

Antibodies to enteric adenoviruses (Ad40 and Ad41) in sera from Iranian children

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Abstract

Background: There is strong epidemiologic and serologic evidence that infection with the enteric adenoviruses can result in severe gastroenteritis in children. **Objectives:** This study was performed to determine the prevalence of enteric adenovirus infection in Iran. **Study design:** One hundred and twenty-seven single sera from children up to 7 years of age, collected from healthy Iranian children in 1993–1994, were tested for antibodies to enteric adenoviruses by neutralization tests. **Result and conclusion:** Antibodies to enteric adenoviruses have been detected in about one-half of sera. It is concluded that infection by these viruses is common among children in Iran. © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

It has been estimated that 5–10 million children die each year in the Third World from diarrheal diseases, many of which caused by viruses (White and Fenner, 1994). Especially, in these regions of the world, it is important to perform epidemiological studies to identify the causative agents of these illnesses. There is strong epidemiologic and serologic evidence that infection with the enteric adenoviruses type 40 (Ad40) and type 41 (Ad41) can result in severe acute diarrhea in young chil-

dren (Grimwood et al., 1995; Horwitz, 1996). The enteric adenoviruses have been recognized as being, after rotavirus, the second most commonly identified agent in stools of infants and young children with viral gastroenteritis in various parts of the world (Cukor and Blacklow, 1984; Uhnou et al., 1984; Albert, 1986; Cruz et al., 1990) In agreement with these observations, the presence of antibodies to Ad40 and Ad41 rise rapidly during the first years of life, indicating that this is the period when most infections occur (Kidd et al., 1983; Albert 1986; Shinozaki et al., 1987).

There are no epidemiological studies performed to detect the incidence of enteric adenovirus infections in Middle Eastern countries like Iran. Such studies are necessary to provide a rational basis for responsible patient management. In the

Abbreviations: Ad40, adenovirus type 40; Ad41, adenovirus type 41.

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present study 127 sera collected from Iranian children have been examined for the presence of neutralizing antibodies to Ad40 and Ad41. The results show that infection by these viruses is widespread among Iranian children.

2. Materials and methods

2.1. Human sera

One hundred and twenty-seven sera were collected in 1993–1994 from healthy Iranian rural children less than 7 years of age. The sera were stored at -20°C until use.

2.2. Cells

Monolayer cultures of HeLa and Graham 293 cell lines were grown in Eagle's minimum essential medium (EMEM) containing 10% and 2% fetal bovine serum (FBS) for growth and maintenance, respectively, and antibiotics (100 U/ml penicillin and 50 $\mu\text{g/ml}$ streptomycin).

2.3. Viruses

Ad40 strain Dugan and ad41 strain Tak were a gift from Dr J.C. De Jong (Rijksinstituut voor de Volksgezondheid Bilthoven, The Netherlands). Strain Dugan (passaged three times in tertiary cynomolgous monkey kidney cells at Bilthoven) was passaged twice in HeLa cells on receipt. Strain Tak (originally passaged in Hep-2 cells) was passaged twice in Graham 293 cells followed by two passages in HeLa cells on receipt.

2.4. Neutralization test

The neutralization tests were performed by a combination of the method of Erdman and Hierholzer (1997) and De Jong et al. (1983) with some modifications. Sera were heated at 56°C for 30 min and 1:20 dilutions were prepared in EMEM without FBS. Virus preparations of Ad40 and Ad41 were obtained by harvesting infected cell cultures when all cells showed cytopathic effect (CPE), rapidly freezing and thawing the cultures three times and clarifying by centrifugation at 3000 rpm for 20 min. Working dilutions of these viruses contained 100 TCID₅₀. Equal volumes of diluted serum and each of the two types were incubated at room temperature for 1 h. The resulting suspensions were tested separately for infectivity in HeLa cell culture tubes, each receiving 0.2 ml. Cultures were incubated at 37°C and were examined daily with an inverted light microscope. The tests were read when in the virus control tubes 90–100% of cells showed CPE which usually took 5–7 days. A serum was regarded as positive for antibodies to the concerned virus type if it inhibited CPE completely or confined it to less than 10% of the cells. A concurrent titration of each test virus was included to determine that the test dose actually contained approximately 100 TCID₅₀. Known positive and negative control sera were also included in each run.

3. Results

At a dilution of 1:20, 65 (51%) of 127 sera were positive for neutralizing antibodies to enteric ade-

Table 1

Results of neutralization tests on 127 children's sera for presence of antibodies to Ad40 strain Dugan and Ad41 strain Tak

| Number of sera tested by neutralization against | Ad40 strain Dugan | | |
|---|-------------------|----------|-------|
| | Positive | Negative | Total |
| Ad41 strain Tak | | | |
| Positive | 40 | 10 | 50 |
| Negative | 15 | 62 | 77 |
| Total | 55 | 72 | 127 |

noviruses (Ad40 and/or Ad41). Forty of the 127 sera reacted with both Ad40 and Ad41 at titers of ≥ 20 , whereas 62 sera neutralized neither type. Twenty-five sera reacted with one type only (Table 1).

4. Discussion

In the present study, neutralizing antibodies to enteric adenoviruses have been detected in about one-half of sera from Iranian children. This high seropositivity rate is similar to that reported by other studies (Kidd et al., 1983; Shinozaki et al., 1987; Jarecki-Khan and Unicomb, 1992) and demonstrates that infection by Ad40 and Ad41 is also common among children in Iran. The true prevalence of enteric adenoviruses in sera is probably higher than those observed. Some children mount a low antibody response after infection and in others serum antibody titer may decline below the titer of 1:20 used in the neutralization test as a screening dilution. A 1:20 dilution of serum was used because the preliminary studies indicated that this dilution was high enough to minimize the possible toxic effects of serum and heterotypic responses.

In neutralization tests, Ad40 and Ad41 exhibit extensive cross-reactivity (De Jong et al., 1983, 1993; Hierholzer et al., 1988). When neutralization tests with human sera are carried out in monkey kidney cell cultures, the results show that there are two distinct types of enteric adenoviruses (De Jong et al., 1983). However, there is a much closer relationship between the two types when continuous human cell cultures are used in neutralization tests. In this case antisera to type 40 at high concentration neutralized type 41 and vice versa (De Jong et al., 1983; Kidd et al., 1983; Albert, 1986) and this may be one of the reasons for the reaction of 40 sera with both types in this study. Another reason may be previous infection with two viruses.

The present study confirmed for Iran the high presence of enteric adenovirus infections among children. This fact justifies the implementation of rapid test to diagnose the concerned infections. A positive rapid test result means high chance for a relatively mild course of the disease followed by a complete recovery (Uhnoo et al., 1984; Horwitz,

1996). Moreover, unnecessary, potentially harmful, and costly administration of antibiotics can be avoided.

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