DOI: 10.4274/tpa.963

Role of Ebstein-Barr virus in children with tonsillar hypertrophy

Sibel Aka[1], Berna Yayla Özker[2], Ebru Demiralay[3], İsmet Ercan Canbay[1]

[1]Başkent University, İstanbul Application and Research Hospital, Pediatrics, İstanbul, Turkey
[2]Başkent University, İstanbul Application and Research Hospital, Otolaryngology, İstanbul, Turkey
[3]Başkent University, İstanbul Application and Research Hospital Pathology Unit, İstanbul, Turkey

Summary

Aim: The aim of this clinical prospective study is to evaluate the relationship between Epstein-Barr virüs (EBV) and asymmetric and symmetric tonsillar hypertrophy in children between 3-14 years old.

Material and Method: Tonsil size of forty two children were evaluated a orophayrngeal inspection preoperatively.Tonsils were grouped according to Brodsky L scala classification (Grade 1-4).Childrens are separated into two groups including the ones that have grade 1-2 and grade 3-4 tonsil size, +1 difference between two tonsils was accepted as tonsillar asymmetry. Viral capsid antigen IgG (VCA IgG), viral capsid antigen IgM (VCA IgM), early antigen and EBV nuclear antigen (EBNA) levels were measured in serum preoperatively. EBV latent membrane protein-1 and EBNA-2 levels were determined with immunohistochemical studies after paraffin sectioning. Chi-square and Fisher's exact tests were used to compare groups. The study was approved by the ethics commite (KA0992).

Results: The 20 of the cases were boy and 22 were girl. The mean age was 7.12±2.07. There were no significant difference between groups, among age and gender distribution.Thirty-two children (76.2%) were sero-positive for VCA IgG. Among 62.5% of them, EBNA IgG was also positive. VCA IgG was significanlty higher in children with tonsillar hypertrophy grade 3-4 and EBNA was significantly higher in children with tonsillar hypertrophy grade 1-2. All tonsil spacements are evaluated as chronic tonsillit that shows lymphoid hyperplasia. 35.7% of EBV is found by immunohistochemical staining.This ratio is determined as 28.6% for latent membran protein-1 and 19% for EBNA.The relationship between EBV and degree of tonsillar hypertrophy was found to be statistically insignificant ($p>0.05$). Asymetric tonsillar hypertrophy were seen among 16.7% of the children.The relationship between EBV and asymetric tonsillar hypertrophy was also found to be statistically insignificant ($p>0.05$).

Conclusions: We found statistical correlation between the grade of tonsillar hypertrophy and viral load in serum. This study points out the reservoir of EBV in tonsil tissue. *(Turk Arch Ped 2013; 48: 30-34)*

Key words: Asymmetric tonsil, Epstein Barr virus, recurrent tonsillitis, tonsillar hypertrophy

Introduction

Tonsillar hyperthrophy (TH) is a common clinical finding in the childhood. It is generally associated with recurrent tonsillitis. It is a condition that causes to inhibition in the upper respiratory tract.

The mucosal lymphoid tissue comprising the Waldeyer's ring is the first defense area of the airway against various microorganisms. Many microorganisms lead to recurrent tonsillitis by infecting this tissue. Although bacterial agents and especially streptococcus strains are primarily blamed, viral agents including especially "Ebstein-Barr virus" (EBV) has been found to be related with chronic recurrent tonsillitis. There are studies which confirm the relation between recurrent tonsillitis and TH and EBV in the literature (1,2,3).

Ebstein Barr virus is a DNA virus belonging to the gamma herpes virus group. It may lead to latent infections. Infection with the virus is very prevalent in the world. More than 90% of the adults are infected with the virus. The primary infection usually occurs in the childhood and is generally asymptomatic. The first area of localization of Ebstein Barr virus is the oropharyngeal epithelial cells and B lymphocytes. The tonsils are the first site of involvement and the source for the virus. When the virus enters the cell, EBV nuclear antigens 1 and 2 (EBNA-1 and EBNA-2) are found in the nucleus of the host cell. As a result of a series of stimuli originating from Ebstein Barr virus nuclear antigens 1 and 2, various proteins are sythesized including mainly latent membrane proteins 1 and 2 (LMP-1 and 2). Complex relation between these proteins leads to formation of cells which contain many EBV gene copies bound to the cellular DNA. Thus, the

Address for Correspondence: Sibel Aka MD, Başkent University, İstanbul Application and Research Hospital, Pediatrics, İstanbul, Turkey
Phone: +90 542 344 33 22 E-mail: sibelaka@hotmail.com **Received:** 04.03.2012 **Accepted:** 10.01.2012

virus is maintained in a latent state in the B lymphocytes here and may be reactivated. This reactivation is especially important in immunosupressed patients (2,3,4,5). Ebstein Barr virus is not only related with infectious mononucleosis disease, but it has also been associated with Hodgkin and non-Hodgkin lymphoma, nasopharengeal cancers and breast cancer. In recents years, some studies proposed that it is associated with autoimmune diseases, lupus erythematosus and multiple sclerosis (6,7,8).

