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ORIGINAL ARTICLE

Application of molecular assay for adenovirus detection among different pediatric patients

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Abstract
Objective: Adenoviruses play an important role in the etiology of severe acute lower respiratory infection, especially in young children. The aim of the present study was to evaluate the Human Adenovirus (HAdV) detection by different methods (Direct Fluorescence Assay - DFA and Nested Polymerase Chain Reaction - nested PCR), among samples collected from different groups of pediatric patients.
Methods: Collection of samples was made in children with congenital heart disease (CHD - 123 nasal aspirates collected in the years of 2005, 2007 and 2008) and in community children (CC - 165 nasal aspirates collected in 2008). Children were eligible if they presented acute respiratory infection (ARI) of probable viral etiology, within up to 7 days of symptoms' onset. All studied samples were evaluated by DFA and nested PCR assay.
Results: Of the 290 samples included during the study period, 43 (14.8%) were positive on at least one test: 17/165 (10.3%) of the CC and 26/125 (20.8%) of the CHD children. The nested PCR detection rates in the community children were 15/165 (9.1%), and for children with CHD, 24/125 (19.2%). Molecular method showed higher detection rates when compared to the DFA test ($p<0.001$). Univariate analysis showed that children with congenital heart disease presented a significantly higher chance for acquiring the HAdV (Odds Ratio 2.3; 95% CI: 1.18-4.43).
Conclusions: Based on data obtained in the present evaluation, we suggest that a routine surveillance should be performed in high risk patients by molecular methods, thus improving diagnostic flow and efficiency.

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Adenovírus e reação em cadeia da polimerase

Aplicação de teste molecular para detecção de adenovírus em pacientes pediátricos distintos

Resumo

Objetivo: Os adenovírus desempenham um papel importante na etiologia da infecção aguda grave do trato respiratório inferior, especialmente entre crianças. O objetivo do estudo foi avaliar a detecção do adenovírus humano (HAdV) por diferentes métodos (imunofluorescência direta – DFA e reação em cadeia da polimerase *nested* - *nested* PCR) em amostras coletadas de diferentes populações de pacientes pediátricos.

Métodos: O material foi coletado de crianças portadoras de doença cardíaca congênita (DCC – 123 aspirados nasais coletados em 2005, 2007 e 2008) e de crianças da comunidade (CC – 165 aspirados nasais coletados em 2008). As crianças eram consideradas elegíveis se apresentas sem infecção respiratória aguda (IRA) de provável etiologia viral, com até sete dias de início dos sintomas. Todas as amostras coletadas no estudo foram avaliadas por meio de DFA e *nested* PCR.

Resultados: De 209 amostras incluídas, 43 (14,8%) foram positivas em pelo menos um dos testes feitos: 17/165 (10,3%) das crianças da comunidade e 26/125 (20,8%) das crianças cardiopatas. As taxas de detecção por *nested* PCR foram 15/165 (9,1%) em crianças da comunidade e 24/125(19,2%) em crianças cardiopatas. O método molecular mostrou maiores taxas de detecção quando comparado com a DFA (p<0,001). A análise univariada mostrou que as crianças portadoras de cardiopatia congênita apresentaram chance significativamente maior de adquirir HAdV (*odds ratio* 2,3; IC 95%: 1,18-4,43).

Conclusões: Baseado nos resultados obtidos na presente avaliação, recomenda-se a vigilância de rotina em pacientes de risco (DCC) por métodos moleculares, que melhora o fluxo diagnóstico e a eficiência da detecção.

Introduction

Adenoviruses play an important role in the etiology of severe acute lower respiratory infection, especially in young children.[1] Human adenoviruses (HAdV) spread rapidly in closed environments, which often cause epidemic disease in crowded communities. Furthermore, adenovirus infection is difficult to clinically distinguish from other viral or bacterial respiratory infections.[2] This combination of factors indicates that improved clinical microbiological methods are necessary for detection of the acute respiratory disease caused by adenoviruses.

Prior to the advent of PCR, DFA and viral isolation were the most sensitive methods that were available for respiratory viruses detection.[3,4] However, a significant number of specimens in patients with clinically compatible viral respiratory infection were incorrectly determined to be negative by DFA and viral culture, implying a failure to identify the causative virus in a significant percentage of cases.[5-7] Molecular methods demonstrate greater sensitivity when compared to conventional assays for detecting adenovirus in respiratory samples.[8,9]

In the pediatric population HAdV is the second most common detected pathogen[10] and the virus is responsible for 5%–15% of respiratory disease.[10,11] Clinical samples from children often present higher viral loads when compared to adults patients.[12] Brazilian studies described an incidence ranging from 3% to 7.1%, depending on the technique used.[13-16] Other group at risk to acquired HAdV respiratory infection was the congenital heart disease children, but there are no published data.

In this context, the aim of the present study was to assess HAdV occurrence by two different methods (Direct Fluorescence Assay – DFA and Nested Polymerase Chain Reaction – nested PCR) in samples collected from different pediatric populations.

Method

This cross sectional study included two different populations assessed from 2005 to 2008:

1. *Congenital heart disease children* (CHD): during 2005, 2007, and 2008, 123 outpatients with CHD assisted in the Congenital Heart Disease Pediatric Service were included. During the year of 2006 the congenital heart disease ward underwent a structural and administrative reform, preventing sample collection. Among these patients, 49.7% were male, the mean and median age 3.9 and 2.9 years old, ranging from1 year and 6 month to 11 years old.
2. *Community children* (CC): in 2008, 165 outpatients seen in a primary care facility by a pediatrician were included. Among these patients, 47.2% were male, the mean and median age 3.9 and 3.0 years old, ranging from 7 months to 11 years old.