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Outbreak of adenovirus serotype 8 conjunctivitis in preterm infants in a neonatal intensive care unit

Y. Ersoy [a,*], B. Otlu [b], P. Türkçüoğlu [c], F. Yetkin [a], S. Aker [d], C. Kuzucu [b]

[a] Department of Infectious Diseases & Clinical Microbiology, Inonu University School of Medicine, Malatya, Turkey
[b] Department of Medical Microbiology, Inonu University School of Medicine, Malatya, Turkey
[c] Department of Ophthalmology, Inonu University School of Medicine, Malatya, Turkey
[d] Infection Control, Inonu University School of Medicine, Malatya, Turkey

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SUMMARY

Background: Adenovirus keratoconjunctivitis outbreaks have rarely been reported in preterm infants. An outbreak of adenovirus conjunctivitis occurred between 15 January and 25 February at a neonatal intensive care unit of a university hospital in Turkey.
Aim: To describe the evolution, investigation and management of the outbreak.
Methods: Adenovirus type 8 was identified in 14 samples by polymerase chain reaction analysis. A case–control study was performed to determine the risk factors.
Findings: Fifteen preterm neonates, five healthcare workers (HCWs) and four parents suffered from conjunctivitis signs such as lacrimation, swelling and redness of the eye. A retinopathy of prematurity (ROP) examination was found to be the most important risk factor for adenovirus conjunctivitis (odds ratio: 17.5; 95% confidence interval: 1.9–163.0; $P=0.012$). The eyelid speculum (blepharostat) used during the ROP examination was not sterilized between each patient and was found to be the cause of contamination.
Conclusion: The outbreak was controlled by measures such as barrier precautions, hand hygiene, sterilization of the blepharostat, suspending patient transfer to other units, and excluding infected HCWs for at least 15 days.
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Introduction

Epidemic keratoconjunctivitis caused by adenovirus is an acute eye infection usually seen as epidemics in the community. It presents with lacrimation, photophobia and pain. It is a highly contagious and serious disease involving the cornea and conjunctiva. Serotypes 4, 8, 19, and 37 have usually been reported as causes of epidemic keratoconjunctivitis in adult patients.[1–4]

Nosocomial adenovirus eye infection outbreaks have previously been reported in adults.[5–9] Most outbreaks have been in ophthalmology departments, and caused by inadequate disinfection of instruments and the environment; reports from neonatal intensive care units (NICUs) are rare.[6,10–13] We describe the evolution, investigation and successful control of an outbreak of conjunctivitis caused by adenovirus type 8 in a university hospital NICU.

Methods

Setting

The outbreak occurred in an 891-bed university hospital in eastern Turkey. The NICU has 16 beds where only preterm

* Corresponding author. Address: Inonu University School of Medicine, Turgut Ozal Medical Centre, Department of Infectious Diseases and Clinical Microbiology, 44280 Malatya, Turkey. Tel.: +90 422 341 10 59; fax: +90 422 341 07 28.
E-mail address: dryasemin@hotmail.com (Y. Ersoy).

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neonates are hospitalized. Prospective active infection surveillance is undertaken, and the NICU is visited by an infection control nurse each day (except weekends). Once an outbreak was suspected, prospective and retrospective analysis was undertaken to determine whether an outbreak was indeed occurring. After a nurse on the NICU was diagnosed with adenovirus conjunctivitis, the investigation was broadened to include healthcare workers (HCWs) who were questioned about eye symptoms. Some parents of neonates also reported eye symptoms during the outbreak, and were also included in the study.

