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Brief Communication

Molecular epidemiology of adenoviral conjunctivitis in Hanoi, Vietnam

Running title: ADENOVIRAL CONJUNCTIVITIS IN HANOI

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Abstract

Purpose: To investigate the serotypes of adenovirus causing conjunctivitis in Hanoi, Vietnam.

Design: Clinical diagnosis of adenoviral conjunctivitis and laboratory based experimental study.

Methods: We collected 21 conjunctival swabs from 21 different patients with a clinical presentation compatible with adenoviral conjunctivitis, in Hanoi, Vietnam. Immunochromatography and real-time PCR were performed to detect human adenovirus (HAdV). The sequence of PCR products was analyzed to determine the serotype of HAdV.

Results: Of 21 samples, HAdV DNA was detected in 14 samples (66.7%) by real-time PCR. The serotype analysis showed HAdV-8 in 11 samples (78.6%), HAdV-3 in 2 samples (14.3%) and HAdV-37 in 1 sample (7.1%). Of 11 HAdV-8 samples, 1 sample (9.1%) was prototype, and the other 10 samples (90.9%) had identical nucleotide sequence and were identified as a variant of HAdV-8.

Conclusions: HAdV-8 was found to be the predominant serotype in Hanoi, Vietnam. Most of the HAdV-8 samples were variants of HAdV-8.

Key Words: adenovirus, conjunctivitis, molecular epidemiology, real-time PCR

Epidemic outbreak of conjunctivitis often appears at different locations of the world. Human adenoviruses (HAdV) are one of the leading causes of acute conjunctivitis seen in daily practice. 1 HAdV are divided into six major subtypes and 51 different serotypes have been identified. HAdV-4, 8, 19, and 37 are commonly responsible for the epidemic keratoconjunctivitis. Rapid identification of these serotypes is helpful in the prevention and control of the epidemics.

Methods

Between June and November 2003, 21 conjunctival swabs were collected from 21 different patients with a clinical presentation compatible with adenoviral conjunctivitis, in Hanoi, Vietnam. All patients had given informed consent, according to the Declaration of Helsinki. The swabbed samples were placed in transport media for immunochromatography and DNA extraction. The immunochromatography test (Adenocheck; Santen, Inc., Osaka, Japan) was carried out according to the manufacturer's instructions. Viral DNA was extracted by using the Sumitest EX-R&D kit (Genome Science Laboratories Co., Ltd., Fukushima, Japan). To quantify the adenoviral gene, real-time PCR was performed on LightCycler instrument (Roche Diagnostics, Mannheim, Germany). The primers were designed to amplify a 554-bp fragment of adenoviral hexon gene. The sequence of PCR products was analyzed and

part of it (350-bp) was compared with the database as described. [2] RT-PCR was performed to amplify enterovirus genome including the entire VP4 region as described.[3]

Results

Of 21 samples, HAdV DNA was detected by real-time PCR in 14 samples (66.7%), in which 13 samples (61.9%) were positive for adenovirus by immunochromatography test also (Table 1). All the samples were negative for enterovirus by RT-PCR.

The HAdV gene in real-time PCR positive samples was quantified and sequenced. The copy number and serotype of HAdV in conjunctival swabs are shown in Table 2. The copy number of HAdV in immunochromatography test positive samples ranged from 9.6×106 to 1.7×109/ml. The copy number of HAdV in that immunochromatography test was negative but real-time PCR was positive was 3.1×105/ml. The serotype analysis of 14 real-time PCR positive samples showed HAdV-8 in 11 samples (78.6%), HAdV-3 in 2 samples (14.3%) and HAdV-37 in 1 sample (7.1%). Of 11 HAdV-8 samples, 1 sample (9.1%) was prototype, and the other 10 samples (90.9%) had identical nucleotide sequence and were identified as a variant of HAdV-8.

Discussion

Adenoviral conjunctivitis and enteroviral conjunctivitis are under the National Epidemiological Surveillance of Infectious Diseases, Japan and are common in Japan. Because of recent active interchange of people among countries, it is important to obtain information on viral conjunctivitis in various foreign countries, especially in the Asian area. [4]

In this study, the epidemiology of adenoviral conjunctivitis in Hanoi, Vietnam was investigated. Of 21 samples, HAdV DNA was detected in 14 samples (66.7%) and HAdV-8 was found to be the predominant serotype in this region. This result was different from that of Japan where HAdV-37 was a dominant serotype in recent years. The result shows that dominant serotype of HAdV changes geographically. Of 11 HAdV-8 samples, 10 samples (90.9%) showed the same mutation and were identified as a variant of HAdV-8. Further phylogenetic analysis is needed to identify whether it is a new genomic variant.

Immunochromatography has been regarded as a rapid and easy test to detect HAdV antigen with high specificity. HAdV DNA was detected in all the immunochromatography positive samples, which testified the high specificity of immunochromatography. HAdV DNA was detected only in 1 immunochromatography negative sample. The result of real-time PCR showed the copy number of HAdV in this sample was far less than that of immunochromatography positive samples. It seems that

the sensitivity of immunochromatography is related to the quantity of HAdV in the sample.

In this study, we investigated the molecular epidemiology of adenoviral conjunctivitis in Hanoi, Vietnam by the combination of real-time PCR and sequencing. HAdV-8 was found to be the dominant serotype in this region. Most of the HAdV-8 samples were identified as a variant of HAdV-8, which needs further phylogenetic analysis.

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Table 1. Result of real-time PCR and immunochromatography test for detection of HAdV in the patients with adenoviral conjunctivitis presentation in Hanoi, Vietnam (n=21)

real-time PCR	immunochromatography test	
	Positive (%)	Negative (%)
Positive (%)	13 (61.9)	1 (4.8)
Negative (%)	0 (0)	7 (33.3)

Table 2. Copy number and serotype of HAdV in adnoviral conjunctivitis samples collected in Hanoi, Vietnam

Sample number	Copy number/ml	Serotype
1	1.8×10^{7}	8
2	1.7×10^{8}	8v
3	9.6×10^{6}	8v
4	1.7×10^{9}	8v
5	4.9×10^{7}	37
6	8.9×10^{8}	8v
7	4.6×10^{7}	3
8	4.7×10^{7}	8v
9	6.8×10^{8}	8v
10	2.2×10^{8}	8v
11	1.6×10^{9}	8v
12	9.4×10^{8}	8v
13	7.6×10^{8}	8v
14	3.1×10^{5}	3

8v: a variant of HAdV-8