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Original Article / Çalışma - Araştırma

Presence of herpesviruses in adenoid tissues of children with adenoid hypertrophy and chronic adenoiditis

Adenoid hipertrofisi ve kronik adenoiditli çocuklarda adenoid dokuda herpesvirüs varlığı

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Objectives: The aim of study was to determine the presence of some of the herpesviruses including herpes simplex virus (HSV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV) in adenoid tissues of children with adenoid hypertrophy (AH) and chronic adenoiditis (CA) and to investigate the potential role of the herpesviruses in patogenesis of AH and CA.

Patients and Methods: A total of 72 patients (41 boys, 31 girls; mean age 4 years and 2 months; range 2 to 9 years) who underwent adenoidectomy or adenotonsillectomy (with or without placement of a ventilation tube) in our clinic between October 2007 and May 2008, were included. The patients were divided into two groups, as AH group (n=42) and the CA group (n=30). Adenoid tissues collected from patients in both groups were analyzed by polymerase chain reaction (PCR) for the presence of HSV, EBV and CMV-DNA.

Results: The results of the PCR indicated that 33.3% in the AH group and 36.6% in the CA group were herpesvirus DNA positive. Among the herpesviruses studied, HSV-DNA was detected at the highest level (14.2% and 16.6%, respectively) in both groups, although the difference between the groups was not significant. EBV-DNA positiveness was 11.9% and CMV-DNA was 4.7% in the AH group, whereas, EBV-DNA positiveness was 13.3% and CMV-DNA was 6.6% in the CA group.

Conclusion: Herpesviruses were determined at a high rate in adenoid tissue of children with AH and CA, suggesting that there may be a potential relationship between the presence of herpesviruses and occurrence of AH and CA in children. However, more extensive studies are required to elucidate the role of herpesviruses in the pathogenesis of AH or CA.

Key Words: Adenoid hypertrophy; chronic adenoiditis; herpesviruses.

Amaç: Bu çalışmanın amacı, adenoid hipertrofisi (AH) ve kronik adenoiditli (KA) çocuklarda adenoid dokuda herpes-simpleks virüs (HSV), Epstein-Barr virüsü (EBV) ve sitomegalovirüs (CMV) dahil olmak üzere, bazı virüslerin varlığını belirlemek ve AH ve KA patogenezinde herpesvirüslerin potansiyel rolünü araştırmaktır.

Hastalar ve Yöntemler: Ekim 2007-Mayıs 2008 tarihleri arasında, kliniğimizde ventilasyon tüpü ile veya olmaksızın adenoidektomi veya adenotonsillektomi yapılan toplam 72 hasta (41 erkek, 31 kız; ort. yaş 4 yıl ve 2 ay; dağılım 2-9 yıl) çalışmaya dahil edildi. Hastalar AH grubu (n=42) ve KA grubu (n=30) olmak üzere, iki gruba ayrıldı. Her iki gruptan alınan adenoid doku örnekleri, HSV, EBV ve CMV-DNA varlığını saptamak açısından, polimeraz zincir reaksiyon (PCR) yöntemi ile analiz edildi.

Bulgular: Polimeraz zincir reaksiyon sonuçları, AH grubunda %33.3 ve KA grubunda %36.6 herpesvirüs DNA pozitifliği olduğunu gösterdi. Çalışılan herpesvirüsler arasında her iki grupta da en yüksek düzeyde bulunan tür, HSV-DNA idi (sırasıyla %14.2 ve %16.6), buna karşın, gruplar arasındaki fark, anlamlı değildi. AH grubunda EBV-DNA pozitifliği %11.9 ve CMV-DNA pozitifliği %4.7 iken, CA grubunda EBV-DNA pozitifliği %13.3 ve CMV-DNA pozitifliği %6.6 idi.

Sonuç: Adenoid hipertrofisi ve KA olan çocukların adenoid dokusunda yüksek düzeyde herpesvirüs varlığı tespit edildi, bu da, çocuklarda herpesvirüs varlığı ve AH ve KA gelişimi arasında muhtemel bir ilişki olabileceğini düşündürmektedir. Ancak, herpesvirüslerin AH ve KA patogenezinde oynadığı rolü açıklığa kavuşturmak için, daha kapsamlı çalışmalara gereksinim vardır.

Anahtar Sözcükler: Adenoid hipertrofisi; kronik adenoidit; herpesvirüsler.