Data on babies, including gestational age, gender, birth weight, the date conjunctivitis started, underlying diseases and the examination for retinopathy of prematurity (ROP) and other ophthalmic procedures were collected from the patient files, electronic patient records and from the medical personnel. A questionnaire form was used to identity possibly affected persons and to collect information about signs and symptoms of conjunctivitis in HCWs and parents. A case–control study was performed to identify risk factors for development of adenovirus conjunctivitis in preterm infants. Neonates with findings of conjunctivitis such as lacrimation, purulent discharge and lachrymal swelling in one or both eyes who were hospitalized in the NICU between January and March 2010 with no other identified cause of conjunctivitis were defined as the cases. The control group comprised infants who had no eye infections in the NICU in the same period.

Microbiological and molecular analysis

Microbiological cultures were obtained from all infants with conjunctivitis in the NICU, from the HCWs with symptoms and the infants' parents with eye symptoms. Conjunctival swabs from both eyes were taken with dry cotton swabs and cultured on 5% blood agar and chocolate agar mediums for bacterial pathogens. In addition, conjunctival swabs were taken from the infants with conjunctivitis, from the HCWs with symptoms and from the infants' parents with eye symptoms for detection of adenovirus DNA by polymerase chain reaction (PCR). We also tested two neonates that did not show any eye symptom in the same unit to be sure of the clinical significance of the test results. Specimen transport medium (Digene, AR MED Ltd, Reading, UK) was used for collection of specimens. Pupil dilatation drops (1.0% phenylephrine and 0.2% cyclopentolate) were also analysed for adenovirus DNA. An in-house PCR method was used for detection of adenovirus DNA.[14] Highly protected AdV hexon 3–4 gene regions were amplified for the detection of adenovirus DNA.[14] The sequences of primer pair 5′-GACATGACTTTCGAGGTCGATCCCATGGA-3′ (hexon 3) and 5′-CCGGCTGAGAAGGGTGTGCGCAGGTA-3′ (hexon 4) were used. Amplification conditions were as follows: initial denaturation at 94 °C for 7 min followed by 40 cycles at 94 °C for 1 min, 57 °C for 1 min, 72 °C for 1.5 min with an additional extension step at 72 °C for 5 min. The amplified product (140 bp) was electrophoresed in 2% agarose gel, stained with ethidium bromide and visualized under UV light. Two different methods were used for genotyping of the adenovirus. The Adenoplex assay (Adenoplex, Prodesse Inc., Waukesha, WI, USA), a PCR-based method, was used for detection of adenovirus subtypes. The procedures for all these processes were performed according to the manufacturer's instructions. The second method was a sequence-based typing method where the primer sequences were selected according to Okada *et al.*[15] Finally, the adenovirus PCR-positive product was purified (QIAquick Gel Extraction kit, Qiagen, Hilden, Germany) and sequenced by using a kit (BigDye Terminator Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA) and an analyser (310 Genetic analyzer, Applied Biosystems). All data were analysed using the Clustal X (ver. 1.83) multiple alignment program. The nucleotide sequences of reference strains of each adenovirus serotype were obtained from the GenBank nucleotide sequences database. All data were analysed by BLAST analysis on the NCBI website.

Statistical analyses

Data were expressed as the mean ± SD for continuous variables and as frequencies (%) for categorical variables. SPSS version 16.0 was used for all data management and analysis. The differences of continuous variables between the groups were assessed using the independent sample *t*-test, and the chi-squared test was performed for categorical variables. Logistic regression analysis was used to examine the relationship between the significant variables. Odds ratios (ORs) were calculated for each explanatory variable. $P < 0.05$ was considered as statistically significant.

Results

A total of 15 preterm neonates, four NICU nurses, four parents, and one ophthalmology resident had conjunctivitis between 15 January and 25 February. The timings of the onset of illness among all affected individuals are shown in Figure 1. The first neonatal case presented on 15 January with lacrimation, purulent discharge and swelling. None of the neonates had fever or systemic complications during the attack, and none developed keratitis. Six patients were initially treated with topical cephazoline 5% four times daily, with no response. Adenovirus conjunctivitis was not suspected until 5 February, when one of the NICU nurses presented at the ophthalmology department with a watery discharge and red eye. Conjunctival follicles and membranes were detected and a clinical diagnosis of adenoviral conjunctivitis was made. All four infected nurses had similar presentations with red eye, watery discharge and pain, while the symptoms of the ophthalmology resident were mild. One nurse had keratitis. Four of the HCWs were free of symptoms and signs within 15 days of onset, and the other after 17 days.

