

Brief Original Article

Prevalence of Epstein-Barr virus DNA in tonsillar tissue from patients with chronic tonsillitis in Mexican population

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Abstract

Introduction: Epstein-Barr Virus (EBV) infection prevails in underdeveloped and developing countries. The tonsils seem to be candidate replication sites for EBV and some studies have exposed a close association among viral infections and chronic tonsillitis. The objective of this study was identifying the EBV prevalence in Mexican patients who had undergone tonsillectomy because of chronic tonsillitis.

Methodology: Frozen tissues and medical records were obtained from 50 Mexican patients. DNA was extracted and subjected to PCR to amplify the EBER-2 region of EBV. Next, the patients were classified according to general and clinical characteristics searching a relation with the EBV-DNA positivity.

Results: EBV genome was detected in 46% (23/50) of the analysed tonsil tissues. Trends were found regarding the relationship of viral presence with lower values in terms of age (6.1 ± 2.8 vs 7.6 ± 3.7), a greater degree of hypertrophy (3.5 ± 0.4 vs 3.0 ± 0.6) and an increase in the number of episodes of tonsillitis (11 ± 7.4 vs 9 ± 6.5).

Conclusions: The prevalence found of EBV-DNA positivity in tonsillar tissues from patients diagnosed with chronic tonsillitis, supports the fact that palatine tonsils can be occupied by EBV and highlights the importance of conducting future studies focused on understanding the role of the EBV infection in chronic inflammatory processes in the population involved in this study.

Key words: Chronic tonsillitis; tonsillectomy; Epstein-Barr virus; PCR detection.

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Introduction

The palatine and pharyngeal tonsils are the most important structures in the collection of lymphatic tissue known as Waldeyer's tonsillar ring. Immunological reaction of the tonsils can adversely affect, especially when there is hypertrophy with upper respiratory airway obstruction or recurrent infections, these situations are often treated by tonsillectomy [1,2].

Epstein-Barr virus (EBV) is an ubiquitous double-stranded DNA virus that belongs to the Herpesviridae family and Gammaherpesvirinae subfamily. EBV infection prevails in underdeveloped and developing countries [3,4]. The tonsils seem to be candidate sites for EBV replication and some studies have exposed a close association among viral infections and tonsillitis [5-7]. The downregulation of the lymphocytic function of palatine tonsils by viral infections would facilitate a secondary bacterial infection [8].

EBV is associated not only with chronic tonsillitis but also with malignancies such as tonsillar cancer, oral

squamous cell carcinoma and lymphoma [9,10]. It is recently being associated with autoimmune diseases, such as lupus erythematosus and multiple sclerosis [11,12]. The objective of this study was to describe the prevalence of EBV-DNA by PCR (polymerase chain reaction) in tonsillar tissues from Mexican patients who had undergone tonsillectomy because of chronic tonsillitis.

Methodology

Patients and tissue samples

This study was realized in accordance with protocols for the use of surgical tissues and medical histories previously accepted by the institutional bioethics committee and the Helsinki Declaration of 1975, as revised in 2008. Tonsillar tissues were obtained from 50 Mexican patients, who had undergone tonsillectomy. All samples were stored at -80°C in an ultra-low freezer.

Extraction and amplification of DNA

DNA was extracted from tonsillar tissue (DNeasy Blood and Tissue kit, QIAGEN) and stored at -20°C until used. DNA samples (100ng) were subjected to PCR using Taq DNA polymerase (AmpliTaqGold 360 Master mix Kit, Applied Biosystems), oligonucleotides GH20 (GAAGA-GCCAA-GGACA-GGTAC) and GH21 (GGAAA-ATAGA-CCAAT-AGGCA-G) to amplify β-globin gene, EBER-2S (CCCTA-GTGGT-TTCGG-ACACA) and EBER-2AS1 (ACTTG-CAAAT-GCTCT-AGGCG) to amplify the EBER-2 region of the EBV-genome. The PCR was performed in a ProFlex PCR System (Applied Biosystem). The standard cycle procedure was a 5-minute denaturation at 95°C followed by 35 cycles of 30-seconds of denaturation at 95°C, 1-minute of annealing at 60°C for EBER-2, 30-seconds at 55°C for β-globin and a 2-minute extension at 72°C. Cycling was followed by a 7-minute extension at 72°C. Visualization of gene amplification was done using an ethidium bromide stained 2% agarose gel in which 12µl of each PCR product was separated by electrophoresis; a 100bp DNA ladder was used as the parameter (Thermo Fisher Scientific). Image of the stained gels was captured using the BioDoc-It Imaging Systems (UVP,Inc).

