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RESEARCH PAPER

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Epstein-Barr virus in tonsillar tissue of Iranian children with tonsillar hypertrophy: Quantitative measurement by real-time PCR

Shirin Kalantari[1] | Sevrin Zadheidar[1] | Zahra Heydarifard[1] | Ahmad Nejati[1] | Kaveh Sadeghi[1] | Somayeh Shatizadeh Malekshahi[2] | Nastaran Ghavami[1] | Talat Mokhtari-Azad[1] | Nazanin-Zahra Shafiei-Jandaghi[1]

[1]Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2]Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Correspondence

Nazanin-Zahra Shafiei-Jandaghi, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
Email: nz-shafiei@tums.ac.ir

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Abstract

Background and Objectives: Epstein-Barr virus (EBV) infection is ubiquitous all around the world. Tonsils seem to be candidate replication sites for EBV, and these tissues can be infected acutely or chronically. Some studies reported an association between EBV infection and tonsillar hypertrophy. In this study, we aimed to evaluate the presence and copy number of the EBV genome in tonsil tissue specimens of patients with tonsillar hypertrophy.

Methods: A cross-sectional study was performed on 50 fresh tonsil tissue samples from children, who underwent tonsillectomy because of tonsillar hypertrophy. Patients' tonsil tissues were evaluated using real-time polymerase chain reaction for EBV genome and viral load. Finally, the results were analyzed using SPSS software.

Results: EBV genome was detected in 58% (29/50) of tonsillar tissues. The relationship between EBV genome detection rate and age groups was in the statistical significance range ($P = 0.051$). Among 29 positive cases, the average EBV viral load was (3.1×10^5) copy/g ± (0.5×10^5) copy/g. No significant difference was observed among different sex and age groups for EBV viral load.

Conclusion: Herein, EBV genome detection could support the colonization of EBV in the tonsils, which may have a direct or indirect association with the pathogenesis of tonsillar hypertrophy.

KEYWORDS

EBV, Iran, tonsillar hypertrophy

[Correction added on 31 January 2024, after first online publication: Second affiliation has been removed from Author Nazanin-Zahra Shafiei-Jandaghi.]

INTRODUCTION

The palatine tonsils are one of the most important secondary lymphoid tissues and provide a new in vivo model for studying lytic and latent virus infections and immune responses.[1] Tonsils are lymphoid tissue situated near the entrance of the digestive and respiratory tracts, and like all lymphoid tissue, play a role in the immunity and body's defense against infections and foreign pathogens. They act as a first-line defense, forming the initial immunological response to inhaled or ingested pathogens.[2] Tonsillar hypertrophy develops as a result of immunologic reactions within the tonsils. Moreover, parenchymal hyperplasia or fibrinoid degeneration brings about hypertrophy of tonsil tissues, leading to obstruction of crypts.[3] Tonsillar hypertrophy is one of the common diseases in children and may lead to several other complications, such as snoring, obstructive sleep apnea, slow feeding, and behavioral problems.[4,5] The exact cause of this common disease has not yet been completely understood. But since palatine tonsils seem to have a tendency to harbor viruses (e.g., herpesviruses) asymptomatically, there are suggestions there might be a causal relationship between viral presence in this tissue and chronic tonsillar diseases, such as tonsillar hypertrophy and recurrent or chronic tonsillitis.[6]