In this study, it was aimed to investigate the relation between the presence of EBV in the tonsillar tissues and symmetrical and asymmetric tonsillar hypertrophy in children who underwent tonsillectomy because of TH.

Material and Method

42 children aged 3-14 years old who were followed up by Başkent University İstanbul Hospital otolaryngology outpatient clinics between October 2009 and October 2010 and who were decided to undergo tonsillectomy because of recurrent tonsillitis and/or obstructive findings were included in the study. Written information about the study was given to the families of all the children and written informed consent was obtained from the families for use of tonsillectomy materials for the study. The study was initiated after obtaining approval from the Başkent Universtiy ethics committee (KA0992).

EBV profile test was performed in the blood samples obtained from the patients for preparation before tonsillectomy (VCA IgG, VCA IgM, Erly Antijen and EBNA IgG) (Euroimmun, Germany).

The tonsillar size was evaluated by the same otolaryngologist with the preoperative oropharyngeal view and was classified as grade 1-4 according to Brodsky L (9) scale. According to this scale, the tonsil is outside the tonsillary fossa and obstructs the airway by 25% in 1+ hypertrophy, 25-50% obstruction is present in 2+ hypertrophy, 50-75% obstruction is present in 3+ hypertrophy and 75% obstruction is present in 4+ hypertrophy. 1+ difference between the tonsils was considered as asymmetric tonsillar hypertrophy.

Tonsillectomy materials were sent to pathology for histopathological examination in a sterile container containing 10% formaldehyde. Tissue processing was performed in the materials fixed in a liquid containing 10% formaldehyde. Hematoxylin eosin staining was performed on the sections prepared from praffin blocks. On light microscopy, all patients were found to be compatible with chronic tonsillitis showing lymphoid hyperplasia. EBV Latent Membran Protein (EBV LMP-1, NCL-EBV-CS1-4, Novacastra, England) and EBNA-2 (EBV Nuclear Antigene-2, NCL-EBV-PE2, Leica, england) antibodies were stained by applying immunohistochemical sterptavidin-peroxidase method to the sections prepared from parafin blocks. The presence of cells which showed nuclear staining for EBNA-2 and which showed cytoplasmic staining for EBV LMP1 was investigated using light micrscope.

Results

20 of the patients (47.6%) were male and 22 (52.4%) were female. The ages of the patients ranged between 3.67 and 14,08 years. The mean age was found to be 7.12±2.07 years. The mean age was found to be 7.74±1.6 years in the group with a grade 1-2 tonsillar size and 6.87±2.15 in the group with a grade 3-4 tonsillar size. No significant difference was found between the two groups in terms of age (p=0.220).

VCA IgG was found to be positive in 76.2% of the patients (n=32). EBNA was also found to be positive in 62.5% (n=20) of the patients with a positive VCA IgG (Table 1).

In the groups with a grade 1-2 tonsillar size (n=12), the VCA IgG positivity was found with a rate of 20.6%, while it was found with a rate of 79.4% in the group with a grade 3-4 tonsillar size (n=30). A significant relation was found between tonsillar size and VCA IgG (p=0.018). The odds ratio of VCA IgG positive patients to be grade 3-4 was found to be 6, 429 (95% CI=1, 22-33.64) (Table 2).

EBNA IgG positivity was found with a rate of 10% in grade 1-2 patients and with a rate of 90% in grade 3-4 patients. The relation between tosillary size and EBNA IgG was found to be significant (p=0.011). The odds ratio EBNA positive patients to be grade 3-4 was found to be 7.50 (95% CI:1.39-40.43) (Table 2).

EBV positivity was found with a rate of 35,7% in the tonsillectomy materials with immunohistochemical method. This rate was 28.6% for latent membrane protein-1 and 19% for EBNA-2. The rate of the patients with a positive latent membrane protein-1 and a positive EBNA-2 was found to be 11.9% (Table 3).

No significant relation was found between tonsillary size and LMP-1 (p=0.280). However, the rate of grade 3-4 hypertrophy in LMP-1 positive patients was found to be higher compared to LMP-1 negative patients. No significant relation was found between tonsillary size and EBV EBNA (p=0.53) (Table 4).