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Adenoid hypertrophy (AH) is a common cause of nasal obstruction in the pediatric population. Complications associated with adenoid hypertrophy include learning difficulties, behavioral changes, and in severe cases, failure to thrive, pulmonary hypertension, and secondary right-side cardiac hypertrophy.[1] In addition, AH can affect patient well-being, causing sleep disturbances, chronic rhinitis, speech and swallowing disorders and emotional distress.[2] Adenoidectomy is among the most common surgical procedures for treatment of AH in children. Adenoidectomy is recommended for the treatment of upper airway obstruction and recurrent or chronic adenoiditis, which may be present as chronic rhinosinusitis or chronic otitis media with effusion.[3]

Adenoids can be infected acutely or chronically by a number of microorganisms due to their anatomical location.[4] In addition, it has been demonstrated that the adenoids may serve as reservoirs of some types of herpesviruses.[5,6] Three herpesviruses including herpes simplex virus (HSV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are widespread among people.[7] Similar to other herpesviruses, following a primary infection, these viruses cause latency in different organs and reactive infection may occur when the immune system of the body is compromised. The face and cranial area are the most important regions where latency occurs.[7,8] Adenotonsillar tissue is often a reservoir for replicating EBV and may enlarge secondary to EBV proliferation, leading to EBV-related lymphoid hyperplasia. The EBV occurs in the nasopharynx, salivary glands, and epithelial cells of the parotid and causes latency in B cells infiltrating the oropharyngeal mucosa.[9] The herpes simplex and Varicella zoster viruses cause latent infections in the cranial nerve ganglia. Reactivation of HSV is a common problem in society and is a predominant cause of orolabial infections. The salivary glands are the most important tissue in which CMV latency occurs.[8,9]

Chronic adenotonsillitis and adenotonsillar hypertrophy are different diseases of the same tissues with different clinical and histopathological features.[10] Although the effect on the occurrence of AH and CA by microbial agents inhabiting the upper respiratory tract is generally accepted, contradictory data, especially about the effect of herpesviruses is available. The aim of the present study was to determine the presence of some of the herpesviruses including HSV, EBV, and CMV in adenoid tissues of children with AH or CA and to investigate the potential role of the herpesviruses in pathogenesis of AH and CA.

PATIENTS AND METHODS

In the study, adenoid samples were collected from 72 children (41 boys, 31 girls; mean age 4 years and 2 months; range 2 to 9 years) who underwent adenoidectomy or adenotonsillectomy (with or without placement of a ventilation tube) between October 2007 and May 2008 at the Department of Otolaryngology, Faculty of Medicine, Firat University, Elazığ, Turkey. Data collected included patient age, sex, indication for surgery, types of operation performed. Informed consent was obtained from parents prior to inclusion in the study in accordance with the ethical standards of the institutional ethics committee.

Forty-two of the children who were experiencing dysphagia, mouth breathing, snoring and hyponasal voice without recurrent adenotonsillitis and/or symptoms of obstructive sleep apnea secondary to adenoid hypertrophy were assigned as AH group. The remaining patients who were experiencing chronic rhinosinusitis or chronic otitis media with effusion were assigned as CA group. Diagnosis of AH and CA were made by history, ear nose and throat (ENT) and radiological examination.

In all cases, adenoids were removed with a curette under general anesthesia by one of several senior otolaryngologists. The adenoid specimen was sent to the microbiology laboratory. Tissue samples were washed with phosphate buffered saline (PBS) three times since herpesviruses are commonly found in saliva. After washing, tissues were homogenized. Each sample was divided into two equal parts and stored at –80 ºC until used for polymerase chain reaction (PCR) and virus-culture PCR analysis.

Isolation of DNA and PCR analysis

Herpes simplex virus-1 McKrae strain, Raji cells transformed with the EBV and serum samples known to contain CMV were used as positive controls. DNAs from a portion of the clinical samples and the positive controls were isolated using as a commercial DNA extraction kit (Wizard Genomic DNA Purification System, Promega Corp., Madison, WI). The rest of the tissue samples were stored at –80 ºC.

Table 1. Herpesviruses primers used for polymerase chain reaction analyses

Pathogens	Sequences of the primers	The amplification products
HSV[a]	5′-CTG GTC AGC TTT CGG TAC GA-3′	342 bp
	5′-CAG GTC GTG CAG CTG GTT GC-3′	
EBV	5′-GGC TGG TGT CAC CTG TGT TA-3′	239 bp
	5′-CCT TAG GAG GAA CAA GTC CC-3′	
CMV	5′CCT AGT GTG GAT GAC CTA CGG GCC A-3′	249 bp
	5′-CAG ACA CAG TGT CCT CCC GCT CCT C-3′	

HSV: Herpes simplex virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; bp: Base pair; a: This target sequence is specific to HSV types 1 and 2 glycoprotein B gene and is known not to code for any other human herpesviruses.