One of the common herpesviruses that can infect the tonsils is Epstein-Barr virus (EBV). EBV is a double-stranded DNA virus belonging to the *Herpesviridae* family, Gammaherpesvirinae subfamily, Lymphocryptovirus genus, and human herpesvirus four species, which infects almost 90% of the world population.[7] It is a ubiquitous human lymphotropic herpesvirus with an acknowledged causal role in several benign and malignant diseases.[8] The mechanism of the EBV infection is defined by a distinct viral life cycle with primary infection, latency, and lytic reactivation phases.[9] After primary infection, the virus maintains a latent state within memory B cells serving as the primary reservoir for EBV.[9] EBV latency is defined by distinct gene expression programs. Viral latency is mediated through promoter silencing, characterized by limited protein expression, and categorized by four latency types (latency 0–III).[9] EBV is associated with nearly all nasopharyngeal carcinoma (NPC), approximately 10% of gastric carcinomas, 30%–40% of classical Hodgkin lymphoma, a subset of diffuse large B-cell lymphoma (DLBCL), and other T/natural killer cell lymphoproliferative disease (T/NK cell LPDs).[9] EBV infections are usually spread via saliva during childhood or adolescence.[1] In developing countries, data indicated that primary infections are generally acquired early in life, which has been attributed to crowded living conditions, often asymptomatic or with mild symptoms.[1,10] It should be mentioned that the frequency of EBV in Iranian patients with tonsillar hypertrophy remained to be explored. For these reasons, this study is designed to detect and quantitatively measure the EBV DNA in the tonsillar tissues of Iranian children with tonsillar hypertrophy.

METHODS

Patients

This cross-sectional study was undertaken for the detection of EBV infection in patients with tonsillar hypertrophy. Fifty fresh human tonsil samples were consecutively obtained from Amir Alam Hospital, affiliated with the Tehran University of Medical Sciences, between June and October 2019. The patients were characterized to have tonsillar hypertrophy if their tonsils occupied more than 50% of the oropharyngeal airway, corresponding to a Brodsky score of 3 or 4.[6] It should be noted that all of these patients who underwent tonsillectomy had sleep-disordered breathing symptoms or obstructive sleep apnea. Among the mentioned patients, those younger than 16 years old were eligible for the study. There was no available information on the patient's history of tonsillitis. At the time of surgery, none of the children showed any characteristics of acute respiratory viral infections. This study was approved by the Tehran University of Medical Sciences ethics committee (IR.TUMS.SPH.REC.1401.071).

Sample collection, processing, and EBV detection

The tissue samples were processed within 3 h after surgery. Immediately after transferring the tonsils from the hospital to the National Influenza Center (NIC) of Iran, several pieces of fresh tonsillar tissue were put into viral transport medium (VTM) tubes and stored at −70°C for later analysis. QIAmp DNeasy Mini Kit Qiagen was used for DNA extraction from tonsil tissues according to the manufacturer's instruction. The quality and quantity of extracted genomic DNA was evaluated. The concentration of total DNA was evaluated by NanoDrop spectrophotometer. The integrity of the extracted DNA of all samples was evaluated by polymerase chain reaction (PCR) using B-ACTIN gene with specific primers and probes. The Real-Time PCR for EBV DNA detection and viral load evaluation in tonsillar tissues was performed using the GeneProof EBV PCR kit with the following cycling conditions: 37°C for 2 min (hold); 95°C for 10 min (hold); 45 cycles of 95°C for 5 s (denaturation), 60°C for 40 s (annealing) and 72°C for 20 s (extension). The mean DNA concentration (Ct values) of each sample was calculated as reported, related to the tonsil's weight. The lower limit of detection was 1.3×10^4 EBV copies per gram of tissue. For statistical analysis of results, SPSS 26.0 for Windows was used. P values < 0.05 were considered statistically significant.

RESULTS

The samples used in this study were obtained from children undergoing tonsillectomy procedures due to tonsillar hypertrophy aged 3–15 years (mean of 8.07 and standard deviation of 2.85 years).

TABLE 1 Epstein-Barr virus (EBV) DNA detection and correlation with age and gender (number of cases).

	EBV-positive cases			EBA negative cases			Total cases		
Age (year)	Female	Male	Total	Female	Male	Total	Female	Male	Total
≤5	1	1	2	3	4	7	4	5	9
6–10	6	15	21	2	8	10	8	23	31
11–15	2	4	6	0	4	4	2	8	10
Total	9	20	29	5	16	21	14	36	50

TABLE 2 Comparison of Epstein-Barr virus (EBV) viral load in tonsillar tissue of children with hypertrophy based on sex and age.

Items	Median (copy/g)	Interquartile range (copy/g)
Sex		
Male	6.6×10^4	2.7×10^5
Female	6.6×10^4	1.1×10^5
Age group		
≤5 (year)	6.6×10^4	-
6–10 (year)	5.3×10^4	2.4×10^5
11–15 (year)	1.1×10^5	3.4×10^5

Note: " - "indicates no data available.

