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Major Article

Human adenovirus detection among immunocompetent and immunocompromised patients presenting acute respiratory infection

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ABSTRACT

Introduction: Human adenoviruses (HAdV) play an important role in the aetiology of severe acute lower respiratory infection, especially in immunocompromised individuals. The aim of the present study was to detect HAdV using two different methods, direct fluorescence assay (DFA) and nested polymerase chain reaction (nested PCR), in samples collected from patients with acute respiratory infection (ARI) within 7 days of symptom onset. **Methods**: Samples (n=643) were collected from patients in different risk groups from 2001 to 2010: 139 adult emergency room patients (ERP); 205 health care workers (HCW); 69 renal transplant outpatients (RTO); and 230 patients in a haematopoietic stem cell transplantation program (HSCT). **Results**: Adenovirus was detected in 13.2% of the 643 patients tested by DFA and/or PCR: 6/139 (4.3%) adults in the ERP group, 7/205 (3.4%) in the HCW group, 4/69 (5.8%) in the RTO group and 68/230 (29.5%) in the HSCT patient group. Nested PCR had a higher detection rate (10%) compared with the DFA test (3.8%) ($p<0.001$). HSCT patients exhibited a significantly higher rate of HAdV infection. **Conclusions**: The adenovirus detection rate of the nested PCR assay was higher than that of the DFA test. However, the use of molecular methods in routine diagnostic laboratory work should be evaluated based on the specific circumstances of individual health services.

Keywords: Human adenoviruses. Respiratory infection, Direct fluorescence assay. Nested PCR. Immunocompromised patients. Immunocompetent patients.

INTRODUCTION

Adenoviruses play an important role in the aetiology of severe acute lower respiratory infection, especially in young children and immunocompromised individuals[1]. Human adenoviruses (HAdV) spread rapidly in closed environments and often cause epidemic disease in high-density communities[2].

Health care workers (HCWs) are in direct contact with HAdV-infected patients, and this population is especially susceptible to infection with the virus, acting as a source of infection during outbreaks. Furthermore, there have been few studies in this population, and little is known about HAdV circulation in HCWs compared to community subjects[3].

In immunocompromised patients, especially stem cell and solid organ transplant patients, HAdV can cause severe infection, with mortalities ranging from 18-83%, depending on the nature of the immunosuppression[4-8]. In Brazil, there is a lack of sufficient information about the immunocompromised population, with detection ranging from 0-3% [3,9].

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Furthermore, it is difficult to clinically distinguish adenovirus infection from other viral or bacterial respiratory infections[10]. Rapid diagnostic methods help to avoid inadequate antimicrobial treatment and support infection control interventions to limit nosocomial infections . Molecular methods demonstrate greater sensitivity than conventional assays for detecting adenovirus in respiratory samples[11,12].

Brazilian studies have described HAdV frequencies of 3 to 7.1%, as measured using different diagnostic techniques[13-16]. Outbreaks have been frequently reported among young patients[17].

The aim of the present study was to assess the rate of HAdV detection achieved by different methods, namely the direct fluorescence assay (DFA) and nested polymerase chain reaction (nested PCR), from samples collected from different risk groups over a period of six years.

METHODS

Study population

In the present study, samples from four different populations were collected from 2001 to 2010.

Adult emergency room patients (ERP): From 2001 through 2003, 139 adult patients from the general community were evaluated by general practitioners at the São Paulo Hospital emergency room.

Health care workers (HCWs): From 2001 through 2003, 205 adult health care workers were evaluated by general practitioners in the Sao Paulo Hospital employee health support centre.

Renal transplant outpatients (RTO): From 2001 through 2003, 69 adult outpatients of the renal transplant clinic were evaluated.

Patients in a haematopoietic stem cell transplantation programme (HSCT): From 2008 to 2010, 230 adult patients attending the haematopoietic stem cell transplantation program of the São Paulo Hospital haematological service were evaluated. Samples were collected from all patients by a member of the medical staff, usually an infectious disease physician. Of the 230 patients in this group, information about transplantation was available for 221 (96%): 55.2% (122/221) of these patients were undergoing transplantation at the time of study enrolment, and 44.8% (99/221) were non-transplanted .

Inclusion criteria

Adults (> 12 years old) presenting acute respiratory infection (ARI) of likely viral aetiology were eligible. Sample collection was performed within 7 days of symptom onset and after evaluation by a physician. The respiratory symptoms assessed were coryza, cough, sore throat, and nasal congestion, and the systemic symptoms assessed were fever, headache, malaise, chills, and fatigue.