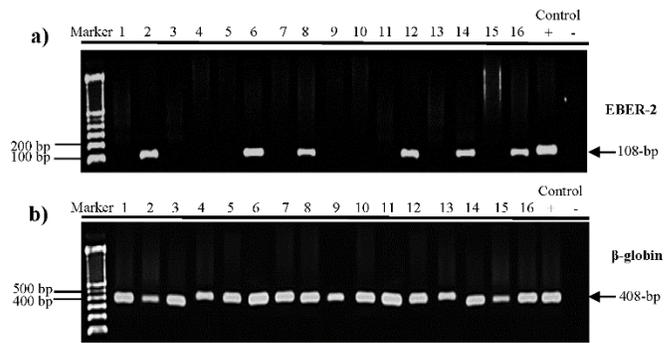
Statistical analysis

Percentage of EBV-DNA prevalence and frequency of clinical parameters were described. Pearson’s correlation coefficient was used to determine the

Table 1. Clinical characteristics of patients.

Characteristic	Patients, N (%)
Gender	
Female	17 (34)
Male	33 (66)
Age (years)	
0-5	16 (32)
6-12	31 (62)
13-18	3 (6)
Tonsillar hypertrophy (Grade)	
I	0 (0)
II	6 (12)
III	28 (56)
IV	16 (32)
Tonsillitis (Episodes per year)	
1-9	32 (64)
10-19	9 (18)
20-24	9 (18)

Figure 1. Detection of EBV DNA by polymerase chain reaction analysis.



A) Representative agarose gel (2 %) electrophoresis showing 100-bp molecular marker, amplification of the 108-bp EBER-2 fragment (Samples 1 – 16, DNA extracted from the line Raji as positive control and Milli-Q water as negative control). B) Representative agarose gel (2%) electrophoresis showing 100-bp molecular marker, amplification of the 408-bp β-globin fragment (Samples 1 - 16, DNA extracted from the line Raji as positive control and Milli-Q water as negative control).

relationship between clinical variables. Differences among variables in EBV positive and negative groups were analysed by student’s T test. Results were considered significant at p<0.05. All statistical procedures were conducted with SPSS version 22 software (IBM).

Results

Clinical characteristics of patients

The age range of the tonsils donors was 2-16 years (mean age 7.3 years) of which 33 (66%) were males and 17 (34%) were females. Tonsillar hypertrophy grade was defined from I to IV according to the space they occupied of the oropharyngeal diameter: Grade I (0-25%), II (26-50%), III (51-75%) and IV (76-100%). There weren’t any grade I cases amongst these samples, 6 (12%) correspond to grade II, 28 (56%) grade III and 16 (32%) grade IV. The tonsillitis recurrence range was of 5 to 24 episodes per year (mean of 9). Patients grouped according to previous variables are enlisted in Table 1.

Detection of EBV in tonsillar tissue

DNA was amplified by PCR with primers covering the EBER-2 region of the EBV-genome and β-globin to check whether the samples contained amplifiable DNA. The expected products were 108bp for EBER-2 and 408bp for β-globin (Figure 1). The samples were considered to be EBV positive if amplification of the gene occurred. We were therefore able to detect the EBV-genome in 23 (46%) tonsillar tissues; all the samples shown amplification to β-globin gene as described in Table 2.

Table 2. Amplification of the EBV and the β -globin genes in tonsils.

