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BRIEF COMMUNICATION

MOLECULAR EPIDEMIOLOGY OF ADENOVIRUS CONJUNCTIVITIS IN RIO DE JANEIRO, BRAZIL, BETWEEN 2004 AND 2007

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SUMMARY

Viral conjunctivitis is a common, highly contagious disease often caused by adenovirus. We investigate the frequency of adenoviral conjunctivitis in the population of Rio de Janeiro, Brazil, between March 2004 and May 2007 and identified the predominant serotype circulating among this population. Seventy-five ocular swabs were collected from 66 patients with clinical presentation of conjunctivitis. The specimens were analyzed for detection of adenovirus (AdV) by polymerase chain reaction (PCR). The PCR products were further analyzed for virus typing by sequence analysis and/or heteroduplex mobility assay (HMA). Forty-five samples (60%) were positive for AdV of which 21 samples were typed as AdV19 (46.7%), 7 AdV8 (15.5%), 3 AdV31 (6.7%), and one each AdV1, AdV2, AdV3, AdV4 and AdV6. For nine samples the serotype was not determined. AdV19 was the predominant serotype circulating in Rio de Janeiro during the studied period.

KEYWORDS: Adenovirus; Conjunctivitis; Ocular infection.

Adenoviruses (AdV) are one of the major causes of conjunctivitis representing 15 to 70% of all conjunctivitis cases worldwide[5,9,13,17,20,22]. These viruses belong to the *Adenoviridae* family, *Mastadenovirus* genus, which is divided into six species (A - F) and 51 serotypes. The serotypes AdV8, 19 and 37 are often associated to epidemic keratoconjunctivitis (EKC) although other serotypes such as 2, 3, 4, 5, 7, 10, 11, 21, 22, 29 and 34 have also been associated to this illness[5,9,13,17,20,22]. Pharyngeal-conjunctival fever, a disease commonly affecting children, is most commonly caused by serotypes 3 and 7 whereas serotypes 1 to 11, 15, 16, 17, 19, 20 and 22 are agents of non-specific follicular conjunctivitis[6,7]. The purpose of this study was to investigate the frequency of adenoviral conjunctivitis in the population of Rio de Janeiro, Brazil, and to identify the predominant serotype circulating among this population.

Between March 2004 and May 2007, 75 ocular swabs were collected from 66 patients with a clinical presentation of conjunctivitis in the city of Rio de Janeiro, Brazil. The protocol was approved by the Committee of Ethics in Research of the involved institution and all patients had given informed consent, in accordance with Resolution 196/96, of the Brazilian Ministry of Health. The swabs were placed in virus transport media and kept at 4 ºC until the moment of DNA extraction. Adenovirus DNA was extracted by using the Wizard® Purification Kit (Promega, Madson, USA) according to the manufacturer's instructions. The extracted DNA was amplified by polymerase chain reaction (PCR) using specific primes for a conserved region of the hexon gene that yield a 301bp fragment[2]. The amplified fragments were subjected to heteroduplex mobility assay (HMA) as described by previously[19] and/or sequencing analysis for serotype identification. For the HMA the samples were hybridized against control viruses belonging to serotypes AdV3, AdV4, AdV7, AdV8, AdV11, and AdV19. For sequencing, the amplified DNAs of positive samples were purified using the Wizard SV gel and PCR Clean-Up system kit (Promega), and the sequences determined using the PCR primers and the BigDye® Terminator Cycle Sequencing Kit and the ABI PRISM® 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequences were assembled and analyzed with the programs SeqMan, EditSeq, and MegAlign in the Lasegene software package (DNASTAR, Madison, WI, USA).

Forty-five samples (60%) were positive for Adv of which 21 samples were typed as AdV19 (46.7%), seven AdV8 (15.5%), three AdV31 (6.7%), and one each AdV1, AdV2, AdV3, AdV4 and AdV6 (Fig. 1). For nine samples the serotype was not determined because of the poor quality of the DNA. Five other samples were positive for Coxsackievirus A24 (data not shown). The seasonal distribution of the positive samples demonstrates that the AdV19 was the predominant strain between March and May of 2004. The other Adv serotypes were detected throughout the studied period (Fig. 2).

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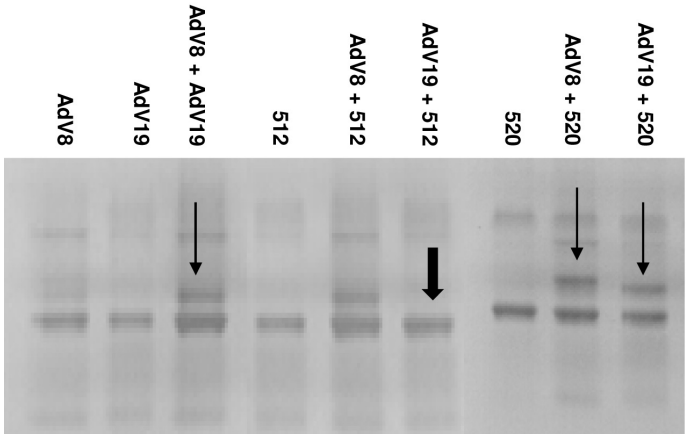

Fig. 1 - HMA profile of a portion of the hexon region of selected adenovirus-positive samples. Lines AdV8, AdV19, 512 and 520 show PCR products. The third line show the hybridization profile of AdV control strains AdV8 with AdV19. Lines AdV8+512, AdV19+512, AdV8+520, AdV19+520, show the HMA product's of adenovirus strains amplified form ocular swab hybridized with control strains AdV8 and AdV19. Strains 512 belong to serotype 19 whereas strain 520 does not belong to neither serotypes 8 or 19. Short thick arrow, homoduplex; long thin arrows, heteroduplexes.

