Detection of Adenovirus in Urine of Immunocompromised Patients by Polymerase Chain Reaction

Hend A. Sharaf, Maha K. Gohar and Enas A. Tantawy
Medical Microbiology & Immunology Department
Faculty of Medicine, Zagazig University

ABSTRACT

Adenovirus (AdV) has been increasingly shown to play a role in the morbidity and mortality of immunosuppressed patients. This work aimed to detect AdV in urine samples of immunocompromised patients of both pediatric and adult oncology units and study some factors that may associate with increased risk of AdV infections and subsequent detection in urine samples. This study was conducted from December 2009 to December 2010 in Medical Microbiology and Immunology Department Faculty of Medicine Zagazig University. Urine samples were collected from 100 patients in oncology wards of both Pediatric and Internal Medicine Departments (50 from each department) in Zagazig University Hospitals. Each sample was subjected to PCR detection of AdV. The characteristics of pediatric and adult immunocompromised patients in whom AdV was detected in urine were compared. Twenty (20%) out of 100 urine samples were positive for AdV by PCR, 15 (30%) out of 50 pediatric and 5 (10%) out of 50 adult immunocompromised patients. The difference was statistically significant (P=0.01). Pediatric patients diagnosed as cases of leukemia were significantly associated with AdV detection in urine (P=0.01). The use of immunosuppressive therapy is significantly associated with AdV detection in urine in both pediatric and adult patients (P=0.02 and 0.05 respectively). In conclusion Adenovirus infection is an important cause of morbidity and mortality in immunocompromised patients, particularly who are receiving immunosuppressive therapy. The need for rapid method of diagnosis and an effective, nontoxic antiviral therapy is apparent.

INTRODUCTION

Adenovirus is an extremely hardy virus. The infectivity of desiccated adenovirus from a nonporous surface was documented for up to 35 days. The shedding of virus especially from the gastrointestinal tract and respiratory tract may continue for months after initial infection. Close contact with individuals in crowded places increases the risk for infection. Prolonged shedding of virus especially from immunocompromised hospitalized patients and its hardy nature make it an ideal agent for nosocomial transmission(1).

Adenoviruses induce latent infections in tonsils, adenoids, and other lymphoid tissues(2). Adenovirus diseases in immunosuppressed patients have been attributed to both primary infection and reactivation of endogenous latent infection. In addition, there is some evidence for transmission of adenovirus infection from donor organ to recipient(3).

There are 51 different serotypes for AdV based on type-specific serum neutralization, and these are classified into six species (A, B, C, D, E, and F) on the basis of hemagglutination, oncogenic and phylogenetic properties(4). Some recent studies have suggested that specific serotypes cause more severe disease, especially in immunocompromised patients(5). Different serotypes have been found to have different tissue tropisms that correlate with different clinical manifestations of infection. Limited epidemiological investigations have revealed that among some specific serotypes, multiple genetic variants exist that often have quite different geographical distributions and associated virulence(6).

Adenoviruses cause a wide range of diseases in humans, including sudden infant perinatal death(7), chronic airway obstruction(8), pulmonary dysplasia(9), myocarditis and dilated cardiomyopathy(10), intussusception (11), upper and lower respiratory illness, urinary tract infections, conjunctivitis, gastroenteritis(12), mononucleosis-like syndromes(13), and obesity(14).

Immunocompromised individuals may develop severe and frequently fatal localized or disseminated disease (14). During the past decade, use of more potent chemotherapy and immunosuppressive agents, improvement of virological diagnostic methods, and better control of cytomegalovirus (CMV) infections have been associated with an increasing appreciation of the role that other viruses, such as AdV, play in the morbidity and mortality of immunosuppressed patients(16). Unfortunately,
AdV infections are usually not recognized until a late stage of disease is reached, because of nonspecific clinical symptoms of infection in an immunocompromised host\(^{(16)}\).

