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Original Article

Molecular Detection of Adenoviruses in the Sinus Tissues of Patient by Nested-PCR in Shiraz, Southwest Iran

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ABSTRACT

Background and Aims: Rhinosinusitis is an inflammation of the mucous membrane that may be caused by infectious agents such as bacteria, fungi and viruses. Few studies have been carried out on the role of viruses in Rhinosinusitis patients. The aim of this study was the molecular detection of Adenoviruses in sinus tissues by nested polymerase chain reaction (PCR) in Shiraz.

Materials and Methods: In the present study, 103 paraffin-embedded biopsy specimens of sinus tissues were subjected to DNA extraction and tested for adenovirus DNA using Nested PCR. The amplification of a *β-globin* gene by PCR-based method was used to confirm the quality of extracted DNA.

Results: A total of 103 samples of sinus tissues were examined. Of these patients, 50 (48.54%) were male and the rest were female (51.46%). The patients' age ranged between 2 and 82 years and the mean age was 42.15±1.56 years. The adenovirus DNA was detected in 13 of 103 (12.6%) samples.

Conclusions: The results of this study showed that Adenoviruses have high prevalence in rhinosinusitis patients. As a results, it is an important to investigate clinical significance of viral infections especially Adeno viruses in these patients.

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Introduction

Rhinosinusitis is one of the most prevalent problems faced in general medical practice. It can be acute or chronic, and it can be caused by infectious agents, allergies, air pollution, or structural problems in the nose [1]. Viral agents play an important role in the development of this disease and acute sinusitis is one of the most common complications of viral infections [2]. Also, It is caused by bacteria growing in the sinuses [3]. Viruses such as human adenovirus (HAdV), human rhinoviruses (HRVs), coronaviruses (CoVs) and parainfluenza virus (PIV) have been isolated from 10% to 13% of the maxillary sinus aspirates in patients with acute maxillary sinusitis [4, 5]. HAdV are non-enveloped viruses containing a double stranded DNA genome. The clinical manifestations of adenoviral disease are variable based on the age and immune competence of the host. These viruses have been recognized as pathogens that cause a broad spectrum of illnesses including gastroenteritis, respiratory disease, conjunc-tivitis, hemorrhagic cystitis, and exanthema [6]. HAdV are an important cause of mild upper respiratory tract illness, however, they can also produce severe pneumonia and other complications. They have a worldwide distribution, and their infections can occur throughout the year [7]. Transmission of these viruses can appear through the conjunctiva, a fecal-oral route, aerosolized droplets and exposure to infected tissues [8].

The role of infectious agents in the development of sinusitis has been scheme for many years. Viral agents can contribute to the development of this disease. Several studies have shown the presence of viruses in the sinuses using viral culture techniques [9-14]. Diagnosis of HAdV infections is currently based on virus isolation in cell culture, antibody detection or antigen detection by immunofluorescence. However, these methods have been limited and the results are sometimes difficult to interpret. Therefore, the PCR-based technique is a rapid and sensitive assay to detect HAdV DNA [15]. The aim of this study was to understand the molecular detection of HAdV in sinusitis patients, using the Nested-PCR.

Materials and Methods

In this cross-sectional study, paraffin-embedded biopsy specimens were collected from 103 patients with sinusitis symptoms, who had referred Khalili hospital affiliated to Shiraz University of Medical Sciences during the years 2012-2014. This study was approved by the Ethics Committee of the Shiraz University of Medical Sciences. Consent was taken from all patients.

DNA extraction

Five sections (10 µm) were cut from each paraffin block and placed in a 1.5 ml micro tube. The samples were deparaffinized by adding 1200 µl of xylene to the 1.5 ml tubes containing the tissue section. After tube vortex and incubation for 5 min at room temperature,

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the tubes underwent centrifugation at 14000 rpm for 5 min. Then, the supernatant was removed and 1000 µl of absolute ethanol was added to each tube. Finally, the tubes were centrifuged at 14000 rpm for 5 min and the supernatant was removed; both steps were repeated. In the next step, the tubes were incubated at 37°C on a heating block until the total evaporation of the ethanol. The DNA was then extracted using a QIAamp DNA minikit (Qiagen, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20˚C until testing [16].

