Adenovirus Infection in Pediatric Liver Transplant Recipients

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A retrospective review of adenoviral infection in pediatric liver transplant recipients was done at Children's Hospital of Pittsburgh to define its epidemiology and clinical importance. Medical records of patients with adenovirus were reviewed and data collected regarding clinical course, microbiologic studies, biopsy results, immunosuppression, concurrent infections, and outcome. Of 484 liver transplant recipients, 49 had 53 episodes of adenoviral infection. The most common sites of adenoviral infection were the liver, lung, and gastrointestinal tract. Serotypes 1, 2, and 5 were recovered most often; type 5 was commonly associated with hepatitis. Invasive adenoviral infection occurred in 20 children, leading to death in 9. Median time from transplantation until isolation of adenovirus was 25.5 days. This timing suggests either reactivation or donor-associated transmission. Prospective studies using molecular epidemiologic techniques will be helpful in evaluating transmission patterns of adenovirus in this population.

Orthotopic liver transplantation (OLT) is done with increasing frequency in the pediatric population for a variety of hepatic disorders. Infectious complications are a major source of morbidity and mortality for these patients. Cytomegalovirus (CMV) and Epstein Barr virus have been well documented as major pathogens after transplantation [1, 2]. Although adenoviral infection has been previously identified as an important cause of hepatitis after OLT in children [3–7], analysis has been limited by the small number of affected patients in each report. An apparent increase in adenoviral infections among pediatric liver transplant patients at the Children's Hospital of Pittsburgh led us to review our experience with this infection.

Methods

Pediatric liver transplant recipients with adenovirus isolated from one or more sites were identified through the virology laboratory records between 1 January 1982 and 30 November 1989. Medical records of these patients were systematically reviewed and data collected regarding clinical course, microbiologic studies, biopsy results, immunosuppression, concurrent infections, and outcome using standardized definitions. Adenoviral isolates during the same time period from nontransplant patients were recorded by date, source of isolate, and serotype.

Viral studies. Surveillance viral cultures of respiratory secretions, urine, and buffy coat were obtained every other week for the first 2 months after liver transplantation from 1985 to 1988. These same studies were routinely sent as part of an evaluation of fever, along with cultures of stool, liver biopsy, and bronchoalveolar lavage (BAL) fluid when clinically indicated. Liver biopsies were routinely sent for viral culture regardless of clinical status after June 1985. Specimens were inoculated into single tubes of African green monkey kidney, rhesus monkey kidney, HEp-2 cells and human foreskin fibroblasts. Starting in May 1987, human neonatal kidney cells were also used. Cultures were maintained for 2 weeks with the exception of human foreskin fibroblasts, which were observed for 4 weeks. Throughout the study, adenovirus was identified by its cytopathic effect. Beginning in 1988, an adenoviral EIA (Adenoclone; Cambridge Bioscience, Worcester MA) was also used to confirm the diagnosis. At least one isolate from each patient was sent to the Allegheny County Health Department for serotyping using adenoviral antisera to types 1–8 and 19 (Centers for Disease Control, Atlanta).

Pathologic studies. Pathologic specimens included tissue from biopsies, BAL fluid, and autopsy specimens. Touch preparation of tissues and frozen and permanent sections were processed for routine histologic studies. Biopsy and autopsy specimens were stained using an avidin-peroxidase method with monoclonal anti-adenovirus antibody (MA805) and anti-CMV antibody (MA810), as previously described [3]. Histologic identification of adenovirus was made by finding typical inclusion bodies, positive antibody stain, or electron microscopic evidence consistent with adenovirus in the tissue.

Definitions of clinical illness. Clinical episodes during which adenovirus was recovered from culture were classified as definite, probable, possible, or indeterminate for adenoviral disease depending on the success of demonstrating tissue damage, viral inclusions, or evidence of concurrent infection. Symptomatic infection was defined as one in which at least some of the patient's symptoms could be (definitely or probably) ascribed to adenovirus. Invasive infections were defined as those with evidence of organ involvement. Fever was considered a temperature >38.0°C.

A diarrheal illness was defined as a new report of diarrhea (at least six liquid stools a day) for ≥2 days (consecutive). Adenovirus was considered to be the probable cause of the illness if

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diarrhea was accompanied by a positive stool culture in the absence of other recognized pathogens. Adenovirus was considered to be the possible cause when the stool was positive but concurrent enteric pathogens were also identified.

Pneumonia was diagnosed when fever, new or worsening lower respiratory symptoms, or hypoxemia accompanied radiographic pulmonary changes. Adenovirus was considered to be the definite cause of pneumonia when isolated from BAL fluid or lung biopsy specimen. Probable adenoviral pneumonia was diagnosed if the organism was isolated from upper respiratory tract secretions in the absence of concurrent pathogens. Otherwise, the illness was classified as possible adenoviral pneumonia (mixed infection) or indeterminate.