The patients were divided into three age groups, including under 5, 6–10, and 11–15 years. Among 50 cases, there were 14 (28%) females and 36 (72%) males.

EBV DNA was detected in 29 (58%) of 50 tonsil samples. The proportions of males and females were (55.6%) and (64.3%), respectively. There were no significant differences in the prevalence of EBV DNA in tonsils between the two sex groups ($P = 0.574$, chi-square test). The results of EBV DNA detection in age groups of children are shown in Table 1. Statistical analyses have shown a borderline significant association between EBV DNA detection and age ($P = 0.051$).

The viral load in tonsils from EBV-positive children, as determined by quantitative real-time PCR, ranged between 1.3×10^4 and 2.7×10^6 copies/g tissues. The median EBV DNA load in the tonsils was similar in the males and females (Table 2). Viral load showed no differences when compared among the age groups, but the median EBV DNA load was higher in the 11–15 years children than others (Table 2).

DISCUSSION

Tonsillar hypertrophy is a common finding in children, but the exact etiology of that remains controversial. Many studies have investigated the prevalence of EBV in the tonsils of children with hypertrophy in developed and developing countries. However, there have been no reports of the prevalence and viral load of EBV in hypertrophic tonsils among Iranian children. Herein, EBV DNA was found in 58% of tonsillar hypertrophic tissues collected from children aged 3–15 years. In a number of studies, the detection rate of the EBV genome in tonsillar tissues has been reported to be higher than in the present study. For instance, in Finland, the EBV genome was detected in 64% of under 16 years old children's tonsillar tissue.[6] In a number of other studies, the detection rate of EBV DNA in tonsil tissue has been reported to be lower than in this study. In a Japanese study, EBV genome presence was reported in the tonsil tissue of 43% of children under 16 years of age with tonsillar hypertrophy.[11] In Sweden, Asadian et al.[12] reported that the EBV genome was identified in 34% of hypertrophic tonsil tissue of patients under 19 years of age. In a 2017 study from China, Liu and Peng[13] identified the EBV genome in 32% of the tonsil tissue of patients with tonsil hypertrophy. A previous study in the same country in 2014 reported a 45.2% of EBV DNA detection rate in hypertrophic tonsillar tissues.[14] In United Arab Emirates nationals in 2010, Al-Salam et al.[15] revealed that 43% of the samples were EBV DNA positive by in situ hybridization (ISH) assay. EBV genome was detected in 50% of children's tonsil tissue from Turkey.[16] While, in another study of this country, 22.7% of under 18 years old patients were EBV positive.[17] In a study conducted between 2012 and 2014 in Turkey, Gunel et al.[4] detected EBV genome among 32% of pediatric hypertrophic tonsils.

So, the expression of EBV DNA can still be seen in the tonsil tissues of children without tonsillitis. The significance of the presence of EBV DNA in the tonsil tissues of children without tonsillitis is not fully understood. However, it was shown that EBV infection is associated with tonsillar hypertrophy and is prevalent in 43% of tonsillectomy specimens, which is found in both the interfollicular regions and follicles of tonsils and adenoids.[15] In children under 24 months of age, EBV infection might be responsible for obstructive hyperplasia.[18] The prevalence of EBV in adenoids (15%) is lower than in tonsils (43%), indicating a lower association between adenoid hypertrophy and EBV infection.[15] The relationship between EBV and asymmetric and symmetric tonsillar hypertrophy was evaluated in a clinical prospective study, but the results were inconclusive.[19] The long-term effects of EBV infection in tonsillar hypertrophy are not yet fully understood.