Molecular detection

All extracted DNA were initially subjected to polymerase chain reaction (PCR) with consensus primers PCO3/PCO4 (*β-globin*) to confirm the quality of the extracted DNA (Table 1). PCR was performed in a total volume of 25 µL, containing 1mM $MgCl_2$, 200 µM (each) deoxyribonucleotide triphosphates solution (dNTPs), 1X reaction buffer10x, 1U Taq DNA polymerase (Sinaclon, Iran) and 1 µM each specific primer. The reaction mixture was subjected to thermal cycler (Eppendorf Mastercycler) with the following program: 1 cycle at 94˚C for 10 min followed by 35 cycles at 94˚C for 1 min, 44˚C for 1 min,72˚C for 1 min, and a final extension at 72˚C for 10 min. Then, amplification of HAdV using specific primers was performed on samples which were positive for β-globin gene (Table 1).

PCR amplification was conducted using 50µl reaction volumes containing 45 µl of reaction mixture containing 1 mM $MgCl_2$1x of PCR buffer 10x, 0.1 mM each deoxy nucleotide tri phosphate (dNTP), 0.4 pmol/µl of each primer, 1U of Taq DNA polymerase and 5 µl of nucleic acid extract with the following setting: 1 cycle at 94˚C for 10 min followed by 35 cycles at 94˚C for 1 min, first round nested PCR 55˚C and two round nested PCR 60˚C for 1 min and 72˚C for 1 min, and a final extension at 72˚C for 10 min. A total of 5 µl of the first-round product was used in the second round of amplifications. Then PCR products were detected on 1.5% agarose gel electrophoresis followed by staining with ethidium bromide.

Table 1. Primers used for internal control and detection of HAdV by nested-PCR

	Primer	5' to 3' Sequence	Size (bp)	Reference
β-globin	PCO3	5′- ACACAACTGTGTTCACTAGC-3′	110	Mahmoudvand, 2017 [17]
	PCO4	5′- CAACTTCATCCACGTTCACC-3′		
Adenovirus	Hexon1-F	5′- GCCGAGAAGGGCGTGCGCAGGTA-3′	161	Moattari, 2014 [18]
	Hexon1-R	5′-TACGCCAACTCCGCCCACGCGCT-3		
	Hexon2-F	5′- TGACTTTTGAGGTGGATCCATGG-3′	107	Moattari, 2014 [18]
	Hexon2-R	5′- GGTCTCGATGACGCCGCGGTGC-3′		

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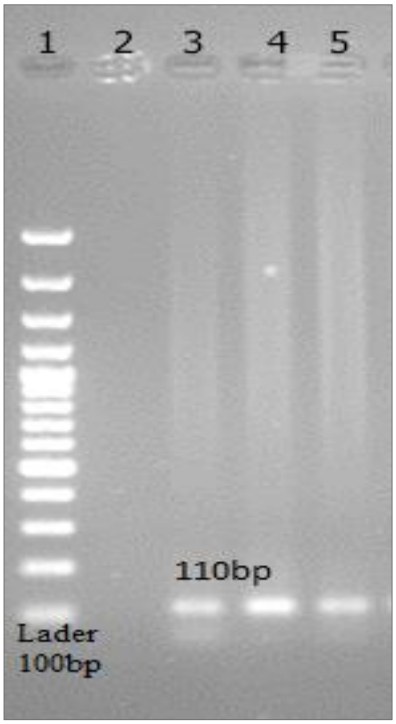

Fig. 1. Photographs of gel electrophoresis, PCR analysis of DNA samples extracted from sinusitis tissue using *β- globin* primers. Lane 1: DNA ladder 100 bp; Lane 2: Negative control; Lane 3-5; Samples 1-3.

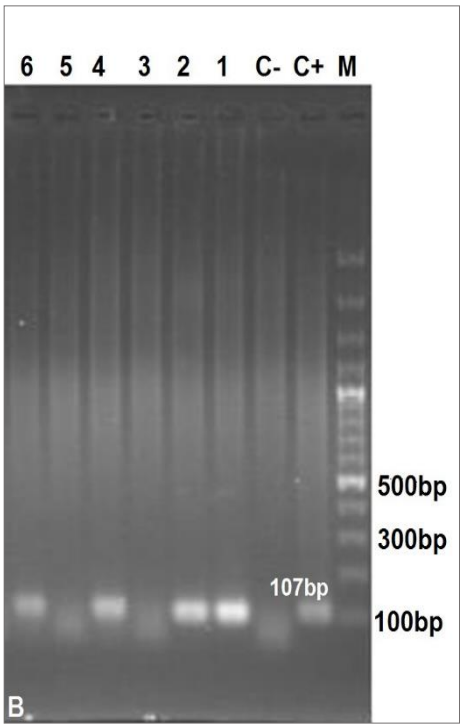

Fig. 2. Photographs of gel electrophoresis: PCR analysis of DNA samples extracted from sinusitis tissue using Adenovirus primers. M: DNA ladder 100 bp; C+: Positive control; C-: Negative control; Lane 1-6: Samples 1-6.

