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ROLE OF VIRUSES IN THE ETIOLOGY OF FOLLICULAR CONJUNCTIVITIS

By

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SUMMARY

Sixty two cases of clinically diagnosed nontrachomatous conjunctivitis were included in this study . All patients were subjected to through clinical and ophthalmoloical examination. Laboratory investigations were carried out for the isolation and identification of the viral agenst. The laboratory confirmed cases of follicular conjunctivitis comprised: Herpes simplex (20), epidemic keratoconjunctivitis due to adenovirus (4), hemorrhagic conjunctivitis due to enterovirus (8) and Newcastle disease (2). Varicella zoster conjunctivitis was clinically diagnosed in 3 cases , but the virus was not isolated in any of them. Moreover, conjunctivitis medicamentosa and conjunctivitis associated with chronic tonsillitis were clinically diagnosed , each in 3 cases.

It is concluded that clinical diagnosis of viral follicular conjunctivitis is sometimes difficult , and appropriate laboratory tests are imperative in establishing the viral etiology.

INTRODUCTION

Folicular conjunctivitis is a common ocular disorder varying cliniclaly fom simple allergic conjunctivitis to severe affections with devastating effect on the eye. Most cases may present in various forms such as watery acular discharge, sandy sensation hemorrhages and others.

Herpetic infections are self-limited, but in some immuno-suppressed patients serious ocular manifestation may be noticed (Darouger et al., 1978). They are frequently associated with keratitis (Pavan - Langston, 1979), or vesicular eruptions (Langston and Gary, 1991).

Outbreaks of acute follicular conjunctivitis were reported by Dawson et al., (1972) in adeno-virus infection causing epidemic keratoconjunctivitis, and by Bahgat and Koka Wahab (1989) in cases of epidemic hemorrhagic conjunctivitis due to entero-virus . Moreover, conjunctivitis may be ascribed to infection with Newcastle disease virus , the agent of a zoonotic virosis contracted from different species of domestic and fural birds (Utteback and Schwartz, 1973 and Calnek et al., 1994).

Apart from viral agents, follicular conjunctivitis may arise from drug intake (atropic , eserin) or it may be associated with the presence of septic foci in the body (Kaski, 1994).

The present study aims at investigating the role of viruses in the etiology of follicular conjunctivitis.

MATERIAL AND METHODS

Materials:

Clinically diagnosed cases (n = 62) of follicular conjunctivitis constitute the materials of this work. These cases were among the outpatients attending the Ophthalmic Clinic of kasr El-Eini Hospitals and Research Institute of Ophthalmology.

Methods:

2.1. Ophthalmic Examination: Vision, tonometry, slit-lamp biomicroscopy, detection of corneal epithelial defects, stromal infiltrations and any changes in the conjunctiva and lids.

2.2 Microscopical Examination; To exclude conjunctivitis due to *Chlamydia trachomatis* , conjunctival scrapings were taken from the site. of maximal infection. After topical anaesthesia, the tarsal conjunctiva was lightly scraped with sterile platinum spatula, avoiding lid margin since it may contain keratinized cells. To avoid drying and to preserve the cytologic features, scrapings were immersed in methyl alcohol and broken into a thin smear for maximum visibility of individual cells. The slides were then immersed in freshly prepared Giemsa stain , incubated at 37°C for 60 minutes and quickly decolorized in ethyl alcohol.

3. Isolation of Viruses:

3.1. Sampling : Samples were taken from the affected palpebral conjunctiva, using sterile applicators moistened in broth. Two swabs were taken for each sampl, put in screw-capped vials containing Hank's or Earle's balanced salt solution in amounts of 0.5 ml as transport media. The media were stored at -70°C for inoculation on tissue culture. Throat swabs were taken from patients with history of poultry contact f the isolation of Newcastle disease virus.

To exclude the bacterial etiology follicular conjunctivitis, blood aga MacConkey's agar and choclate agar we plated for aerobic bacteria, where thioglycolate broth was used for anaerobics

3.2. Cell Cultures: Two types of cell lines we used for isolation trials of the virus : th African monkey cell line (Vero) and th Hella cells.

