER using in situ hybridization protocol described earlier. After nuclear visualization, mouse antihuman monoclonal antibodies for CD20 (DAKO Cytomation, Glostrup, Denmark) were added and visualized by a commercially available detection kit (DAKO EnVision Plus-HRP, DAKO, Glostrup, Denmark) and 3-3′-diaminobenzidine (DAKO, Glostrup, Denmark) as a chromogen substrate to obtain a brown end-product. Subsequently, mouse antihuman monoclonal antibodies for CD3 (DAKO Cytomation, Glostrup, Denmark) were added and visualized using the EnVision Plus-alkaline phosphatase kit (DAKO, Glostrup, Denmark), and New Fuchsin (Merck, Darmstadt, Germany) as a second substrate to yield a red end-product. Finally, sections were mounted by water soluble mounting media.

2.5. Statistical analysis

The statistical analysis was performed using SPSS for windows version 18 (SPSS Inc, Chicago, USA) and analyze it (Analyze-it software Ltd., Leeds, UK). Student's *t*-test was used to compare continuous variables. Quantitative variables were analyzed with the χ^2-test and correlations of ordinal variables using the Spearman rank correlation coefficient. P value <0.05 was considered significant.

2.6. Research ethics

The project has been approved by Al Ain district Human research Ethical committee (Protocol No. 07-145).

3. Results

3.1. Age and gender distribution

In total, 46 cases of tonsillectomy and adenoidectomy, due to tonsillar and adenoid hypertrophy, were selected. All cases were in the 1st and 2nd decade of life with predominant clustering (63%) in the 1st decade. Twenty-seven cases were females and 19 cases were males.

3.1.1. EBV expression correlation between age and gender

Although EBV-positive cases were more ($n = 14$) in the 1st decade than in the 2nd decade ($n = 6$), Cross tab and logistic regression show no significant association between EBV expression and the age ($P = 0.324$) and gender ($P = 0.442$) distributions (Table 1).

Table 1
EBV expression and correlation with age and gender.

Age	EBV[a] positive cases			EBV[a] negative cases			Total cases		
	Female +	Male +	Total +	Female −	Male −	Total −	Female	Male	Total
0–9	6	8	14	6	8	14	12	16	28
10–19	5	1	6	10	2	12	15	3	18
Total	11	9	20	16	10	26	27	19	46

[a] Epstein–Barr virus.