Among patients with invasive AdV diseases, the virus was most commonly detected in the urinary tract, gastrointestinal tract, and lung. Among patients who had asymptomatic AdV infections, positive samples were obtained especially from the urinary tract and the gastrointestinal tract\(^{(19)}\).

The isolation of Ads, as well as the determination of serotypes via neutralization tests or hemagglutinin inhibition with type-specific antisera, is a process that may take several weeks. The rapid diagnosis of adenoviral infections, as well as the determination of serotypes, is crucial to epidemiological surveillance and to decisions regarding optimal treatment strategies. The shell vial method improves isolation in tissue cultures by increasing viral adsorption to the cells. However, it still requires an assay time of approximately 2 days. Recently, the PCR-based assay has become a popular alternative method for the detection and typing of AdVs, offering rapid and sensitive detection and precise molecular identification\(^{(17)}\).

This work aimed to investigate AdV presence in urine of immunocompromised patients of both pediatric and adult oncology units using PCR and study some factors that may associate with increased risk of AdV infections and subsequent detection in urine samples.

**MATERIAL & METHODS**

**Study group and specimens.**

This study was conducted from December 2009 to December 2010 in Medical Microbiology and Immunology Department Faculty of Medicine Zagazig University. Urine samples were collected from 100 patients in oncology wards of both Pediatric and Internal Medicine Departments (50 from each department) in Zagazig University Hospitals. Demographic data for each patient were registered including age, sex, disease and use of immunosuppressive therapy. The age range in pediatric patients was 0.3-17.7 years while in adults it was 18.6-56.3 years. Pediatric patients included 25 males and 25 females, 35 of them were using immunosuppressive treatment while 15 were not using immunosuppressive treatment, 30 were having leukemia, 11 with Non-Hodgkin’s lymphoma and 9 with Hodgkin’s lymphoma. Adult patients were 20 males and 30 females, 25 of them were using immunosuppressive treatment while 25 were not. Leukemia, Non-Hodgkin’s lymphoma and Hodgkin’s lymphoma accounted for 20, 15 and 15 adult patients respectively.

**Urine sample processing.**

Urine samples from each patient were processed within 60 min of collection. Briefly, 10 ml of urine was centrifuged at 3,650 \(\times\) g for 30 min. All but 0.5 ml of supernatant and sediment was discarded. One milliliter of viral transport medium (Eagle minimum essential medium with 5% fetal bovine serum, 50 \(\mu\)g of gentamicin per ml, and 5 \(\mu\)g of amphotericin B per ml) was added to the sediment, and the pH was adjusted to 7.0 with 7.5% sodium bicarbonate. The processed sediments were stored in aliquots at -70°C until PCR was performed\(^{(18)}\).

**PCR.**

Primers (Pioneer, Germany) for AdV detection were selected based on sequences in the hexon gene, a part of the AdV genome that is highly conserved among different serotypes. The primers were as follow: 5’-GACATGACTTTGCAGGTCGATCCCATGG A-3’ and 5’-CCGGCTGAGAGGTTGTGGCAGGTA-3’. A PCR-based assay method initially developed to detect AdV from conjunctival samples\(^{(19)}\) was modified to apply to different specimen types\(^{(18)}\). Unlike some previously described primer sequences used for detection of AdV from clinical samples\(^{(20)}\), these primers detected all types of AdV tested, including different genotypes of serotype 11\(^{(18)}\).

Urine samples in viral transport medium were tested by PCR without prior extraction of nucleic acids. Two microliters of processed urine samples, was added to 40 µl of master mix (final concentrations of 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM MgCl\(_2\), 200 µM [each] dNTP, 0.2 µM [each] primer, and 2.5 U of Taq polymerase (Qiagen, GmbH, Germany), and each reaction mixture was adjusted with water to a final volume of 50 µl. Amplification in a thermocycler (Perkin Elmer- Cetus, USA) consisted of an initial round at 94°C for 7 min, 55°C for 1 min, and 72°C for 1.5 min, followed by 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. Each run included negative controls (distilled water). The presence of a 139-bp band on an ethidium bromide-stained gel was considered a positive result\(^{(18,21)}\).