Sample collection

Nasal wash (NW) specimens were collected from patients presenting with acute respiratory symptoms who were referred by physicians from São Paulo Hospital to the Clinical Virology Laboratory, São Paulo, Brazil[18].

Adenovirus detection

All samples were maintained at 4°C and transported immediately to the laboratory. An aliquot (1 mL) was separated for molecular analysis and stored at -70°C. The remaining specimen volume was evaluated on the same day by direct fluorescence assay, as described in a previous study[19]. One aliquot from each stored sample was subjected to DNA extraction using the QIAamp DNA Blood extraction kit (Qiagen, USA) according to the manufacturer's instructions, followed by nested PCR for the detection of all adenovirus serotypes, as previously described[20].

Direct fluorescence (immunofluorescence) assay: All studied samples were evaluated using the direct fluorescence assay (DFA) . The tests were performed using the *SimulFluor Respiratory Screen and Panel* (Chemicon Int., USA), in accordance with the manufacturer's instructions.

Nested PCR assay: For the first amplification reaction (PCR product – 301 bp), 5 µL of extracted DNA was added to a tube with a reaction mixture consisting of 2.5 µL of 10X buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 3.5 mM $MgCl_2$, 0.5 µM of the primers Hex1deg and Hex2deg[20] (Hex1deg - 5′- GCC SCA RTG GKC WTA CAT GCA CAT C - 3′, Hex2deg - 5′- CAG CAC SCC ICG RAT GTC AAA - 3′), 1 µL of a dNTP mixture containing 20 mM of each nucleotide, 2.5 U of Platinum® Taq DNA Polymerase (Invitrogen, Brazil) and autoclaved MilliQ water to a final volume of 25 µL.

For the second amplification reaction (PCR product - 171 bp), 2 µL of the amplicon produced by the first reaction was added to a tube with a reaction mixture consisting of 2.5 µL of 10X buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 3.5 mM of $MgCl_2$, 0.5 µM of the primers Nehex3deg and Nehex4deg[20] (Nehex3deg - 5′- GCC CGY GCM ACI GAI ACS TAC TTC - 3′, Nehex4deg - 5′- CCY ACR GCC AGI GTR WAI CGM RCY TTG TA - 3′), 1 µL of a dNTP mixture containing 20 mM of each nucleotide, 2.5 U of Platinum® Taq DNA Polymerase (Invitrogen, Brazil) and autoclaved MilliQ water to a final volume of 25 µL.

A positive control (Adenovirus Serotype 3) and a redundant negative control (autoclaved MilliQ water) were included in each series. A sample was considered positive when an amplicon could be visualised by 2% agarose gel electrophoresis.

The sensibility of the reaction was standardised and showed a detection limit of 10^{-4} $TCID_{50}$/mL.

Statistical analysis

Between-group comparisons of different categorical variables were performed using the Chi-square test and non-conditional multiple logistic regression (SPSS, version 11.5). A p value < 0.05 was considered to be statistically significant.

Ethical considerations

The present study was approved by the Ethics Committee of São Paulo Hospital and the Federal University of São Paulo (1654/09).

RESULTS

Samples from 643 symptomatic patients were analysed (40.4% females and 59.6% males). The demographic characteristics of all studied patients are shown in **Table 1**.

TABLE 1 - Demographic data of the study populations

	Samples		Gender male		Age (years)		
	n	%	n	%	mean±SD	median	range
HCWs	205	31.9	154	75.1	36.2±11.7	36.0	16-68
ERP	139	21.6	62	44.6	32.7±13.8	29.0	12-83
RTO	69	10.7	35	50.7	38.8±11.7	38.5	15-60
HSCT	230	35.8	132	57.4	43.7±15.2	47.0	18-80
Total	643	100.0	383	59.6	38.3±14.12	37.0	12-83

HCWs: health care workers; ERP: emergency room patients; RTO: renal transplant outpatients; HSCT: patients in a haematopoietic stem cell transplant program; SD: standard deviation.