Statistical analysis

Data were analyzed using SPSS 21(SPSS Inc., Chicago, IL, USA) software. Fisher's exact test was used for data analysis. A p-value below 0.05 was considered statistically significant.

Results

A total of 103 samples of sinus tissues were examined for the presence of HAdV in our investigation. Of these patients, 50 (48.54%) were male and the rest were female (51.46%). The patients' age ranged between 2 and 82 years and the mean age was 42.15±1.56 years. The HAdV DNA was detected in 13 out of 103 (12.6%) samples (Figures 1, 2). Of the 13 samples that were positive for HAdV genome, 8 (61.53%) cases were male and the rest were female (38.47%). The age range of the samples containing the HAdV genome was between 18 and 82 years and the mean age was 40.07±2.03 years. Of 13 samples, 12 cases (92.30%) ranged between 20 and 60 years old. The difference was not statistically significant in terms of gender (p=0.38).

Discussion

Sinusitis refers to infection, inflammation or swelling of the sinuses and nasal cavity. They affect millions of adults in the United States

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annually [19]. There are many factors and processes that may play a role in the etiology of this disease including infectious agents [20]. Viruses are considered as the major etiological agents in sinusitis and directly implicated in about 10% of sinusitis cases [4, 5]. Upper respiratory tract viral infections and allergicrhinitis are the most common initiating factors in sinusitis. Among the various families of viruses, HRVs, CoVs and influenza viruses (flu) are most pathogenic in the creation of this disease; others are caused by HAdV, PIV, respiratory syncytial virus (RSV) and human metapneumovirus [2, 21]. This condition may also be caused by bacteria, although this is rare and occurrs in roughly 0.5 to 2 percent of the cases [22]. Recent worldwide epidemics of respiratory infections due to HAdV have resulted in renewed interest in this virus [23]. Respiratory infections included by HAdV cause significant morbidity and mortality, with case fatality rates as high as 12%. The increase in cases of HAdV infection has also been reported in Asia and a viral infection fails to subside by taking antibiotics [24]. Therefore, a rapid detection and accurate identification of pathogenic agents is needed for selecting an appropriate treatment. In this study, the use of nested-PCR method has indicated around 12.6% incidence of associated HAdV infections, but in previous studies using viral culture techniques it has revealed about 13% including HAdV, RSV, and PIV [4, 5]. This finding showed that the frequency of HAdV is equal with the whole frequency of HAdV, RSV, and PIV in the study performed by culture techniques. Moreover, in a study performed by Ramadan et al., none of the 20 samples tested were positive for HAdV [25]. The cause of this difference may be due to differences in the method used for diagnosis.

The frequency of HAdV in Shiraz was 22% in throat swabs (72 out of 328 samples) that is slightly higher than the frequency of 14.4% reported by another study from Iran and several parts of the world including Oman (15%), Kenya (14%), Brazil (10%), Korea (10.3%), Australia (7.3%), Hung Kong (5.3%), Mejia (5.2%), and China (4.9%) [26]. However, the importance of our study is that we collected samples from sinus tissues. In the present study, out of 13 HAdV positive samples, 8 (61.53%) cases were male and the rest were female (38.47%). Although the frequency of HAdV DNA was higher in the sinus tissue from females than males, the difference was not statistically significant. Of 13 samples, 12 cases (92.30%) ranged between 20 and 60 years. This finding showed that the presence of HAdV in patients is not likely associated with a weakened immune system whereas epidemic of HAdV pneumo-nia have been reported since 2011, in both children and adults [24]. In our study, of 13 patients with sinusitis that were positive for HAdV DNA, 8 (61.53%) and 5 (38.47%) were affected with chronic and acute sinusitis, respectively. This finding indicated that although the frequency of HAdV DNA was higher in chronic sinusitis than acute cases, the difference was not statistically significant. Over the last few years, HAdV-3 has become the major agent of acute respiratory infection worldwide encompassing 15 to 87% of adenoviral respiratory infections [27].

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Conclusion

This study revealed that HAdV frequency in sinus tissue is very high. More accurate studies with larger sample sizes are needed to explore the prevalence of HAdV in sinusitis patients. Also, accurate identification of pathogenic agents is needed to find out a more effective treatment for this disease.

Conflict of Interest

No conflict of interest.

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