Hepatitis was diagnosed by the presence of fever, elevated transaminase levels, and characteristic histopathology. Definite adenoviral hepatitis was defined histologically by the finding of adenoviral inclusions, positive antibody stain or positive electron microscopy. In addition, suggestive histopathology (consisting of microabscesses and cell dropout without inclusions [3]) was also considered definite adenoviral hepatitis if the culture from the liver was positive. Diagnosis of probable adenoviral hepatitis was made when the liver culture was positive but histopathology was not confirmatory. Possible adenoviral hepatitis was the classification applied to patients when histopathology of the liver was suggestive and adenovirus was isolated from a site other than the liver. Indeterminate adenoviral hepatitis was defined when adenovirus was isolated from the liver but antibody stains were negative and concurrent infection was present.

Concurrent bacterial or fungal infections were identified when organisms were isolated from a normally sterile site within 1 week of the adenoviral isolation. Contaminants were excluded by the lack of clinical correlation, negative repeat cultures before therapy, or both. Viral infections within 2 weeks of the adenoviral isolate were also considered concurrent infections.

Immunosuppression. Immunosuppressive regimens varied slightly over the course of the study. All children received cyclosporine and steroids as previously described [7]. In addition, azathioprine was given to 17 children. Suspected or proven mild to moderate rejection was treated with steroid augmentation. Steroid-resistant rejection was treated with a cycle of OKT3 (a murine monoclonal antibody to T lymphocytes).

Statistical methods. Yates's corrected χ^2 test was used for analysis of continuous variables, and Student's t test was used for categorical variables. Fisher's exact test was used for small samples. $P < .05$ was considered significant, and $.05 < P \leq .1$ was considered indicative of a trend.

Results

Patient population. During the study period, 484 children underwent 656 OLTs. Fifty children had adenovirus isolated from one or more sites. The virus was found preoperatively in one child on a surveillance culture, thereby eliminating him from analysis. Of the remaining children, 20 were boys and 29 were girls. Ages at the time of OLT ranged from 0.8 to 17.2 years (median, 3.0) and were similar to the ages of all children undergoing OLT. Biliary atresia was the most common (71%) disorder leading to transplantation.

Fifty-three episodes of adenoviral infection occurred in 49 patients. Four children had two different serotypes isolated. In one patient the different strains were isolated only 1 day apart from stool and urine. The other three patients had temporally separate episodes 8, 30, and 255 days after the initial infection.

Timing. The median time of adenoviral isolation was 25.5 days after liver transplantation (range, 4–888). Only four episodes of the infections occurred >3 months after transplantation and none of these were invasive. Severe infections, such as hepatitis or pneumonia, occurred within the first 50 days except in two cases, 64 and 69 days after transplantation. The latter patient had concurrent rejection and posttransplant lymphoproliferative disease. Apparent clusters of adenoviral infection occurred at several times, but not all isolates from each cluster were of the same serotype. No patient shared the same or adjacent rooms, and none of the children shared primary nurses.

Serotypes and sites of isolation. Table 1 lists the adenoviral serotypes and sites of isolation in the OLT patients as well as adenoviral isolates from 85 non-OLT patients during the study period. Two OLT patients had adenoviral strains that were not typeable against available antisera, and one patient's isolate was not available for serotyping. Serotypes 1 and 2 were found commonly among both groups of patients. However, the study patients had a high prevalence of adenovirus type 5, while the non-OLT patients were often found to have disease caused by types 3 and 8. No apparent temporal relationship was noted between isolates recovered from OLT recipients and those from the community.

Urine was the most common site of adenoviral isolation; 23 infectious episodes were documented in 20 patients. Signs and symptoms of disease accompanied 11 of the 23 episodes. Two children had hemorrhagic cystitis. Nine had adenovirus isolated from other sites with symptoms reflective of their primary area of involvement. Three episodes of adenovirus associated with concurrent infection were classified as indeterminate. Eight children with only a urine isolate had asymptomatic shedding.

Twenty-one children had adenovirus isolated from respiratory secretions: 19 from throat or nasopharyngeal swabs and 3 from BAL fluid in children with severe respiratory illness and high fever. Two of these 3 children died with adenoviral infection as a contributing factor. Five children had probable and 2 had possible adenoviral pneumonia (table 2). The rest had upper respiratory infections (2), extrapulmonary symptoms (3), or no symptoms.