A total of 16 swabs were taken for PCR and 22 for bacterial culture from the infected persons. Bacterial cultures were negative in all cases (Table I).

Infection control interventions

After the nurse was diagnosed with adenovirus conjunctivitis on 5 February 2009, contact precautions and cohorting of the patients were immediately implemented. More rigorous infection control measures were implemented on 12 February, after the first positive adenovirus PCR results were obtained. Disposable gowns and gloves were worn by HCWs and parents used for all contacts with all patients, whether infected or not. ROP examinations were temporarily discontinued. None of the materials on the bedside of a patient was transferred to others.

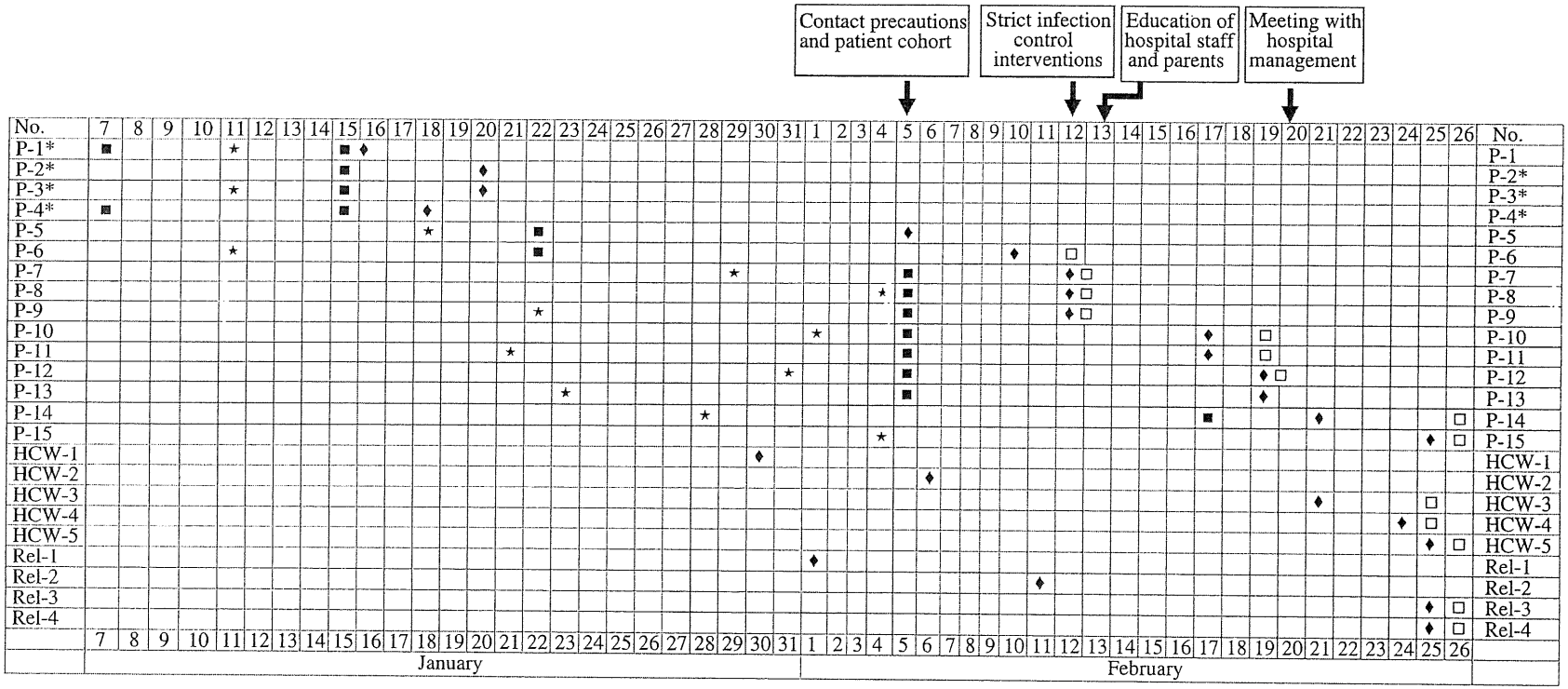


Figure 1. Temporal relationship between admission, retinopathy of prematurity (ROP) examination, onset of symptoms and polymerase chain reaction results during the outbreak. *, patients were hospitalized on 21 December 2008; ★, admission to the unit; ■, date of ROP examination; ◆, date of onset of symptoms; □, positive result of polymerase chain reaction; HCW, healthcare worker; Rel, relative; P, patient.

Table I
Results of bacterial culture and adenovirus polymerase chain reaction (PCR) in cases

Groups	Conjunctival swab for bacterial culture	Bacterial culture positivity	Conjunctival swab for PCR	PCR adenovirus positivity
Preterm neonates ($n=15$)	15	–	9	9
Healthcare workers ($n=5$)	5	–	5	3
Relatives ($n=4$)	2	–	2	2
Total ($N=24$)	22	–	16	14

Environmental surfaces such as staff desks, telephones and computer keyboards were cleaned daily with 10% hypochlorite. Patient transfers to other units in the hospital were stopped. HCWs with conjunctivitis were excluded from work for at least 15 days. Additional training on hand hygiene, barrier precautions and disinfection of equipment was given to HCWs. Family members who had contact with the neonates received education about hand hygiene and contact precautions (Figure 1). The hospital infection control committee organized a meeting on 20 February with hospital management, the head of the paediatric unit, the NICU nurse manager and the ophthalmologist who performed ROP examinations. Additional blepharostats were procured so that there were sufficient numbers to allow sterilization or high level disinfection between examinations.