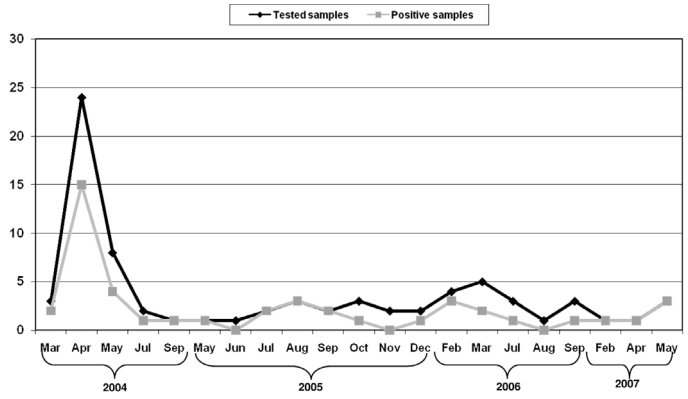

Fig. 2 - Seasonal distribution of adenovirus (AdV) infections in Rio de Janeiro, 2004-2007.

Conjunctivitis is the most frequent ocular disorder observed in ophthalmic clinics. Although several viruses are associated to conjunctivitis such as members of *Enterovirus* genus, particularly enterovirus 70 (EV70) and a variant of Coxsackievirus A24 (vCA24), adenoviruses are still the leading causes of acute conjunctivitis[11,18,23]. Adenovirus conjunctivitis is mainly associated with serotypes AdV8, 19 and 37. Conjunctivitis epidemic outbreaks occur typically in the summer although sporadic cases have been reported throughout the year in some localities such as Taiwan and Japan[1,3,5]. During 2003, Brazil faced the worse conjunctivitis epidemic in 20 years[10]. According to the Ministry of Health's National Health Institution (FUNASA), more than 200,000 cases were reported in seven Brazilian states[10]. CARMONA *et al.* identified the vCA24 as the causative agent of the epidemic[4]. In the summer of 2004, another conjunctivitis epidemic occurred in the city of Rio de Janeiro, and the health authorities believed that it was a continuation of the 2003 epidemic caused by vCA24. In the present study, although only a low number of samples were analyzed, the high prevalence of the AdV19 between March and May 2004 suggests that this virus could have been the etiologic agent of this second epidemic.

Epidemiological data on AdV ocular infections are scarce in Brazil. However, adenoviruses belonging to serotypes AdV1, 2, 3, 4, 5, 7, and 8, had already been described as etiological agents of conjunctivitis in the country[12,14,21]. In this study we detected the presence of serotypes AdV1, 2, 3, 4, 8, that had previously been detected in Brazil in association with conjunctivitis, and demonstrated the circulation of AdV19 that had never been described in the country before. We also detected the serotypes AdV6 and AdV31 which are not commonly associated with conjunctivitis.

Cases of chronic conjunctivitis caused by AdV3, 4, 5 and 19 have been reported previously[8,15,16] and can be related to the treatment with corticosteroids that would prolonge the adenovirus infection[16]. We identified two cases of chronic ocular infection caused by AdV. One patient presented conjunctivitis symptoms for more than two months but only provided one ocular swab that was positive for AdV1. The second patient presented the symptoms of papillary conjunctivitis for almost two years, and had received topic treated with corticosteroids, antibiotics and anti-inflammatory drugs. This patient provided eight ocular swabs collected along the two years period, and all had tested positive for AdV2.

In this study we investigated the molecular epidemiology of adenoviral conjunctivitis in Rio de Janeiro, Brazil between 2004 and 2007. AdV19 was the predominant serotype circulating in Rio de Janeiro during the studied period.

RESUMO

Epidemiologia molecular de adenovírus associados à conjuntivite no Rio de Janeiro, Brasil, no periodo de 2004 a 2007

A conjuntivite viral é doença ocular comum, altamente contagiosa, frequentemente causada por adenovírus. Neste estudo, investigamos a frequência de conjuntivite por adenovírus na população do Rio de Janeiro, Brasil, entre março de 2004 e maio de 2007, e identificamos o sorotipo predominante circulando nesta população. Setenta e cinco *swabs* de secreção ocular foram coletados de 66 pacientes com conjuntivite. As amostras foram analisadas para detecção de adenovírus (AdV) por reação em cadeia da polimerase (PCR). Os produtos da PCR foram caracterizados por sequenciamento e/ou ensaio de mobilidade do heteroduplex (*Heteroduplex Mobility Assay* - HMA) para identificação do sorotipo viral. Quarenta e cinco (60%) amostras foram positivas para AdV das quais 21 foram identificadas como pertencentes ao sorotipo AdV19 (46,7%), sete AdV8 (15,5%), três AdV31 (6,7%), e uma de cada: AdV1, AdV2, AdV3, AdV4 e AdV6. Para nove amostras o sorotipo não pode ser identificado. O AdV 19 foi o sorotipo predominante circulando no Rio de Janeiro durante o período estudado.

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The study protocol was approved by the Ethics Committee of the Instituto de Puericultura e Pediatria Martagão Gesteira of the Federal University of Rio de Janeiro and is in accordance with the Brazilian Ministry of Health Regulation 196/96. Informed Consent for research was obtained from the patients after explaining the nature of the study.

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