Among the 643 samples collected during the study period, 85 (13.2%) were found to be HAdV positive by at least one test: 7/205 (3.4%) from the HCW group, 6/139 (4.3%) from the ERP group, 4/69 (5.8%) from the RTO group, and 68/230 (29.6%) from the haematological patient group . **Table 2** shows the differences in detection rates between assays in each of the different studied populations. Overall, 24 (3.8%) of 643 samples

TABLE 2 - Adenovirus detection by direct fluorescence assay and nested PCR assay among different studied populations from São Paulo from 2001-2010

	Number of samples	DFA positive		Nested-PCR positive		p	Total positivity	
		n	%	n	%		n	%
HCWs	205[a]	0	0.0	7	3.4	0.015	7	3.4
ERP	139	0	0.0	6	4.3	0.030	6	4.3
RTO	69	0	0.0	4	5.8	0.12	4	5.8
HSCT	230[b]	24	10.4	47[c]	20.4	0.005	68	29.6
Total	643	24	3.8	64	10.0	< 0.001	85	13.2

PCR: polymerase chain reaction; DFA: direct fluorescence assay; HCWs: health care workers; ERP: emergency room patients; RTO: renal transplant outpatients; HSCT: patients in a haematopoietic stem cell transplant program. [a]DFA could not be performed in 8 samples; [b]DFA could not be performed in one sample; [c] Three samples tested positive in both tests and were counted as positive for nested PCR.

TABLE 3 - Time from symptom onset to sample collection, stratified according to direct fluorescence assay and nested PCR assay results

	Days					
	≤ 5			> 5		
	positive	%	total	positive	%	Total
DFA	17	3.4	501	7	4.9	142
Nested-PCR	42	8.4	501	22	15.5	142
p-value		< 0.01			< 0.01	

PCR: polymerase chain reaction; DFA: direct fluorescence assay.

were DFA positive (10 had insufficient material for DFA), and 64 (10%) of 643 samples were nested PCR positive (one sample had insufficient volume for nested PCR).

The nested PCR assay achieved a significantly higher detection rate than the DFA assay among all studied populations, except for the renal transplanted outpatients group. When the entire studied population was analysed, the molecular method showed a higher detection rate than DFA ($p < 0.001$). The highest detection rate was found in HSCT patients ($p < 0.001$) . The distribution of positive samples along the period of study is shown in **Figure 1**.

The time from symptoms onset to sample collection was analysed and was found to be significantly different between DFA and nested PCR **(Table 3)**. Compared to DFA, nested PCR detected a greater number of cases both in samples collected within five days of symptom onset and in samples collected between days 5 and 7. Within the HSCT group, nested PCR assay detection was higher in patients with more than five days between symptom onset and sample collection ($p<0.001$).

HSCT patients exhibited a 12 times higher rate of HAdV infection than HCWs ($p<0.001$; CI: 5.37-26.9).