Sixteen adenoviral episodes involved the liver. Of these children, 14 had definite adenoviral hepatitis (table 2) occurring 15–69 days after transplantation (mean, 27). All had fevers >40.0°C. The number of febrile days ranged from 6 to 44 (mean, 16). Eight children with hepatitis recovered, 4 without loss of their graft. Immunosuppression had been decreased in 3. Four surviving children required retransplanta-

Table 1. Serotypes and source of adenoviral isolates in orthotopic liver transplant recipients and others during 1982–1989.

Serotype	Source of adenovirus						Total episodes with serotype, no. (%)	Nontransplant patient with serotype, no. (%)
	Urine	Throat	BAL	Liver	Stool	Blood		
1	6	2	0	3	5	0	12 (22)	16 (19)
2	6	10	3	4	4	3	18 (34)	19 (22)
3	0	0	0	0	0	0	0	16 (19)
4	0	1	0	0	0	2	2 (4)	2 (2)
5	7	5	0	9	3	3	16 (30)	4 (5)
6	1	0	0	0	0	0	1 (2)	0
7	0	1	0	0	0	0	1 (2)	2 (2)
8	0	0	0	0	0	0	0	13 (15)
19	0	0	0	0	0	0	0	2 (2)
Not 1–7a, 8, or 19	2	0	0	0	0	0	2 (4)	2 (2)
Not typed	1						1 (2)	9 (11)
Total	23	19	3	16	12	8	53 (100)	77 (99)*

NOTE. BAL, bronchoalveolar lavage.
* Only 99% of patients are accounted for because of rounding errors.

tion; fever resolved immediately in each. Six children died. Adenovirus contributed to the death in 5 patients, three of whom died during retransplantation.

Stool cultures were positive for adenovirus in 13 children; 9 were symptomatic. Seven children had gastroenteritis without other identifiable pathogens. One child had concurrent CMV hepatitis, while another child had concurrent *Pseudomonas* bacteremia.

Eight children had adenovirus isolated from buffy coat specimens. This was the sole site of isolation in only two patients. In the rest it was a confirmatory culture in children with pneumonia or hepatitis.

Symptoms. Of the 53 episodes of adenoviral infection, 34 were associated with symptomatic illnesses; 20 patients had invasive disease (table 2). Fifteen children were asymptomatic, and 6 patients were classified as indeterminate. Evaluation of risk factors for symptomatic and invasive disease was done. Thirteen of 20 children with invasive disease compared with 3 of 28 with noninvasive disease had adenovirus isolated from more than one body site ($P < .001$).

Serotype 5 was associated with symptomatic disease more often than other serotypes ($P < .05$). Fifteen of 17 episodes of adenoviral infection with serotype 5 were symptomatic; 10 were invasive.

The use of OKT3 showed a trend toward an association with severe infection. OKT3 was used in 12 (60%) of 20 patients with invasive disease compared with 9 (32%) of 28 without invasive disease ($P = .1$). Other factors including age, time to infection, use of azathioprine, concurrent CMV infection, and number of rejection episodes were not significant risk factors for symptomatic or invasive disease.

Discussion

The incidence of adenoviral infection after pediatric OLT at our institution was 10.1%. Although this study was retrospective, viral cultures were submitted for all patients with significant fevers and illnesses. Accordingly, serious adenoviral infections should not have been overlooked. The median time from OLT until adenoviral infection was 25.5 days, with all symptomatic disease occurring within the first 3 months. Nonetheless, one of our recent transplant recipients presented with a rapidly fatal adenoviral hepatitis 6 months after OLT. This child was heavily immunosuppressed, providing the probable risk factor for severe late infection.

Serotypes 1, 2, and 5 were most common, with serotype 5 causing most of the cases of hepatitis. Serotype 5 is a recognized cause of hepatitis in both immunocompromised and normal hosts [8, 9]. An earlier review of adenoviral hepatitis from our institution implicated only serotype 5 [3]. In this series we found hepatitis caused by serotypes 1 and 2 as well.

The most common invasive disease associated with adenovirus in our population was hepatitis. This contrasts with bone marrow transplant and renal transplant recipients, in whom the most common expressions of adenoviral infection were pneumonia and hemorrhagic cystitis, respectively [8, 10, 11]. Solid organ transplant recipients are known to have a propensity to develop infections at or near the site of the transplanted organ. It is hypothesized that allograft reactions such as graft-versus-host or host-versus-graft disease may make the organ a susceptible target for infection [2]. Alternatively, the donor organ may be the source of the viral infection and thus the site of initial activity.

Table 2. Orthotopic liver transplant (OLT) patients with invasive adenoviral infection.