**Statistical analysis.**

Qualitative data are presented as number and relative percentage and Chi (X\(^2\)) square test used for comparison between qualitative data.
*P* value was calculated using the Epi Info software, *P*<0.05 is considered significant (22).

**RESULTS**

Adenovirus was detected in 20 (20%) out of 100 urine sample. Fifteen (30%) out of 50 pediatric and 5 (10%) out of 50 adult immunocompromised patients were diagnosed as having the 139-bp band of the AdV hexon gene in urine using PCR (Figure 1). The difference was statistically significant (*P*=0.01) (Table 1).

![Figure 1](image)

**Figure (1):** Agarose gel shows amplified products. Lane 1 and 8 show MW marker, 100 to 1000 bp DNA ladder (Bioron, Germany). Lane 2 was the negative control. Lanes 3 and 6 shows 139-bp bands of AdV. Lanes 4, 5 and 7 shows negative samples.

<table>
<thead>
<tr>
<th></th>
<th><em>Pediatric patients (n=50)</em></th>
<th><em>Adult patients (n=50)</em></th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>15 (30%)</td>
<td>5 (10%)</td>
<td><em>P</em>=0.01*</td>
</tr>
<tr>
<td>Negative</td>
<td>35 (70%)</td>
<td>45 (90%)</td>
<td></td>
</tr>
</tbody>
</table>

* *P* showing statistically significant difference

Table (2) shows distribution of AdV detected in urine according to disease. In pediatric patients AdV was detected in 13 (43.33%) out of 30 patients with leukemia, 1 (9.1%) out of 11 cases of Non-Hodgkin’s lymphoma and 1 (11.11%) out of 9 cases of Hodgkin’s lymphoma. Leukemia was significantly associated with AdV detection in urine of pediatric patients (*P*=0.01). While in adults AdV was detected in 4 (20%) out of 20 leukemia patients, 1 (6.6%) out of 15 Non-Hodgkin’s lymphoma and non was detected in Hodgkin’s lymphoma patients. There was insignificant association between clinical diagnosis and AdV detection in adult immunocompromised patients.
Table 2. Distribution of AdV detection according to disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Pediatric patients</th>
<th></th>
<th>Adult patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Positive cases n.</td>
<td>P value</td>
<td>Study group</td>
<td>Positive cases n.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. (%)</td>
<td></td>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Leukemia</td>
<td></td>
<td>30 13 (43.33)</td>
<td>0.01*</td>
<td>20 4 (20.0)</td>
<td>0.14</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>11 1 (9.1)</td>
<td>0.14</td>
<td>15 1 (6.6)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>9 1 (11.11)</td>
<td>0.24</td>
<td>15 0 (0.0)</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant difference

Table (3) shows some factors associated with AdV infection in pediatric and adult immunocompromised patients. In pediatric patients 7 (28%) out of 25 males and 8 (32%) out of 25 females were proven positive for AdV in urine, while in adults 3 (15%) out of 20 males and 2 (6.6%) out of 30 females were proven positive for AdV in urine the differences in both departments were statistically insignificant (P=0.75 and 0.63 respectively). Considering the use of immunosuppressive treatment, 14 (40%) out of 35 pediatric patients were using the treatment, while only 1 (6.66%) out of 15 pediatric patients were not using the treatment, the use of immunosuppressive treatment was significantly associated with AdV detection in urine of pediatric patients (P=0.02). In adults, 5 (20%) out of 25 patients were using immunosuppressive treatment while no detection for AdV was documented in patients not receiving immunosuppressive treatment, the use of immunosuppressive treatment was significantly associated with AdV detection in urine of immunocompromised adults (P=0.05).