The age of developing primary EBV infection varies in different countries of the world, because exposure to EBV is likely to be

influenced by social and economic factors. After the primary infection, EBV establishes indefinite permanent infection within the host, especially in lymphoid tissues. In developing countries, primary infection transmitted through saliva often occurs in childhood asymptomatically. While in developed societies, mostly delayed until the second decade of life or even later, and in 25%–75% of cases, it manifests as infectious mononucleosis. These different clinical symptoms is due to the different immune response in children and adolescents.[20] In the current study, the virus genome was detected in 22.2% of children aged 5 years and younger, 67.7% of 6%–10% and 60% of 11–15 year-old children. In general, statistical analyses have shown an association between host age and EBV DNA detection rate. This association is affected by socioeconomic conditions of each country. In this study, the EBV genome was detected in 55.6% of boys and 64.3% of girls with tonsillar hypertrophy. There was no statistical correlation between sex and EBV detection rate in tissues. This result was in agreement with most other studies.[14–16,21,22]

According to the results of this study, the EBV viral load in hypertrophic tonsillar tissues of children was $\leq 1.3 \times 10^5$ copy/g to 2.7×10^6 copy/g. It should be noted that the viral load in 69% of EBV-positive children was less than 1.3×10^5 copy/g tissue. In the study in Switzerland in 2001, the EBV viral load in the tonsillar tissue of 43 children aged 2–12 years from two groups with tonsillar hypertrophy and frequent tonsillitis who were EBV-Seropositive was investigated. The virus load in the tonsil tissue of children with hypertrophy was reported to be between 2×10^3 copy/g and 1.3×10^7 copy/g.[23] It is worth noting that a higher viral load in tonsil tissue can indicate a higher number of virus-infected cells, the conditions of viral replication, or both.[23] In a study, the expression of EBV lytic and latent genes was evaluated in tonsil of hypertrophic children. According to the results, Sieshima et al.[11] have concluded that the EBV latent phase is a frequent condition in the tonsil tissue of children with hypertrophy. Therefore, a low viral load in the tonsillar tissue of children with tonsillar hypertrophy can be expected. Some studies have suggested that a high viral load in EBV-related malignancies can determine disease status and stage,[24] as simple hypertrophy is not recognized as a lymphoid malignancy. Therefore, the low load in the high sample number is justified. However, further studies on the role of viral load in the pathogenesis of lymphoid tissue, especially in tonsillar tissue, are needed. There was no statistical correlation between EBV copy number in tonsil tissue and the sex of children; in the search that was done to compare this part of the results with other studies, there were not enough studies in this field for EBV. But a study conducted on adenovirus load in tonsil tissue reported no significant relationship between gender and adenovirus viral load.[25] No correlation was found between EBV copy number in tonsil tissue and patients' age, this data was in agreement with Assadian et al.[12] and Vistarop et al.[20] studies. One limitation of this study is that there was no information on the patient's tonsillitis history. Expanding the scope of the study to include clinical aspects would enhance overall impact and provide a more comprehensive understanding of the relationship between EBV infection and tonsillar pathologies.

CONCLUSIONS

In conclusion, this study provides a cross-sectional report of EBV infection in nonacutely ill tonsillectomy children. With the increasing roles of EBV in both benign and malignant diseases, and due to the high prevalence of EBV-infected individuals around the world, tonsils have gained attentiveness as a new in vivo model to study lymphatic organs as virus reservoirs. Identification of a high prevalence (58%) of the EBV genome in tonsillectomy specimens in Iranian children suggests EBV may be involved in tonsillar enlargement. Many aspects of latent and lytic EBV infection remain unclear and are possible points for future studies.

AUTHOR CONTRIBUTIONS

Shirin Kalantari, Sevrin Zadheidar, Zahra Heydarifard, and Kaveh Sadeghi contributed to tests performing. Nastaran Ghavami and Sevrin Zadheidar performed the extraction. Somayeh Shatizadeh Malekshahi and Ahmad Nejati contributed to reviewing the paper critically, and the comments were included. Nazanin-Zahra Shafiei-Jandaghi and Sevrin Zadheidar contributed to drafting the manuscript. Talat Mokhtari-Azad and Nazanin-Zahra Shafiei-Jandaghi designed and supervised the study. All authors have approved the final version of the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets collected and analyzed during this study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Tehran University of Medical Sciences with the approval code (IR.TUMS.SPH.REC.1401.071).

ORCID

Nazanin-Zahra Shafiei-Jandaghi http://orcid.org/0000-0002-6988-4321

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