Disease, patient no.	Serotype	No. of days after OLT	Sites infected	Concurrent infections	Intervention	Outcome
Hepatitis						
1	5	17	Liver	None	Second OLT	Survived: improved after second OLT
2	5	26	Blood, liver, urine	CMV pneumonia and esophagitis	Decreased immunosuppression	Survived: severely ill, slow gradual improvement
3	5	14	Liver, urine	None	Decreased immunosuppression	Survived: mild hepatitis, self-limited illness
4	5	18	Blood, liver, resp (autopsy)	Abdominal abscess	Decreased immunosuppression	Died: severe disseminated disease
5	5	45	Liver	*Klebsiella* bacteremia	None	Died: severe hepatitis and rejection; died during second OLT
6	5	23	Liver, stool	AV type 2 in urine	Decreased immunosuppression, second OLT	Survived: improved after second OLT
7	1	24	Liver (autopsy)	PTLD	IVIG, second OLT	Died: severe illness; died during second OLT
8	1	69	Liver, resp, urine	PTLD, fungal wound infection	Decreased immunosuppression	Died: hemodynamic instability
9	5	32	Liver	None	Decreased immunosuppression	Survived: limited illness that improved by 10 days
10	5	44	Liver	None	None	Survived: self-limited illness
11	1	15	Bile, liver, pleura	Bacterial cholangitis	Second OLT	Survived: severe illness that resolved after second OLT
12	2	17	Blood, liver, resp, stool, urine	CnS bacteremia	Decreased immunosuppression, second OLT	Survived: biphasic with fevers and AV^+ diarrhea; fever resolved by 1 week, then progressive severe disease, followed by rejection and second OLT
Hepatitis and probable pneumonia						
13	5	20	Blood, liver, resp, urine	None	Decreased immunosuppression	Died: severe illness with respiratory distress and hepatitis
14	2	16	Blood, liver, resp, urine (autopsy)	None	Decreased immunosuppression	Died: progressive disseminated disease leading to death in 15 days
Pneumonia						
15	2	9	BAL fluid, resp	Rotavirus	Decreased immunosuppression	Survived: severe 16-day illness that required mechanical ventilation
16	2	4	BAL fluid, resp, stool	CnS bacteremia	Decreased immunosuppression	Died: progressive respiratory illness leading to death in 10 days
17	2	13	BAL fluid	None	Decreased immunosuppression	Died: progressive respiratory illness leading to death in 11 days
Probable pneumonia						
18	2	14	Resp	CnS line infection	None	Survived: self-limited illness
19	4	64	Blood, resp	None	Decreased immunosuppression	Died: severe disseminated illness, leading to death in 9 days
20	5	17	Resp	CMV hepatitis, probable CMV pneumonia	Decreased immunosuppression	Died: progressive respiratory illness after disseminated CMV disease, leading to death in 23 days

NOTE. CMV, cytomegalovirus; resp, respiratory system; AV, adenovirus; PTLD, posttransplant lymphoproliferative disease; IVIG, intravenous immunoglobulin; CnS, coagulase-negative staphylococci; BAL, bronchoalveolar lavage.

Review of potential risk factors suggested that neither *CMV infection*, number of rejection episodes, nor azathioprine use correlated with severe disease. A trend was seen between OKT3 use and invasive adenoviral infection. The potential significance of OKT3 is supported by other studies of liver transplant recipients, which showed an increased risk of invasive viral disease after its use [7].

The usual occurrence of serious adenoviral infections within the first several months after transplantation has been noted by others [8, 10–12]. This timing of adenoviral infection suggests reactivation of latent virus, donor organ–associated transmission, or nosocomial transmission. Adenovirus has been shown to have a capacity for latency, so both donor transmission and reactivation are possible. Shields et al. [8] suggested reactivation as the most likely mode of transmission of adenovirus in bone marrow transplant patients, while Koneru et al. [13] found the most severe disease in liver transplant recipients who were seronegative for adenovirus before transplant. Donor serology was positive in five of six evaluated patients, suggesting donor transmission as the likely source. A recent case report also suggested donor transmission but implicated acute viremia in the donor at the time of his death [6]. Donor transmission of other infections such as CMV and Epstein-Barr virus has been documented [14, 15], demonstrating biologic plausibility for this mode of transmission.

Nosocomial acquisition of adenoviral infection is also a consideration, as several patients with the same strains were found temporally clustered. Although patients did not share primary nurses or rooms, the physician team was identical for all transplant recipients hospitalized at the same time. In addition, nurses commonly cross-cover on a floor, and a communal playroom is available. Accordingly, nosocomial transmission may account for some of the observed infections. Prospective studies using molecular epidemiologic techniques may be able to clarify the role of donor transmission for adenovirus in the future.

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