3.2.1. growth and Maintenance media: Eagle minimal essential medium supplemente with 0.1% gentamycin sulfate 50 mg/ml, 2% sodium bicarbonate and 5% foetal calf serur (2% for the maintenance medium).

The media were inoculated with th samples , incubated at 37°C and observe daily for CPE. Samples of cultures showin CPE were inoculated on cell lines two othe times to confirm the viral cause of CPE.

4. Identification of viruses: The viruses coming ir question as agents of follicular conjunctivitis were screened as follows:

4.1. Herpes simplex virus: CPE positive cell lines were frozen and thawed three times, then worked in ELISA test, using human IgG antihuman IgG immunoperoxidase conjugate at a working dilution of 1: 1600.

In addition, inoculated cell lines were detached by trypsin, cold centrifuged, resuspended in PBS and distributed onto spotted slides for indirect immunofluorescence.

4.2. Adeno-and Entero-Viruses: Using antiserum for each virus the following tests were

carried out according to the procedure described by Peacock and Tomar (1980):

4.2.1. Neutralization test.
4.2.2. Complement fixation test.

4.3. Varicella zoster virus: Complement fixation test was performed using specific antiserum and the culture fluid as an antige.

4.4. Newcastle disease Virus; Antibiotic treated suspensions of throat swabs were inoculated in amounts of 0.1ml in cell lines and into the allantoic chamber of embryonated hen's eggs (9-11 days old embryos). The eggs were incubated for 5 days of until embryonic death. Candling of the eggs was performed daily and embryos dying in the first day of incubation were excluded and embryonic death was considered to be traunatic or due to non specific causes. The allantoic fluid of each egg was harvested for hemoagglutination test using 1% suspension of washed chicken RBCs and known Newcastle disease virus immune serum

RESULTS

The cases presented in this study comprised 62 patients with clinical manifestation suggestive of follicular conjunctivitis. The associated symptoms differentiated these cases into 26 herpes simple conjunctivitis, 6 epidemic keratoconjunctivitis, 10 hemorrhagic conjunctivitis 3 Varicella zoster conjunctivitis, 8 Newcastle disease virus conjunctivitis, 3 atropine topical application and 3 chronic tonsillitis (Table 1).

Herpes simplex cases were accompanied by fever, malaise, nasopharyngitis and preauticular lymphadenopathy. Ophthalmic approach evidenced the presence of follicles at the upper and lower fornices . Upper conjunctival papillae were noticed in 4 cases, pseudomembrane in 2 cases, subconjunctival hemorrhage in another 2 cases, fine epithelial punctate keratitis in 8 cases and subepithelial infiltrations in 3 cases.

Adenovirus follicular conjunctivitis manifested its appearance in the form of the epidemic type in 4 cases, and pharyngo- conjunctival type in 2 cases. Generally, the patients suffered from the constitutional symptoms of fever . Examination of the eyes revealed unilateral follicular conjunctivitis in the lower fornix. The following ocular manifestations were moreover noticed: pseudomembrane (1 case), edema of the semilular fold and caruncle (1 case), subconjunctival hemorrhage (1 case), small rounded foci of subepthelial infiltrations at te center of the cornea with involvement of the other eye within few days, but a milder from (4 cases) and periauricular lymphadenopathy (3 cases).

In all the ten cases of hemorrhagic conjunctivitis, nasopharyngitis was the first signs noticed followed by fine petechiae of the bulbar conjunctiva and the presence of fine follicles of the palpebral (Fig.1) Punctate epithelial erosions and subepithelial infiltration were noticed in 2 of 4 corneal affections. All cases were unilateral but the other eye was also involved in 3 cases.

Varicella zoster cases were manifested in the from of unilateral vesicles along the branches of the trigeminal nerve. Conjunctival follicles and pustules occurred at the upper tarsal part (2 cases) and the bulbar part (1 case). Small few dendritic ulcers were seen at the periphery of the cornea (2 eyes). The ulcers showed dull irregular fluorescent staining with diffuse corneal haze, whereas those of herpes simplex were centeral showing bright fluorescence and localized corneal haze.