Table 3. Factors associated with AdV infection in pediatric and adult immunocompromised patients

<table>
<thead>
<tr>
<th></th>
<th>Pediatric patients</th>
<th></th>
<th>Adult patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Positive cases n.</td>
<td>P value</td>
<td>Study group</td>
<td>Positive cases n.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. (%)</td>
<td></td>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>25 7 (28)</td>
<td>0.75</td>
<td>20 3 (15)</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>25 8 (32)</td>
<td></td>
<td>30 2 (6.66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of immunosuppressive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using</td>
<td>35 14 (40)</td>
<td>0.02*</td>
<td>25 5 (20.0)</td>
<td>0.05*</td>
<td></td>
</tr>
<tr>
<td>Not using</td>
<td>15 1 (6.66)</td>
<td></td>
<td>25 0 (0.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant difference

**DISCUSSION**

In the majority of immune-competent patients, adenovirus infections are often limited to single organ presentation associated with its strong tissue tropism to epithelial surfaces. However in immuneocompromised patients, disseminated infections are common and can be difficult to diagnose due to the often associated multiorgan dysfunction arising secondary to the patho-physiological processes that involved the allogeneic transplant. Adenovirus is responsible for several self-limited diseases of childhood, including acute hemorrhagic cystitis, febrile respiratory illness, epidemic keratoconjunctivitis, tonsillitis, and gastro-enteritis. In contrast, significant mortality has been associated with AdV infections in immunocompromised patient populations.

In immunocompromised patients AdV infection most likely results from reactivation of latent adenoviruses in tonsils, adenoids, and other lymphoid tissues, but primary infections cannot always be excluded.

This work aimed to investigate AdV presence in urine of immunocompromised patients of both pediatric and adult oncology units and study some factors that may associate with increased risk of AdV infections and subsequent detection in urine samples.

In this study 20 (20%) out of 100 patients were positive for AdV in urine samples by PCR. Other studies using PCR for detection of AdV in urine of immunocompromised patients
documented variable percentages, 18% (25) and 11% (26). Other studies using culture for isolating AdV from urine samples of immunocompromised patients showed different results, 3%, 12% and 40% in studies done by La Rosa et al. (27), Bordigoni et al. (28) and Kros et al. (29) respectively.

These results are difficult to compare because each study was testing different categories of immunocompromised patients, some tested bone marrow transplantation patients, other tested solid organ transplant recipients, patients with AIDS or cancer. Also each study tested different ages for patients and used different detection methods and this might contribute to the explanation of the various rates recorded by different investigators.

This study is among several studies which have noted a higher incidence of AdV detected in pediatric than in adult immunocompromised populations (table 1). Adenovirus was detected in 15 (30%) out of 50 pediatric patients while only 5 (10%) were found in 50 adult patients, this was statistically significant (P=0.01). Similarly, in a study done by Flomenberg et al. (3), they noted a higher incidence of AdV isolated from pediatric than from adult immunocompromised populations (31.3% vs. 13.6%, P = 0.003). Also, Howard et al. (15) they documented that pediatric patients were also more likely than adults to have a positive AdV (23% vs. 9%; P < 0.0001). Gerber et al. (30) documented that children aged <7 years were found to be at increased risk for illness, 76.8% of detected AdVs were from children aged <7 years. In a multivariable risk factor modeling done by Gray et al. (31) for AdV disease severity, they found that age <7 years (odds ratio [OR], 3.2; 95% confidence interval [CI], 1.4–7.4) is significantly associated with AdV severe presentation. Bordigoni et al. (28) stated that pediatric patients appear to be infected by AdV more frequently and earlier than their adult counterparts. The higher overall incidence of AdV in pediatric immunocompromised patients is consistent with the epidemiology of AdV infections. Most primary AdV infections are acquired in childhood, and virtually all adults have serologic evidence of past infection with common AdV serotypes.