Table 1. Serologic markers of Ebstein Barr virus

Serologic markers of Ebstein Barr virus	Positive n (%)	Negative n (%)	Total n (%)
VCA Ig G	32 (76.2%)	10 (23.8%)	42 (100%)
VCA Ig M	0	42 (100%)	42 (100%)
"Early antigen"	0	42 (100%)	42 (100%)
EBNA	18 (42.9%)	24 (57.1%)	42 (100%)

VCA: Viral capsid antigen, EBNA: EBV nuclear antigens

The rate of asymmetric TH was found to be %16,7 (Table 5) in the study. No statistically significant relation was found between the state of symetric and asymmetric TH and serum VCA IgG and EBNA IgG levels (p=0.482) (p=0.691).

No statistically significant relation was found between asymmetric TH and LMP-1 which is one of the EBV markers belonging to the tissue samples (p>0.05). However, a significant relation was found between asymmetric TH and EBNA-2 (p=0.005)

Logistic regression analysis was used to determine the risk factors of TH. The effects of VCA IgG and EBNA in blood samples and LMP-1 ve EBNA-2 in the tissue which are risk factors of TH were evaluated using enter logistic regression analysis; the sample was found to be significant (p<0.05) and Negelkerke R square value was found to be 0.282. It was found that the coefficient of determination was high (83.3%). Positivity of VCA IgG and EBNA in association which was the most effective factor on TH was found to be statistically significant in the study (p<0.01). It was observed that VCA IgG and EBNA positivity had an effect of increasing TH by 36.75 fold (95% CI:2.77-486).

Discussion

Ebstein Barr virus infection is observed commonly worldwide and the seropositivity in adults is 90% (2). Studies have shown an increasing seropositivity with advanced age.

Table 2. Comparison of serum Ebstein Barr virus markers by tonsillar size

		Tonsillar hypertrophy		
Serologic markers of Ebstein Barr virus		**Grade 1-2 n (%%)**	**Grade 3-4 n (%%)**	**p**
VCA IgG	Positive Negative	7 (20.6%) 5 (62.5%)	27 (79.4%) 3 (37.5%)	0.018*
EBNA	Positive Negative	2 (10.0%) 10 (45.5%)	18 (90.0%) 12 (54.5%)	0.011*
VCA IgG ve EBNA	Ig G (+%) EBNA (+%) Ig G (+%) EBNA (-%) Ig G (-%) EBNA (-%)	2 (10.0%) 4 (33.3%) 6 (60.0%)	18 (90.0%) 8 (66.6%) 4 (40.0%)	0.016*

* p<0,05, VCA: Viral capsid antigen, EBNA: EBV nuclear antigens

Table 3. Ebstein Barr virus markers in tissue samples

		All patients (n=42)		IgG (+) patients (n=32)	
Epstein Barr virüsü belirteçleri		**n**	**%**	**n**	**%**
LMP 1	Positive Negative	12 30	28.6 71.4	12 20	37.5 62.5
EBNA 2	Positive Negative	8 34	19.0 81.0	8 24	25.0 75.0
LMP 1 and EBNA 2	(+) and (+) (+) and (-) (-) and (+) (-) and (-)	5 7 3 27	11.9 16.7 7.1 64.3	5 7 3 17	15.6 21.8 9.4 53.2

LMP: Latent membrane protein, EBNA: EBV nuclear antigens

Table 4. Comparison of tissue Ebstein Barr virus markers by tonsillar size

		Tonsiller hipertrofi		
Markers of Ebstein Barr virus		**Grade 1-2 n (%)**	**Grade 3-4 n (%)**	**p**
EBV LMP1	Pozitif Negatif	2 (22.2%) 5 (21.7%)	7 (77.8%) 18 (78.3%)	0.976
EBNA-2	Pozitif Negatif	3 (50.0 %) 4 (15.4%)	3 (50.0%) 22 (84.6%)	0.101
EBV LMP1 & EBNA-2	(+) & (+) (-) & (-)	4 (33.3%) 3 (15.0%)	8 (66.7%) 17 (85.0%)	0.379

* p<0.05, LMP: Latent membrane protein, EBNA: EBV nuclear antigens

Morris et al. (10) reported that VCA IgG level was 35% between 1 and 4 years of age, 54% between 10 and 14 years of age and 73% between 15 and 19 years of age. In a study performed in our country, VCA IgG was found to be positive with a rate of 66,7% in patients with recurrent tonsillitis (11).