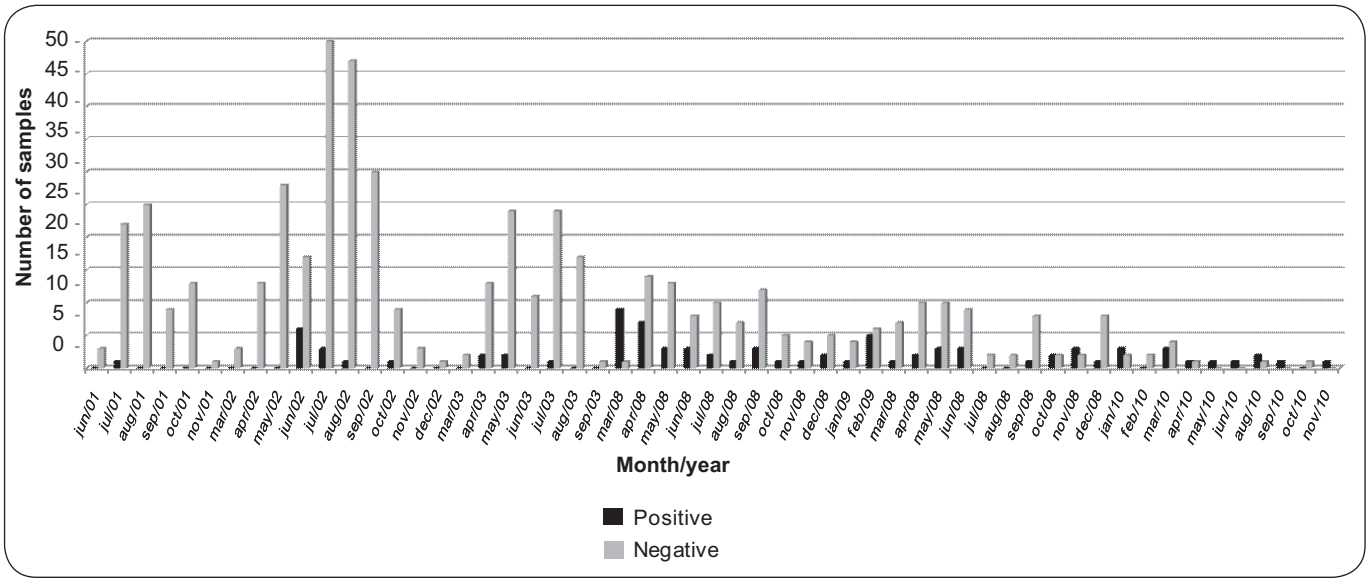

FIGURE 1 - Human adenovirus detection according to month/year of study.

DISCUSSION

In Brazil, few studies have investigated adenovirus infection among immunocompetent non-hospitalised adults. The detection rates among the adult populations in this study (emergency room patients, 4.3%; health care workers, 3.4%) were similar to those described in the literature (2.5% - 12.8%)[21,22]. Lessa et al.[22] reported that 19% of HCWs in a military hospital were infected with HAdV, compared with 5.3% of non-HCWs.

Adenovirus infection is an important cause of morbidity and mortality among stem cell transplant recipients[23,24]. Hospitalised patients presenting haematological diseases, with prolonged hospital stays, are at a high risk of severe HAdV infection and represent a potential source of nosocomial outbreaks[25]. Studies have reported rates of HAdV infection ranging from

2 to 21%, with a mortality rate of 10-60%, depending on the immunosuppressive regimen used[26].

The evaluation of these two tests raised many questions that are important for both clinicians and hospital administrators: What is the ideal time of sample collection after sample onset? Is there a need for repeat testing? What is the ideal number of samples for a routine laboratory flow? What are the implications of negative or positive tests in high-risk patients? Some of these questions were addressed in the present study. HAdV was detected by at least one of the tests in 13% of the samples. Cases of HAdV occurred throughout the year, with higher detection rates obtained by DFA during 2008 March and April. This peak could be explained by an HAdV outbreak in the haematological ward that occurred during that period, as well as by the clinical re-evaluation to which these patients were submitted, i.e., the analysis of multiple samples from the same patients within the same month could have led to an overestimate of the virus occurrence.

The evaluation of patient symptom onset and the tests used showed that the molecular method was always more sensible than the DFA, and this difference was more evident for samples collected after the fifth day of symptom onset. Another study reported similar results, with molecular methods presenting higher sensitivity than DFA in adult patients[27,28]. The lower detection of DFA was related to the low viral load in respiratory samples[29].

Higher detection rates among patients with a longer period of symptom onset were expected due to the higher sensibility of the nested PCR[30,31]. The success of the DFA test relies on key factors such as good sample collection, experienced laboratory staff and high viral load[29,32], while nested PCR, based on nucleic acid amplification, can detect a very low number of viral particles[20]. Among HSCT patients, nested PCR presented higher sensibility after the fifth day, suggesting that the molecular method is best for HAdV detection among patients with a prolonged infection history.

Adenoviruses can produce asymptomatic[33,34] and prolonged[34] infections in humans. These two parameters must be considered when the diagnostic technique is selected. Carraro[18] reported that there was no difference in influenza virus detection between DFA and RT-PCR up to the fifth day of symptom onset, indicating a reasonably good performance of the serological test during the acute phase of the disease. On the other hand, a positive result yielded by the molecular method does not necessarily indicate a symptomatic infection, mainly due to high sensibility of this test[20]. The use of quantitative PCR (real time PCR) could address this issue; the viral load can indicate the presence or absence of disease[11].

All patients enrolled in the present study had acute respiratory infection, which reduces the possibility of asymptomatic infection by adenovirus. Moreover, all samples were collected within 7 days of symptom onset, and it was therefore not possible to analyse the duration of viral shedding.

One limitation of our study was the lack of analysis of possible co-infections with HAdV and other respiratory viruses. However, data from several studies showed low rates, ranging from 1.04% to 3.5%[35-37].

Based on data obtained in the present evaluation, we concluded that the nested PCR assay had a higher HAdV detection frequency than the DFA, mainly among high-risk patients (haematopoietic stem cell transplant recipients). However, the choice to use molecular methods for HAdV detection in routine diagnosis should consider the economic reality of the specific health service and the demand for HAdV testing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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