Gene	PCR amplification		
	No. positive (%)	No. negative (%)	Total
EBV	23 (46)	27 (54)	50
β -globin	50 (100)	0 (0)	50

Relation of EBV DNA presence with clinical characteristics

After EBV detection, we describe the variation observed in the positivity of the tissue samples according to the patients’ medical histories. The difference in mean values of age, hypertrophy grade and tonsillitis were compared in the EBV-DNA positive versus the negative group (Figure 2). The patients in the positive group showed lower values in terms of age (6.1 ± 2.8 vs 7.6 ± 3.7), a greater degree of hypertrophy (3.5 ± 0.4 vs 3.0 ± 0.6) and an increase in the number of episodes of tonsillitis (11 ± 7.4 vs 9 ± 6.5). Previous observations were not statistically significant ($p \geq .05$). However, we encounter that a major number of tonsillitis episodes were correlated with a decrease in age ($p=.038$, $r=-.362$).

Discussion

The involvement of viral infections in the recurrence of tonsillitis has been described previously and according to these authors, if the lymphocytic function of the palatine tonsils were down regulated during a viral infection a secondary bacterial infection would easily be induced [8]. Studies using *in-situ* hybridization found an EBV prevalence of 29% [3] and 65% [13] in patients with tonsillitis. Other studies using PCR report a prevalence of 11% [14] and 54.1% [15]. These results, confirm good sensitivity of PCR for molecular detection and suggest an important association between the EBV and tonsillitis.

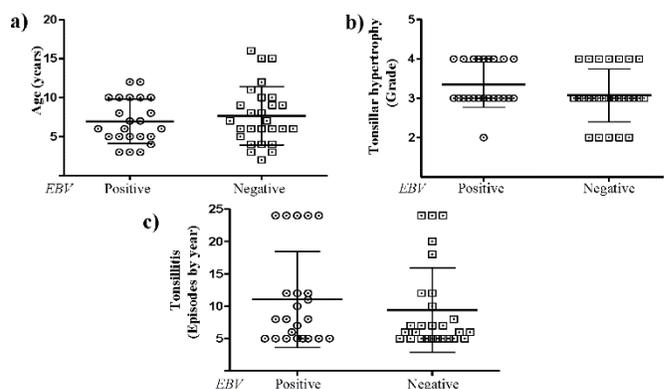
Our results indicate that EBV-DNA remained present in 46% of the tonsillar tissues from Mexican patients diagnosed with chronic tonsillitis, supporting the fact that the palatine tonsils can be occupied by EBV. Regardless of the detection method it is important to consider that the infection prevalence varies considerably worldwide and exposure to EBV is likely to be influenced by socioeconomic factors [16]. Moreover, some authors propose that a significant number of recurring tonsillar infections during childhood may be associated with the presence of EBV [17]. Although, the results obtained not reflect a significant relation of EBV-DNA presence with the patient’s life stages, we encounter that major number of tonsillitis episodes were correlated with a decrease in

the age. This finding could reflect a reduction in the load of viral particles due to the changing immune balance of the host in older children.

The lack of significance in the relationship of EBV infection with clinical parameters, could be explained by substantial differences in transcription activity related with the EBV viral cycle and its impact over the host immune response. It has been described that EBV persists in resting, latently infected memory B cells where the virus maintains a null level of protein expression [18,19]. This absence of viral proteins is the main reason why the infected cells evade the immune response from the host, allowing the infection to persist for a long period before it becomes chronic. Also, has been exposed that the tonsillar lymphocytes are not only reservoirs but also a replication site for the EBV [17]. For this, it would be interesting to identify viral proteins expressed in latent and replicative infections using immunohistochemical methods [20].

Furthermore, authors suggest that the association is not restricted only to tonsillitis when describing the involvement of EBV infection in cancer and autoimmune diseases [9-12]. The considerable prevalence found in this study, gives rise to the development of studies dedicated on investigate the relationship of EBV in the development of chronic diseases that affects Mexican population.

Figure 2. Relation of EBV DNA presence with clinical characteristics.



Values of age (a), tonsillar hypertrophy (b) and tonsillitis (c) were grouped according to the positive or negative amplification of EBV DNA. Mean values with Standard deviation are represented.

Conclusion

The prevalence found of EBV-DNA positivity in tonsillar tissues from patients diagnosed with chronic tonsillitis, supports the fact that palatine tonsils can be occupied by EBV and highlights the importance of conducting future studies focused on understanding the role of the EBV infection in chronic inflammatory processes in the population involved in this study.

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