In our study, we found positive serum VCA IgG with a rate of 76.2% and positive serum VCA EBNA with a rate of 47.6%. Although our results were compatible with the literature, they are sligthly higher compared to seropositivity rates in developed countries and this supports the view that EBV infection occurs at an earlier age in our country compared to developed countries.

The primary infection in the childhood is asymptomatic. However, there are studies supporting the relation between recurrent tonsillitis and TH and EBV in children in the literature. Endo et al. (2) found EVB RNA (EBER) to be positive using in situ hybridization in 10 of 43 patients with TH and in 15 of 42 patients with chronic tonsillitis. Endo et al. (12) compared EBER positivity in adenoid tissues in children below and above the age of two in another study and reported that the frequency of EBV expression in the adenoid tissue increased after the age of two.

Köseoğlu et al. (13) from our country found positive EBV LMP-1 with a rate of 14%, positive ENBNA 2 with a rate of 6% using immunohistochemical method and positive EBV LMP-1 with a rate of 8% and positive EBNA-2 with a rate of 8% using in situ hybridisation in the adenoid and tonsillar tissues in 50 patients with ages ranging between 4 and 32 years. In a similar study performed by Dias et al. (1), EBV DNA was found to be positive with a rate of 54.1% with PCR and EBV LMP1 was found to be positive with a rate of 37.5% with immunohistochemical method. These rates are similar to our results.

The study performed by Hug et. al. (14) is similar to our study and shows that the tonsillar tissue is an important reservoir for pediatric EBV carrier state in TH cases. They screened EBV genome with PCR by obtaining throat swabs from children with and without TH and from children who had had acute infectious mononucleosis. Ebstein Barr virus DNA was found with a similar rate in children with TH compared to children with infectious mononucleosis and with a higher rate compared to children without TH.

Doğan et al. (11) found positive EBV DNA with a rate of 75% in the tonsillar tissues of children who underwent tonsillectomy because of recurrent tonsillitis and could not find a statistically significant relation between serum VCA IgG level and EBV DNA positivity in the tonsillar tissue in these patients. They reported that Ebstein Barr virus was colonized in the palatine tonsils of children and thus the tonsillar tissue was a reservoir.

Al-Salam et al. (15) found EBV with a rate of 43% in tonsillectomy materials and with a rate of 13% in adenectomy materials and reported that the tonsillar tissue was the main reservoir for EBV and this caused to TH. In the same study, it was reported that all cells infected with EBV in the tonsillar tissue were B lymphocytes and they were mostly localized in interfollicular areas.

In our study in situ hybridization method was used instead of PCR which can show false positivity because of its high sensitivity. However, this method may show false negativity becasue of its low sensitivity. In spite of this, we found positive EBV LMP-1 with a rate of 37.5% and positive EBNA-2 with a rate of 25% in the tonsillar tissue. These results support that the tonsillar tissue is an important reservoir for EBV in children. In addition, we found serum VCA IgG and serum EBNA levels to be significantly higher in patients with marked (grade 3 and 4) TH compared to patients with grade 1-2 TH. This result is parallel to the studies showing a positive relation between recurrent tonsillitis and TH which develops in relation with recurrent tonsillitis and EBV in children. Although we could not show this relation at the tissue level, the rate of grade 3-4 TH in EBV LMP-1 positive patients was found to be higher compared to EBV LMP-1 negative patients. We think that a significant relation may be found in a larger series.

In our study, the rate of asymmetric tonsils was found to be 16.7%. In the literature, Cinar F (16) reported the rate of asymmetric tonsils to be 6.69% in 792 tonsillectomy patients and Van Lierop et al. (17) reported the rate of asymmetric tonsils to be 7.6%. Harley et al. (18) found the rate of asymmetric tonsils to be 18.2% in 258 TH patients aged between 2 and 18 years. This rate is similar to our rate. In this study, objective volume measurements were used in contrast to our study.

In contrast, the study performed by Howard et al. (19) compared specific measurements and transparent volume measurements in 34 children aged between 2 and 9 years and showed that the measurements were compatible with each other.

We found no significant relation between the state of tonsillary hyperplasia (symmetric or asymmetric) and VCA IgG and VCA EBNA. Since the number of patients with asymmetric TH was low, more patients and supportive studies are needed to obtain statistically significant results.

Conclusively, our study shows that EBV infection occurs at an earlier age in our country compared to developed countries and a positive relation is found between TH and previous EBV infection and draws attention to the importance of the tonsils as a reservoir for pediatric carrier state. It may be thought that this importance will increase further when it is evaluated together with recent studies emphasizing the relation of EBV virus with many diseases.