The investigated risk factors for adenovirus conjunctivitis in neonates are shown in Table II. ROP examination was found to be the most important risk factor for adenovirus conjunctivitis in the NICU (OR: 17.5; 95% CI: 1.9–163.0; $P=0.012$). One week before the start of the outbreak, a number of blepharostats were lost in the main sterilization unit. As a result, ROP examinations were being undertaken using only one blepharostat which was decontaminated between patients by soaking in 10% povidone-iodine for a few minutes followed by rinsing with sterile distilled water. At the end of the working day, the blepharostat was sterilized by steam. The index case (patient 1) was discharged from the hospital on January 10 and came from home on 15 January for ROP examination, when signs of eye infection were present. Only one of the patients (patient 15) who had adenovirus conjunctivitis had not been examined using a blepharostat. Inadequate compliance with hand hygiene rules after contact with infected neonates was considered to be the most likely cause of infection for this newborn.

Conjunctival samples of all preterm neonates with conjunctivitis were positive for adenovirus, whereas two asymptomatic babies were PCR negative. Three samples from HCWs were positive: the other two HCWs may have had negative swabs because they were not sampled until the end of the second week of their illnesses. All adenoviruses were found to be genotype D and showed high homology with serotype 8. Pupil dilatation solutions were negative for adenovirus DNA. After initiation of infection control measures, six neonates, two parents and three HCWs became symptomatic, but all were judged to have been infected before control measures were implemented. There were no new cases after 25 February. During the outbreak there were no cases of pneumonia or death linked to adenovirus.

Discussion

Epidemic keratoconjunctivitis attack rates have been reported as high as 25% in medical facilities during outbreaks.[16] Most reported outbreaks have been in ophthalmology units and nursing homes.[1,16] However, our experience demonstrates that adenovirus should be kept in mind when investigating outbreaks of conjunctivitis in NICUs.

