Role of Adenoviruses in Viral Conjunctivitis

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To assess the incidence of adenoviruses in viral conjunctivitis, 40 cases diagnosed clinically as viral conjunctivitis were studied. Adenoviruses detection by conjunctival swabbing and scraping was done using the indirect immunofluorescent technique. Results have shown that 75% of cases were positive for adenoviruses, and that both types of sampling methods were efficient in detecting the intranuclear viral antigens in the infected conjunctival cells. The peak of adenoviruses conjunctival infection was observed in November, December and January. It is recommended to use the conjunctival swab method for direct detection of adenoviruses in the infected conjunctival cells by indirect immunofluorescent method.

Adenoviruses infections occur worldwide in humans, in all age groups and can occur in endemic, localized epidemic and sporadic patterns (Foy, 1989).

Adenoviruses can infect and replicate at various sites in the eye but it was found that the epithelial cells are the primary target for adenoviruses cytopathology in vivo (Horwitz, 1990).

Clinically, infections of the eye with adenoviruses may appear either in the form of pharyngoconjunctival fever, acute follicular conjunctivitis as a separate entity or epidemic keratoconjunctivitis. After an incubation period of 7-10 days, the disease begins as a mild follicular conjunctivitis with swelling of both bulbar and palpebral conjunctiva in some cases, Subepithelial corneal keratitis can occur in 5 to 10% of patients that can persist for many months and interfere with vision (Dawson et al., 1970; Lehrman, 1984; Kasel, 1992).

According to Jawetz et al. (1989) and Kasel, (1992), adenoviruses exhibit a single type of morphology, a similar chemical composition, replicate in the cell nucleus, and are species-specific. They are nonenveloped and are 65-80 nm in diameter. The capsid proteins are arranged in an icosahedron, having 20 triangular faces in which the 240 hexons are located and 12 vertices carrying the 12 pentons. The virion contains a single molecule of double-stranded DNA in a linear form.

In human beings, 42 serotypes have been recognized but most human diseases are associated with only one third of these types (Wigand et al., 1987).

It is important to confirm the clinical diagnosis by laboratory diagnosis so as to prevent the spread of infection and treat the cases properly. Our study aimed to direct detection of adenoviruses in the infected conjunctival epithelial cells by a rapid indirect immunofluorescent (IF) technique.
in cases diagnosed clinically as viral conjunctivitis.

**MATERIALS AND METHODS**

Forty cases diagnosed clinically as viral conjunctivitis were selected, from Kasr El-Aini ophthalmic outpatient clinic during the period from October 1992 to September 1993. All patients, were complaining of symptoms and signs pointing to viral affection as burning sensation, redness, photophobia, watery scanty secretions and conjunctival and/or corneal follicles. Patient's age ranged from 7 to 50 years, they were 25 males and 15 females.

From each case two samples were taken: the conjunctival swab specimen, done by stroking the lower conjunctival sac of the affected eye 4-5 times with a very fine sterile saline pre-moistened cotton swab. A second specimen was taken by scraping the palpebral conjunctival surface in order to get a thin layer of epithelial cells. Both specimens were then dipped in sterile tubes containing 2 ml. of sterile phosphate buffer saline (PBS) pH 7.2.

Control samples were collected in the same manner from 20 healthy persons matched for age and sex and without any past history of conjunctivitis, and 18 patients with mucopurulent conjunctivitis.

Preparation of antigen spot slides for examination by indirect I.F. technique

(Riggs, 1979): The swabs were squeezed on the inner walls of the tubes and then removed. The cell suspension was centrifuged and the supernate was discarded. The epithelial cells pellet was suspended homogenously in one drop of PBS. The cell suspension was dropped onto the circles of the teflon coated slides (ERIE Scientific Company No 295, USA). Four wells per case were prepared, two for the conjunctival swabs and two for the scraping. Slides were air dried, fixed in cold acetone for 5-10 min. and then stored at -20°C.

Test procedure (Gardner & Mc Quillin, 1980): Specific monoclonal antibodies for adenoviruses (Baxter Health Care Corporation, Micro Scan Division, CA 95691 USA) were dropped on the antigen slides, and incubated 30 min. at 37°C, then washed and dried. Goat anti mouse IgG labeled serum (Kirkigaard & Perry, USA) was dropped onto the slides, incubated, washed and dried. Slides were mounted with buffered glycerol covered with coverslips and examined under the fluorescent microscope.

**RESULTS**

Our results are summarized in Tables (1&2): In this study, it was found that out of 40 cases diagnosed clinically as viral conjunctivitis, 30 cases (75%) proved to be due to adenoviruses infection, by indirect IF technique. Of these 30 positive cases, 18 (60%) were males and 12 (40%) were females.

The apple green fluorescence of intranuclear adenoviruses in the infected conjunctival cells against the dark red non fluorescence colour denoted a positive reaction. All the 38 control samples were negative.
Table (1) : Clinical patterns of cases.

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>IF Positive Cases</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>- Cases with conjunctivitis and respiratory illness</td>
<td>12</td>
<td>40.0%</td>
</tr>
<tr>
<td>- Cases with conjunctival follicles only</td>
<td>26</td>
<td>86.6%</td>
</tr>
<tr>
<td>- Cases with both conjunctival and corneal follicles.</td>
<td>2</td>
<td>6.6%</td>
</tr>
<tr>
<td>- Cases with corneal follicles only</td>
<td>-</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

It was found that there were no difference in the IF results (either positive or negative) in the samples collected by swabbing or scraping the conjunctival surfaces i.e. the 30 positive cases in which the samples were collected by conjunctival swabbing were also positive by conjunctival scraping and the rest of cases were negative by both methods.