Conflict of interest: None declared.

References

1. Dias EP, Rocha ML, Carvalho MO, Amorim LM. Detection of Epstein-Barr virus in recurrent tonsillitis. Braz J Otorhinolaryngol 2009; 75: 30-34.
2. Endo LH, Ferreira D, Montenegro MC, Pinto GA, Altemani A, Bortoleto AE Jr, Vassallo J. Detection of Epstein-Barr virus in tonsillar tissue of children and the relationship with recurrent tonsillitis. Int J Pediatr Otorhinolaryngol 2001; 58: 9-15.
3. Nadal D, Blasius M, Niggli FK, Meier G, Berger C. Epstein-Barr virus (EBV) DNA levels in palatine tonsils and autologous serum from EBV carriers. J Med Virol 2002; 67: 54-58.

4. Chagas CA, Endo LH, Dos-Santos WL, Pinto GA, Sakano E, Brousset P, Vassallo J. Is there a relationship between the detection of human herpesvirus 8 and Epstein-Barr virus in Waldeyer's ring tissue? Int J Pediatr Otorhinolaryngol 2002; 70: 1923-1927.
5. Sahin F, Gerceker D, Karasartova D, Ozsan TM. Detection of herpes simplex virus type 1 in addition to Epstein-Barr virus in tonsils using a new multiplex polymerase chain reaction assay. Diagn Microbiol Infect Dis 2007; 57: 47-51.
6. Lawrense SY, Rickinson AB. Epstein-Barr: 40 years on. Nat Rev Cancer 2004; 4: 757-768.
7. Ulff-Møller CJ, Nielsen NM, Rostgaard K, Hjalgrim H, Frisch M. Epstein-Barr virus-associated infectious mononucleosis and risk of systemic lupus erythematosus. Rheumatology (Oxford) 2010; 49: 1706-1712.
8. Levin LI, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Ann Neurol 2010; 67: 824-830.
9. Brodsky L. Modern assessment of tonsils and adenoids. Pediatr Clinic N Am 1989; 36: 1551-1569.
10. Morris MC, Edmunds WJ, Hesketh LM, Vyse AJ, Miller E, Morgan-Capner P, Brown DW. Sero-epidemiological patterns of Epstein-Barr and Herpes simplex (HSV-1 and HSV-2) viruses in England and Wales. J Med Virol 2002; 67: 522-7.
11. Dogan B, Rota S, Gurbuzler L, Bozdayi G, Ceyhan MN, Inal E. The correlation between EBV viral load in the palatine tonsils of patients with recurrent tonsillitis and concurrent serum titers of VCA-IgG. Eur Arch Otorhinolaryngol 2010; 267: 143-148.
12. Endo LH, Vassallo J, Sakano E, Brousset P. Detection of Epstein-Barr virus and subsets of lymphoid cells in adenoid tissue of children under 2 years of age. Int J Pediatr Otorhinolaryngol 2002; 66: 223-226.
13. Koseoglu RD, Filiz N, Aladag I, Güven M, Eyibilen A. Kronik tekrarlayıcı tonsillit-adenotonsiller hipertrofide Epstein-Barr virus ve herpes simpleks virüs tip 1 analizi. Ankara Üniversitesi Tıp Fakültesi Mecmuası 2007; 604: 156-162.
14. Hug M, Dorner M, Fröhlich FZ, Gysin C, Neuhaus D, Nadal D, Berger C. Pediatric Epstein-Barr virus carriers with or without tonsillar enlargement may substantially contribute to spreading of the virus. J Infect Dis 2010; 202: 1192-1199.
15. Al-Salam S, Dhaheri SA, Awwad A, Daoud S, Shams A, Ashari MA. Prevalence of Epstein-Barr virus in tonsils and adenoids of United Arab Emirates nationals. Int J Pediatr Otorhinolaryngol 2011; 75: 1160-1166.
16. Cinar F. Significance of asymptomatic tonsil asymmetry. Otolaryngol Head Neck Surg 2004; 131: 101-103.
17. van Lierop AC, Prescott CA, Fagan JJ, Sinclair-Smith CC. Is diagnostic tonsillectomy indicated in all children with asymmetrically enlarged tonsils? S Afr Med J 2007; 975: 367-370.
18. Harley EH. Asymmetric tonsil size in children. Arch Otolaryngol Head Neck Surg 2002; 128: 767-769.
19. Howard NS, Brietzke SE. Pediatric tonsil size: objective vs subjective measurements correlated to overnight polysomnogram. Otolaryngol Head Neck Surg 2009; 140: 675-681.