Guidelines recommend that infants with a birth weight of <1500 g or gestational age of ≤ 30 weeks and selected infants with a birth weight of 1500–2000 g or gestational age of >30 weeks with an unstable clinical course should have retinal screening examinations for ROP.[17] ROP examinations have been performed for several years in our hospital without any

Table II
Demographic parameters of the preterm neonates and controls and analysis of the risk factors

	Cases ($N=15$)	Controls ($N=18$)	P
Male	8 (53.3%)	9 (50%)	NS
Gestational age (weeks)	30.1 ± 2.5	30.5 ± 3.4	NS
Birth weight (g)	1365.3 ± 333.1	1314.9 ± 417.4	NS
Underlying diseases			
Intrauterine growth restriction	0	1 (5.6%)	NS
Respiratory distress	1 (6.7%)	2 (11.1%)	NS
Intracranial bleeding	1 (6.7%)	0	NS
Hyperbilirubinaemia	1 (6.7%)	1 (5.6%)	NS
Prematurity	11 (73.3%)	10 (55.6%)	NS
Pneumonia	1 (6.7%)	4 (22.2%)	NS
ROP examination	14 (93.3%)	8 (44.4%)	0.012

ROP, retinopathy of prematurity; NS, not significant.

previous documented or suspected adenovirus outbreaks. However, adenovirus type 8 is very contagious and resistant to most disinfectants.[18] The adenovirus was spread between preterm infants especially during the ROP examination via the blepharostat in this outbreak.

The clinical symptoms of adenovirus conjunctivitis in our patients were lachrymal swelling, lacrimation and purulent discharge; however, we did not find keratitis in any neonates. Similarly Chaberny *et al.* reported that adenovirus eye infection usually causes eyelid swelling, mucoid discharge and redness of the eye in infants.[12] Purulent discharge and oedema of the eyelids were reported in neonates during another outbreak.[13] Keratitis usually begins 3–4 days after the corneal opacities in adenovirus keratoconjunctivitis.[16] It was reported in 50% of infected adults in another outbreak, whereas only one adult had keratitis in our outbreak.[19] Adenovirus conjunctivitis in neonates may be following a different clinical course than in adult patients and it can be confused with bacterial conjunctivitis. Systemic manifestations of adenovirus type 8 conjunctivitis were reported in three of the seven infected neonates.[11] None of the neonates in the currently reported outbreak had systemic manifestations.

ROP examination was found to be a most important risk factor in this adenovirus outbreak, with a high OR (17.5; 95% CI: 1.9–163.0). The one infected patient (no. 15) who did not undergo ROP examination was probably infected via contact with the hands of a HCW.

The instruments used for eye examination and the equipment that touch the conjunctiva are classified as semi-critical devices according to Centers for Diseases Control and Prevention (CDC) guidelines.[18] Disinfection protocols for eye examination equipment usually focus on tonometers. In the 1985 CDC guideline, it was recommended that tonometer tips are wiped clean and disinfected for 5–10 min with either 3% hydrogen peroxide, 5000 ppm chlorine, 70% ethyl or isopropyl alcohol.[20] However, 3% hydrogen peroxide and 70% isopropyl alcohol were found not to be effective against adenovirus.[21] The 2008 CDC guideline recommends either 5000 ppm chlorine or 70% ethyl alcohol.[18] Chaberny *et al.* reported that critical equipment such as diagnostic instruments were sterilized or disinfected with 90% ethanol in the management of their outbreak.[12] Blepharostats contact a greater area of the conjunctiva than tonometers, increasing the risk of infection. Woodman *et al.* reported that 70% isopropyl alcohol swabs were not adequate for disinfection of a blepharostat against bacteria and adenoviruses.[22] Autoclaving of blepharostats was recommended in that study.[22] Hutchinson *et al.* reported an alternative way for the disinfection of blepharostat using chlorhexidine gluconate.[23] We could not find any special disinfection recommendation for a blepharostat in guidelines.[18,24,25] The fact that this instrument is now a well-documented cause of outbreaks indicates that evidence-based guidelines are required on cleaning and disinfecting blepharostats.