Polymerase chain reaction analysis was performed in a total reaction volume of 50 μl containing 10 μl of 10X PCR buffer (100 mM Tris-HCl, pH 8.0, 500 mM potassium chloride, 15 mM magnesium chloride), 250 μm of each of the four deoxynucleotide triphosphates, 1 U *Taq* DNA polymerase (Promega Corp., Madison, WI), 1 pM each of the primers described in Table 1 and 3 μl template sample DNA.[11] Amplifications were performed at 94 ºC for 5 min and then 34 cycles of denaturation at 94 ºC for 1 min, annealing at 60 ºC for 1 min and synthesis at 72 ºC for 2 min. The amplification products were visualized on a 2% agarose gel by ethidium bromide staining. For assurance of accuracy of the PCR results, the herpesviruses positive samples were double-checked by a second PCR.

For determination of the detection limit of PCR, the PCR product was amplified from HSV-1 McKrae strain, as described above. The amplified fragment of 342 bp was purified using DNA purification system (Promega). Then, 4 μl of the purified DNA was ligated to 10 ng of pGEM-T Easy Vector System (Promega). Tenfold dilutions of the recombinant plasmid were tested with PCR.

We included negative (sterilized dH_2O) and the positive controls (the control viruses) in each experiment to exclude false positive and false negative results.

Virus culture-PCR analysis

The PCR-positive tissue samples were inoculated to the Vero cells grown in 24-well plates. After 62 hour from inoculation, the cells were frozen at –20 °C and thawed at 37 °C. After three blind passages, the supernatants were collected and clarified by centrifuging at 3000 g/5 min. DNA extraction and PCR analysis of the supernatants were carried out for only HSV, as described above.

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, Chicago, Ilinois, USA) for Windows version 10.0 computer software. The relationship between positivity for herpesviruses and groups (42 samples from adenoid hypertrophy group and 30 samples from chronic adenoiditis group) was analyzed using Chi-Square test. *P*-values below 0.05 were considered statistically significant.

RESULTS

The patients with adenoid hypertrophy and those with chronic adenoiditis had similar demographical features, except for the types of operation performed. There was no significant difference in the age and sex distribution between the groups. In all the cases, adenoidectomy was performed. In addition to adenoidectomy, 36 patients had tonsillectomy, 18 had placement of a ventilation tube and 14 patients had tonsillectomy and placement of a ventilation tube (Table 2).

In the PCR carried out with the recombinant pGEM-T plasmid, the detection limit of PCR was about 50 copies per run.

Results of PCR analysis of the 72 samples showed that genomes of HSV in 11 (15.2%), EBV in nine (12.5%) and CMV in four (5.5%) were present. One (1.3%) of these samples was positive with both HSV and EBV-DNA. Therefore 25 (34.7%) of the samples were infected with any of the herpesviruses.

In the adenoid tissue samples, any of the herpesviruses genomes were determined in 33.3% (14/42) of the AH group and in 36.6% (11/30) of the CA group ($p>0.05$). The most common detected virus genomes in both groups were HSV. More specifically, the HSV genomes were detected from

Table 2. Patient demographics

	Adenoid hypertrophy group (n=42)		Chronic adenoiditis group (n=30)	
	n	Mean±SD	n	Mean±SD
Variable				
Age, (years)		4.1±3.6		4.4±4.5
Gender				
Female	19		12	
Male	23		18	
Types of operation performed, no. of patients				
Adenotonsillectomy	27		9	
Adenoidectomy	4		0	
Adenotonsillectomy + ventilation tube	6		8	
Adenoidectomy + ventilation tube	5		13	

SD: Standard deviation.

16.6% (7/42) in the AH and 16.6% (5/30) in the CA. The CA group did not significantly differ in the presence of HSV genome from the AH group. The least detected genomes of virus in both groups were CMV (Table 3). In both AH and CA groups, there was no significant difference between the numbers of the adenoid tissue samples infected with EBV and CMV ($p>0.05$).

In the cell culture–PCR analysis assay performed with Vero cells, seven of the HSV PCR-positive 11 samples were positive for HSV.