From the 30 cases diagnosed by indirect IF to be adenoviruses conjunctivitis, clinically 26 (86.6%) cases of them had conjunctival follicles, 2 (6.6%) cases had both corneal and conjunctival follicles and 2 (6.6%) cases were free of follicles. Also, it was found that out of the 30 IF positive cases 12 (40%), had suffering from respiratory tract infections (4 females and 8 males).

The incidence of adenoviruses conjunctivitis in the study period between October, 1992 till September, 1993, varied from 0% up to 91% as shown in table (2).

Table (2) : Incidence of adenoviruses conjunctivitis during the study period.

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of positive cases for adenoviruses conjunctivitis by indirect IF technique.</th>
<th>Positive %</th>
<th>No. of negative cases by indirect IF technique.</th>
<th>Total No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>3</td>
<td>60%</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>November</td>
<td>8</td>
<td>89%</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>December</td>
<td>10</td>
<td>91%</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>January</td>
<td>7</td>
<td>88%</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>February</td>
<td>2</td>
<td>40%</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>March</td>
<td>-</td>
<td>0%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>April-September</td>
<td>-</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>75%</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>
DISCUSSION

Adenovirus-associated conjunctivitis is a communicable disease and its transmission may be disseminated by towels, hands, swimming pools, contaminated eye instruments and ophthalmic eye solutions, to susceptible persons (Kasel, 1992).

Although adenoviruses infections may be subclinical, the clinical findings should direct attention to the possible diagnosis of adenoviral conjunctivitis.

Many investigators as Kasel, (1979); Krisher and Menegus, (1987); Wadell, (1988); Horwitz, (1990) concluded that cell culture neutralization test although is a reliable and accurate method in diagnosing both the virus or its specific antibodies in the serum, yet it is costly and requires special tissue culture facilities which may not be available in all laboratories, moreover it needs specialized personnel and it is time consuming because the typical adenovirus cytopathic effect (CPE), although may be seen in 2-7 days yet it could require several weeks to appear.

In a study done by Grayston et al. (1958) they found that the continuous epithelial lines as HEP-2; HeLa, and KB cells are highly sensitive but are difficult to maintain for the long period of time (28 days) that may be required to isolate some of the ocular adenovirus strains. They are also more likely to be contaminated with mycoplasma.

Krisher and Menegus (1987), found that primary human embryo kidney (HEK) cells are the best host for adenoviruses but they are expensive and may be contaminated with the adeno-associated virus.

Rapid diagnosis by direct examination of the infected epithelial cells has been tried either by the electron microscopy or by IF technique. Electron microscopy is ideal for detecting the characteristic 80 nm icosahedral particle in the infected cells. Aggregation of adenoviruses by hyperimmune sera can be effectively observed by electron microscopy (Norrby et al. 1969; Wadell 1972; Kasel 1992). But, this method is costly and requires the presence of electron microscope.

Riggs, (1979) found that the IF test has been used for rapid diagnosis of rabies, influenza, herpetic keratitis, and infections due to the respiratory syncytial virus and other viruses. Pal et al. (1983) used the indirect IF method in diagnosing acute haemorrhagic conjunctivitis caused by enterovirus type 70, and they concluded that, in developing countries lacking virus isolation facilities, the IF technique is a promising method for use with direct smear preparations.

In our study, IF technique could detect the virus in 75% of cases presumptively diagnosed clinically to have viral conjunctivitis (Table 2). In (1972) McCormick et al., reported that the IF technique used to detect adenovirus-infected cells is as sensitive as tissue culture growth. The above finding is in line with our results. On the contrary, Belshe and Mufson (1974), concluded that the IF test has not been useful in diagnosis of conjunctival adenovirus lesions. This conclusion is contradicted with our results, but this contradiction may be due to differences in the time of sample collection, number of cells present, as antigen, in the prepared antigen spot slides, the monoclonal antibodies, the conjugate used and the indirect IF technique had different sensitivities in (1974).

Moreover, in our study, there were no statistical differences in sampling techniques either conjunctival swabing or scraping. As, all cases detected as positive cases for adenovirus infection by swabing were also positive by scraping (p-0.0001). So, we recommend the use of conjunctival swabing...
A new human adenovirus of subgroup D: Candidate adenovirus type 42
Arch. Virol. 94: 283-286.

دور الفيروس الخدسي في التهاب الملتحمة الفيروسي

يرمى هذا البحث إلى دراسة دور الفيروس الغدسي في التهاب الملتحمة عن طريق التعرف المباشر عليه بواسطة اختبار الفيروست الماناعي غير المباشر، لهذا تم أخذ عينات من 20 مريضاً يعانون من التهاب فيروسي بالملتحمة بحسب التشخيص الإكلينيكي وذلك بطرقتيتين الأولى وهي عن طريق مسحة من العين من الملتحمة المبطنة للجن والثانية عن طريق كحت سطحي لخلايا الملتتحمة.

وقد تم التعرف على الفيروس الغدسي في 95% من هذه الحالات المشخصة إكلينيكيوياً وأيضاً أثبت كل من مسحة العين والكحت السطحي للملتحمة قد كلاهما المشتركة على التعرف المباشر للفيروس وقد لوحظ أن هذا الفيروس يكثر في شهور نوفمبر وديسمبر ويناير من السنة.