In this study the distribution of cases proven positive for AdV among different diseases were studied (table 2). Pediatric patients suffering from leukemia were significantly associated with AdV infection in urine (P=0.01) but, patients with Non-Hodgkin’s and Hodgkin lymphomas showed insignificant relation to AdV infection (P= 0.14 and 0.24 respectively). On the other hand, non of the studied diseases were associated with AdV infections in adult patients. Flomenberg et al. (3), Howard et al. (15), La Rosa et al. (27) and Robin et al. (32) documented that leukemia was the major disease of immunocompromised patients from whom AdVs were detected in urine from different age groups in their studies. The explanation of that may be due to the fact that patients with acute leukemia takes intensive immunosuppressive therapy more than patients with chronic leukemia and lymphomas that render the patient more susceptible to primary infection or reactivation of latent AdV.

In this work some factors that may associate with AdV infections and subsequent detection in urine were studied (table 3). The sex was compared in both pediatric and adult immunocompromised patients and the differences were statistically insignificant (P=0.75 and 0.63 respectively). Similarly, Flomenberg et al. (3) and La Rosa et al. (27) documented that there were no significant differences in regard to sex in pediatric and adult patients documented positive for AdV in urine. Gerber et al. (30) documented AdV infection in 59% male patients and 41% in female patients.

Considering the use of immunosuppressive therapy, AdV was detected in 40% of pediatric patients using immunosuppressive treatment and in only 6.66% in those not using the therapy, the difference was statistically significant (P=0.02). Also, adult patients showed significant relation between AdV infection and use of immunosuppressive therapy (P=0.05). In a study done by La Rosa et al. (27) they documented that the use of immunosuppressive agents were associated with dissemination of AdV (P<0.001).

In this work screening for AdV in immunosupressed patients was done using urine samples. This choice was guarded by studies done by different investigators about the sites of shedding of AdV in invasive and asymptomatic cases of AdV infections. Howard et al. (15) documented that among patients with invasive disease, AdV was most commonly detected from the urinary tract (urine; bladder or kidney biopsy or autopsy), gastrointestinal tract (saliva or stool; endoscopic biopsy or autopsy), and lung (sputum or bronchoalveolar lavage fluid; lung biopsy or autopsy). Among patients who were asymptomatic, positive samples were obtained especially from the urinary tract (urine) and the gastrointestinal tract. Also Flomenberg et al. (3) documented that AdV was
most commonly detected in stool and urine specimens.

PCR-based detection methods for AdV have been developed. These techniques are fast and can detect co-infections when used in a multiplex assay (33), thus reducing cost, labor, and sample volume needed for analysis. Current PCR assays identify the six subgenera (A to F) or up to three serotypes per reaction mixture (34). Real-time PCR also exists for generic detection of all 51 serotypes (35), and sequence analysis of the genomic region coding for the seven hypervariable loops of the hexon (the primary antigenic determinant) can identify and discriminate all 51 serotypes with a single assay, albeit a relatively time-consuming and complex one (36).

It is widely recognized that not all patients who have AdV detected in any sample develop symptoms attributable to AdV infection (37). Furthermore, not all patients who develop symptoms of a clinical syndrome associated with AdV will have the same clinical course. Although some patients manage to eradicate their infection, others die of disseminated infection with multiorgan failure. It is possible that part of understanding the variable clinical course of AdV infection lies in understanding more about the source of the infection or previous exposure to the virus (15).

An improved understanding of the incidence and outcomes of AdV infections in immunocompromised patients can serve as a basis for developing strategies for more effective treatment.

In conclusion, AdV infection is an important cause of morbidity and mortality in immunocompromised patients, particularly who are receiving immunosuppressive therapy. The need for rapid method of diagnosis and an effective, nontoxic antiviral therapy is apparent.

Acknowledgment

We are grateful to all staff of Oncology Units of Pediatric and General Medicine Departments of Zagazig University Hospitals participating in this study, without whom this work would have been impossible.

REFERENCES