Adenovirus can be isolated from conjunctival swabs up to 9–14 days after the onset of the symptoms.[9,26] Therefore two weeks of exclusion time is usually recommended for infected personnel to prevent the transmission of adenovirus conjunctivitis.[12] Kimura *et al.* investigated the presence of adenovirus DNA in the conjunctiva before the onset of symptoms and could not demonstrate any positivity with PCR.[27] Adenovirus DNA was also not detected on the conjunctiva of healthy and asymptomatic persons.[28] Chaberny *et al.* reported that HCWs were free of signs 20–25 days after the onset of symptoms, and that PCR test results were negative at this time.[12] In our study, conjunctivitis symptoms disappeared within 15–17 days and we did not find adenovirus DNA in two nurses two weeks after the onset of symptoms.

The interventions that successfully controlled this outbreak were barrier precautions, hand hygiene, sterilization of the blepharostat, exemption of infected HCWs from work, and prevention of patient transfer to other units. Chaberny *et al.* extended the duration of alcohol based hand-rubbing to 2 min during their outbreak.[12] However, we did not change the hand hygiene protocol that had been used for several years in our hospital that was based on CDC recommendations.[29]

To conclude, adenovirus type 8 should be considered in conjunctivitis outbreaks in the NICU, and the blepharostat used during ROP examination should be sterilized between patients. Patient relatives and all personnel must obey hand hygiene rules.

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Conflict of interest statement
None declared.

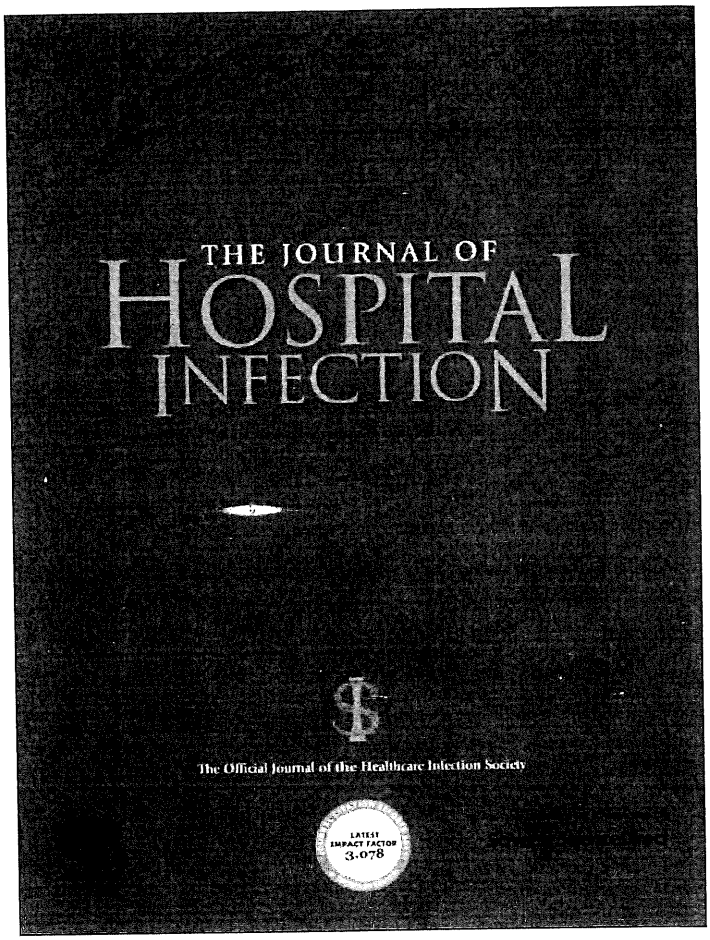
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