The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Molecular identification of viral agents associated with acute conjunctivitis: a prospective controlled study

Emine Akçay[a], *Ahmet Çarhan*[b], *Gözde Hondur*[c], *Zeliha Koçak Tufan*[d], *Necati Duru*[e], *Selçuk Kılıç*[f], *Ezgi Naz Ensari*[a,*], *Nagihan Uğurlu*[a], *Nurullah Çağıl*[a]

[a] *Yıldırım Beyazıt, University Faculty of Medicine, Department of Ophthalmology, Ankara, Turkey*
[b] *Yıldırım Beyazıt, University Faculty of Medicine, Department of Medical Biology, Ankara, Turkey*
[c] *Ulucanlar Eye Training and Research Hospital, Ankara, Turkey*
[d] *Yıldırım Beyazıt University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey*
[e] *Kayseri Education and Research Hospital, Ophthalmology Clinic, Kayseri, Turkey*
[f] *Turkey Public Health Institute, Ankara, Turkey*

ARTICLE INFO

Article history:
Received 8 October 2016
Accepted 29 March 2017
Available online 20 May 2017

Keywords:
Adenovirus
Conjunctivitis
Viral
PCR

ABSTRACT

Background: Viral conjunctivitis are the most frequent infections in ophthalmology clinics. The diagnosis is usually relying on clinical findings and medical history. However, topical antibiotics are often used unnecessarily addition to symptomatic treatment because of unsure agents. We aimed to detect the Adenovirus, Coxsackievirus and Enterovirus from conjunctiva and pharyngeal samples of patients.

Methods: The conjunctiva and pharyngeal samples of the patients with conjunctivitis were taken by Virocult transport media and kept at −80 °C up to study day. *Adenovirus* spp, Enterovirus 70 and Enterovirus 71, Coxsackie A24 and Coxsackie A16 were detected by real-time PCR. Samples from healthy health care workers of ophthalmology clinic were used for control group.

Results: A total of 176 samples (conjunctival and pharyngeal samples of 62 patient and 26 healthy subjects) were included. The mean age of 34 (55.7%) male and 27 (44.3%) female patients was 34 ± 17. Twenty five (40.3%) of the patients were receiving antibiotic drops at first visit. The main etiologic agent in conjunctival samples was found to be Adenovirus (46/62, 74.2%) followed by Enterovirus 70 (4/62, 6.4%) and Enterovirus 71 (4/62, 6.4%). Coxsackievirus 16 and 24 were also found in 2 patients (1/62 each, 1.6%). Pharyngeal samples were also positive for Adenovirus (20/62, 32.3%), Enterovirus 70 and 71 (7/62, 11.3% and 5/62, 8.1% respectively), Coxsackievirus 16 and 24 (2/62, 3.2% and 1/61, 1.6%).

* *Corresponding author.*
E-mail address: enazensari@gmail.com (E.N. Ensari).
http://dx.doi.org/10.1016/j.bjid.2017.03.016

Conclusions: It is very difficult in viral conjunctivitis to make clinical differentiation caused by different agents because of common clinical signs and symptoms. In routine clinical work, the viral conjunctivitis usually related with Adenovirus. But almost one fourth of the patients' conjunctivitis were not related to Adenovirus, which shows the importance of the laboratory diagnostics. True diagnosis plays an important role on prevention of contamination and unnecessary use of antibiotics in viral conjunctivitis.

Introduction

Adenoviruses, a major cause of viral conjunctivitis,[1–3] are double-stranded non-enveloped DNA viruses belonging to the family *Adenoviridae*, genus *Mastadenovirus*. Fifty-one serotypes of human adenoviruses have been recognized and classified into seven species (A-G) based on genome sequencing, phylogenetic, and biological characteristics.[4] Adenoviruses are implicated in a wide range of human diseases including pharyngoconjunctival fever, an acute and highly infectious disease characterized by fever, pharyngitis, acute follicular conjunctivitis, and regional lymphoid hyperplasia with tender, enlarged preauricular adenopathy.[1,3] The serotypes 3, 4, and 7 are frequently associated with the pharyngoconjunctival fever. Epidemic keratoconjunctivitis (EKC) is a highly contagious, severe form of conjunctivitis, which may lead to outbreaks worldwide. Adenovirus serotypes 8, 11, 19, and 37 are common etiologic agents of epidemic keratoconjunctivitis with severe symptoms, such as severe discharge, lacrimation, membrane formation, and multiple subepithelial corneal infiltrates. Acute nonspecific follicular conjunctivitis is mostly caused by human adenovirus serotype 3, 4, 7, and 14, but it does not involve the cornea and the resulting conjunctivitis is typically mild. Nonspecific follicular conjunctivitis usually resolves within a week to 10 days without treatment. Chronic keratoconjunctivitis is the rarest form of ocular adenoviral infection and is caused by adenovirus type 2, 3, 4, and 53.[3,4] Acute hemorrhagic conjunctivitis (AHC) is an epidemic form of highly contagious conjunctivitis mostly caused by Enterovirus 70 and Coxsackievirus A24 variant.[3,5] Herpes simplex virus, rubella, rubeola, varicella zoster, Epstein–Barr and Newcastle viruses are the other agents for viral conjunctivitis.[6]

The diagnosis of viral conjunctivitis is usually performed on the basis of patient history and clinical findings, although serologic and molecular laboratory diagnoses are also currently available. Unnecessary antibiotic therapy is frequently administered as a result of unknown etiology. The aim of this study was to identify the most common etiologic agents of acute conjunctivitis and determine the relationship between the etiologic agents and the clinical findings, complications, and systemic findings among acute conjunctivitis cases.

Methods

Clinical definition

A prospective, controlled clinical study was conducted to evaluate 62 consecutively enrolled patients that were admitted to the Ankara Atatürk Training and Research Hospital between July 2013 and September 2014 with suspected viral conjunctivitis (who had at least one of the following complaints and findings: hyperemia, lacrimation, foreign body sensation, discharge, burning, follicular conjunctivitis, conjunctival hemorrhages, membrane formation, eyelid swelling, or conjunctival hemorrhages) or keratoconjunctivitis (those who had punctate corneal defects or subepithelial infiltrates in addition to viral conjunctivitis findings). Twenty-six healthy volunteers working in the ophthalmology department at all times were included as controls.

This study followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from the study participants before sample collection.

Sample collection

Pharyngeal and conjunctival samples were collected from patients and controls by two experienced ophthalmologists. The samples were placed in viral transport medium (Virocult, BD) with swabs and stored at −80 °C until analysis.

Sample processing and virus identification

Molecular identification was used for Enterovirus 70/71. Coxsackievirus A16/A24v and adenovirus were determined using a real-time PCR kit (DaAn Gene Co., Ltd, Guangzhou, China) using virus-specific primers and fluorescein labeled probes. Viral DNA and RNA extraction were performed in a 200 μl sample volume using PureLink Viral DNA and RNA kit (Invitrogene, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After elution of viral DNA and RNA samples in a 50 μl elution buffer, the concentration of DNA/RNA was confirmed with Qubit® 2.0 Fluorometer (Invitrogene, Thermo Fisher Scientific, Waltham, MA, USA). The real-time PCR conditions for Enterovirus 70/71,