Serodiagnosis of Adenovirus Infections at a Tertiary Care Centre in Saudi Arabia

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Objective: To determine the seroprevalence of adenovirus infection in tertiary care patients.

Design: Retrospective analysis of adenovirus infections by serology.

Setting: King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.


Results: 13 patients had titres > 1:16 in the absence of other pathogens; 11 patients were immunocompromised with five deaths; seven patients experienced pulmonary distress and two had persistent diarrhoea.

Conclusion: Adenovirus infection should be considered in the aetiology of severe illness in immunocompromised children.

Keywords: Adenovirus. Serodiagnosis. Immunocompromised.


Of the known respiratory viruses, the human adenoviruses cause the widest variety of illnesses and comprise at least 47 distinct serotypes. Pertussis syndrome, acute haemorrhagic cystitis, and gastroenteritis are primarily illnesses of young children due to adenoviruses, whereas acute respiratory disease, epidemics and keratoconjunctivitis are primarily adult afflictions. They are responsible for >50% of cases of acute respiratory disease in military recruits, for 6–13% cases of gastroenteritis in young children, and for 2–15% cases of upper and lower respiratory infection in all populations.1 Serodiagnosis of adenovirus infections can be performed for all syndromes since they share group specific antigen epitopes. These can be detected by complement-fixation (CF) or enzyme immunoassay.2,3 Acute and convalescent sera are submitted 2–4 weeks apart. Serology can also be of epidemiologic importance or of clinical interest in establishing an association between an unusual or severe illness and adenovirus infection. Single

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Materials and Methods

Serum samples from 565 patients were analysed for the presence of adenosivirus antibodies during 1987–1992. All sera were tested by complement fixation test (CFT) using a standard microplate method. A 1:4 dilution of serum in barbital buffered diluent (BBD) (Flow Laboratories, High Wycombe, UK) was prepared and heat inactivated at 56 °C for 30 min. Serial dilutions in ‘U’ well microplates (Dynatech, Chantilly, VA, USA), starting at 1:8 to 1:2048 were made, and a second dilution of 1:8 to 1:32 was prepared on the same plate to test for anticomplementary activity (control cells).

Diluted antigen (Whittaker MA Bioproducts, Walkersville, NJ, USA) in BBD was added to test wells and BBD was added to control wells to maintain equal volumes. Guinea pig complement (Whittaker, MA Bioproducts) diluted in BBD was added to all wells, the plates were mixed and incubated overnight at 4 °C. The following day washed sheep red blood cells, sensitized with haemolysin (Whittaker, MA Bioproducts), were added to each well, mixed and incubated at 37 °C for 45 min with mixing after 15 and 30 min.

Plates were centrifuged for 3 min at 300 × g and read with the endpoint at 25% haemolysis. Optimal antigen, complement and haemolysin dilutions were determined by chessboard titrations. Sera not immediately tested were stored at −20 °C until such testing could be performed. Acute and convalescent specimens were tested on the same day.

Results

Single and paired sera from 565 patients were examined during the period January 1987 to January 1992. A titre of <1:8 was considered negative since 98% of our patient population had titres less than this, a titre of 1:8 or 1:16 as exposure to the virus and >1:16 as indicating recent or current infection. There were 13 patients positive for adenosivirus antibodies with titres >1:16 (Table 1). Of these, 11 patients were immunocompromised; five of them died. There was one primary diagnosis of adenosivirus pneumonia and another patient with adenosivirus haemorrhagic cystitis. Seven patients had respiratory distress with fever and two patients presented with persistent diarrhoea.

Eleven of the 13 patients had negative bacterial cultures, three patients were negative for fungal cultures and two patients were negative for mycobacteria. Viral serologies were carried out on eight patients—of whom five were negative for influenza, parainfluenza, respiratory syncytial virus and mycoplasma. A further three patients were negative for herpes simplex, cytomegalovirus and Epstein-Barr virus. Three of the patients failed to respond to multiple antibiotic therapy and two patients failed to respond to acyclovir.

Only four cases had follow-up serology, where one patient had a four-fold rise from 1:64 to 1:256, one patient had from 1:128 to 1:256 and two patients had stationary titres of 1:128 and 1:32. Although the mere demonstration of a high antibody titre in the remaining patients is of limited diagnostic value, the data were evaluated in the light of the clinical findings (Table 1). Twelve of the patients were under 8 years old and 10 of the 13 patients were male. There was only one adult with serological evidence of adenosivirus and he had been admitted for a renal transplant (Table 2).

Discussion

The diagnosis of causative agents such as adenosivirus is often sought by serological methods because of the time, cost and technical expertise needed for cultivation of viruses. Although rapid immunoassays are available for direct antigen detection in clinical specimens (enzyme linked immunosorbent assay, latex agglutination, polymerase chain reaction), they are time-consuming and beyond the scope of many small laboratories, especially when screening for multiple pathogens. The quality of the clinical specimen is also a major factor; some viruses cannot be cultivated and specimens must be collected during active viral shedding. In contrast, screening of large numbers of sera, for antibodies to multiple organisms and for epidemiological purposes remains both time- and cost-effective. A previous report has shown that adenosivirus accounted for 13% of viral respiratory disease between 1967 and 1976. An incidence of 6% was previously reported in Saudi Arabia but the study examined only a very small patient population. Another study on tonsillitis and respiratory infections of viral aetiology in Saudi Arabia showed adenosivirus to be the most dominant respiratory pathogen in
both adults and children. In our population evidence of recent infection was found in only 2.3% but evidence of exposure in 21% of patients.

Shinozaki et al. examined 322 serum samples for enteric adenoviruses in various age groups. They found the incidence to be 20% in children between 1 and 6 months old and 50% in those 37–48 months old; 48% of adults and 10% of the aged had antibodies using a neutralization test. Another study showed that viral pulmonary infections are a major cause of morbidity and mortality in solid organ transplant recipients. The herpes group of viruses caused most viral infections in such patients but adenoviruses, RSV and HIV remained major causes of pneumonitis.

Although serological changes are important for definitive diagnosis, it must be emphasized that serological investigations have their limitations. For example, Landry et al. reported a bone marrow transplant recipient with rapidly fatal gastroenteritis. Adenovirus infection was confirmed by electron microscopy but CFT was negative. The main disadvantage of the CFT test is its low sensitivity; it can detect infection in only 50–70% of cases in general and <50% of cases in young children.

The low number of specimens sent for serodiagnosis causes underestimation of adenoviral infection as a serious illness as our low rate of convalescent specimens after an initial positive specimen shows. However, absence of other infecting organisms in these cases indicates that greater emphasis should be given to this virus by clinicians since these figures may represent diagnoses of adenovirus infection that were overlooked. The low rate of positivity may be a result of patients not having viral serology performed, but instead having the diagnosis made on the basis of electron microscopy and viral culture. The diagnosis of infection is best performed by a combination of culture and serology, but in the absence of specimens for culture and convalescent sera, we examined the results available to establish the extent of adenovirus infection in our patient population. A previous study on adenovirus-associated gastroenteritis at this institution found the virus antigen in 4% of the patients using a combination of electron microscopy, enzyme immunoassay and cell culture techniques.

Our results are similar to the study of Zahradnik et al. who described 15 immunocompromised patients with evidence of adenovirus infection. All cases were associated with high fever, 11 cases had pulmonary complaints, and nine patients died. We concur with them that adenovirus infection should be considered in the aetiology of severe overwhelming illness, especially in the immunocompromised host.
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References