DISCUSSION

Adenoids or pharyngeal tonsils and palatine tonsils are components of the Waldeyer's ring, which is strategically located to perform regional immune functions. The adenoids are believed to play a role in several infectious and non-infectious upper airway illnesses. Due to their hypertrophy, they may be implicated in the etiology of otitis media, rhinosinusitis, adenotonsillitis, and chronic nasal obstruction. Infection and hypertrophy are the parts of the immunological reaction of the palatine and pharyngeal tonsils.[12] The pathogenesis of AH and CA has not been completely understood. However, the relatively high incidence of microorganism isolation confirms that a number of pathogens could be responsible for the occurrence of the disease.[4-6,13]

The three herpesviruses studied in the present research are widespread in society, as all other herpesviruses are. These viruses frequently cause latency in different organs and tissues.[7,8] The HSV causes latent infections in sensory ganglia in the head. Reactivation of HSV is a general problem in society and is a predominant cause of orolabial infections. The salivary glands are the most important tissue where CMV latency occurs. The EBV multiplies in the nasopharynx,

Table 3. Distribution of herpesviruses genomes isolated with polymerase chain reaction analysis of the adenoid tissue samples

	Adenoid hypertrophy group (n=42)		Chronic adenoiditis group (n=30)	
	n	%	n	%
HSV	6	14.2	5	16.6
EBV	5	11.9	4	13.3
CMV	2	4.7	2	6.6
HSV+EBV	1	2.3	0	0
Total	14	33.3	11	36.6

HSV: Herpes simplex virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus.

salivary glands, and epithelial cells of the parotid, resulting in latency in B cells infiltrated into the oropharyngeal mucosa.[8]

In previous studies, the EBV was detected in tonsils and adenoids in about 30% of cases, while in no case was HSV was found in these tissues.[5,6,12] However, in a recent study, the presence of HSV-1 in tonsil tissues with hyperplasia was confirmed in 7.4% of the samples.[14] In another study with 42 patients with acute tonsillitis, Tanaka[15] found that four cases (9.5%) had been caused by HSV. In five (11.9%) of the cases, EBV was shown to play a role in the etiology of tonsillitis. In a recent report, EBV and CMV were localized in both adenoids and tonsils in 92% and 37% of children, respectively, with the virus detectable by quantitative PCR.[16] In our study, the most frequently isolated virus genome in both groups was HSV. The HSV genomes were detected from 16.6% (7/42) in the AH group and 16.6% (5/30) in the CA group ($p>0.05$). The least isolated genomes of virus in all groups were CMV (4.7% in AH 6.6% in CA). The EBV was detected in AH group and in CA group 14.2% and 13.3%, respectively. We think that the difference between the results of the studies may have resulted from the particular characteristics of populations or the diversity of the methods employed.

It should be noted that the PCR assay relies on the detection of genetic material, which is very sensitive in detecting herpesviruses and other viruses, regardless of the agents' viability. It could be argued that the herpes virus DNA might have remained from an earlier infection. Thus, virus culture-PCR analysis for HSV was carried out on PCR-positive samples. The results of the assay revealed the presence of infective viruses in most of the PCR-positive samples. Lack of live HSV in all of HSV PCR-positive samples could be explained by the fact that sensitivity of virus detection might have been lower in cell culture than PCR or live viruses contained in the tissues had become inactive until the time of analysis.

There are certain important limitations to our study. We did not evaluate the status of the herpesviruses in adenoid tissues of the healthy control subjects. In the study, adenoid samples were collected from children who underwent adenoidectomy due to AH or CA. If herpes virus ranges could be detected in healthy children's adenoid tissues as the control group, a more informative comparison could have been made between healthy and infected tissues. Nevertheless, we could not build the control group from healthy children's adenoid tissues in view of ethical problems. Both in the AH or CA groups, there were similar ranges of herpesvirus positivity. Thus, it can be argued that herpesviruses may have a partial share in the pathogenesis of AH or CA and they may have a similar influence in the occurrence of these two clinical conditions.

As noted above, although there is a consensus about the presence of EBV and CMV in adenoid or tonsil tissues and the possible roles of these viruses in diseases, the presence and role of HSV are open to discussion. We believe that the most important origins of the presence of EBV and CMV in the tonsil and adenoid tissues are the lymphocytes infected with EBV and CMV. Because adenoid and tonsil tissues are components of the Waldeyer's ring, these tissues of the lymphatic system can be infected via the lymphocytes infected with these viruses. HSV, particularly HSV-1, is a virus commonly found in saliva and saliva is most common transmission route of infection. Herpes simplex virus has several different cell surface receptors. Therefore, HSV has a wide tissue tropism and may infect a large number of different tissues. However, we still lack information about whether adenoid tissue has HSV receptors or these tissues are infected by the saliva containing HSV.

In conclusion, herpesviruses were detected at a high rate in the adenoid tissues of children with AH or CA. Therefore, it can be suggested that there may be a potential relationship of the presence of herpesviruses with AH and CA. However, the present study is a preliminary study and more extensive studies are required to elucidate the role of herpesviruses in the pathogenesis of AH or CA. Futhermore, for more solid results, we believe that determination of the presences of the herpes virus proteins by immunohistochemical methods and the viral mRNAs by reverse transcription-PCR, especially HSV, in infected tissues will be more meaningful.

Declaration of conflicting interests

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