Cases of Newcastle disease conjunctivitis were clinically suspected in 6 employees in a polutry

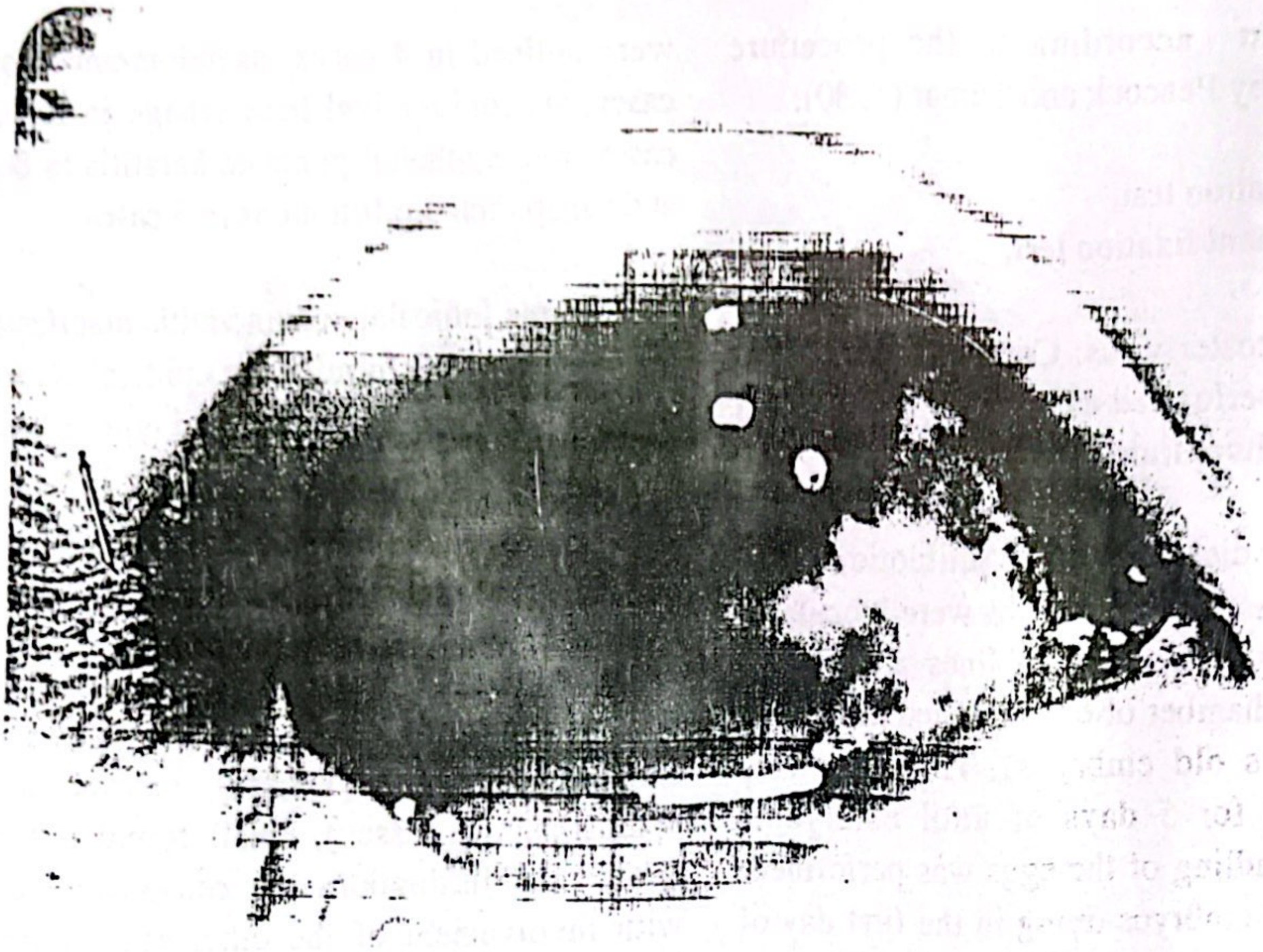

Fig. (1): A case of hemorrhagic conjunctivitis with follicular reaction at the lower palpebral conjunctiva.

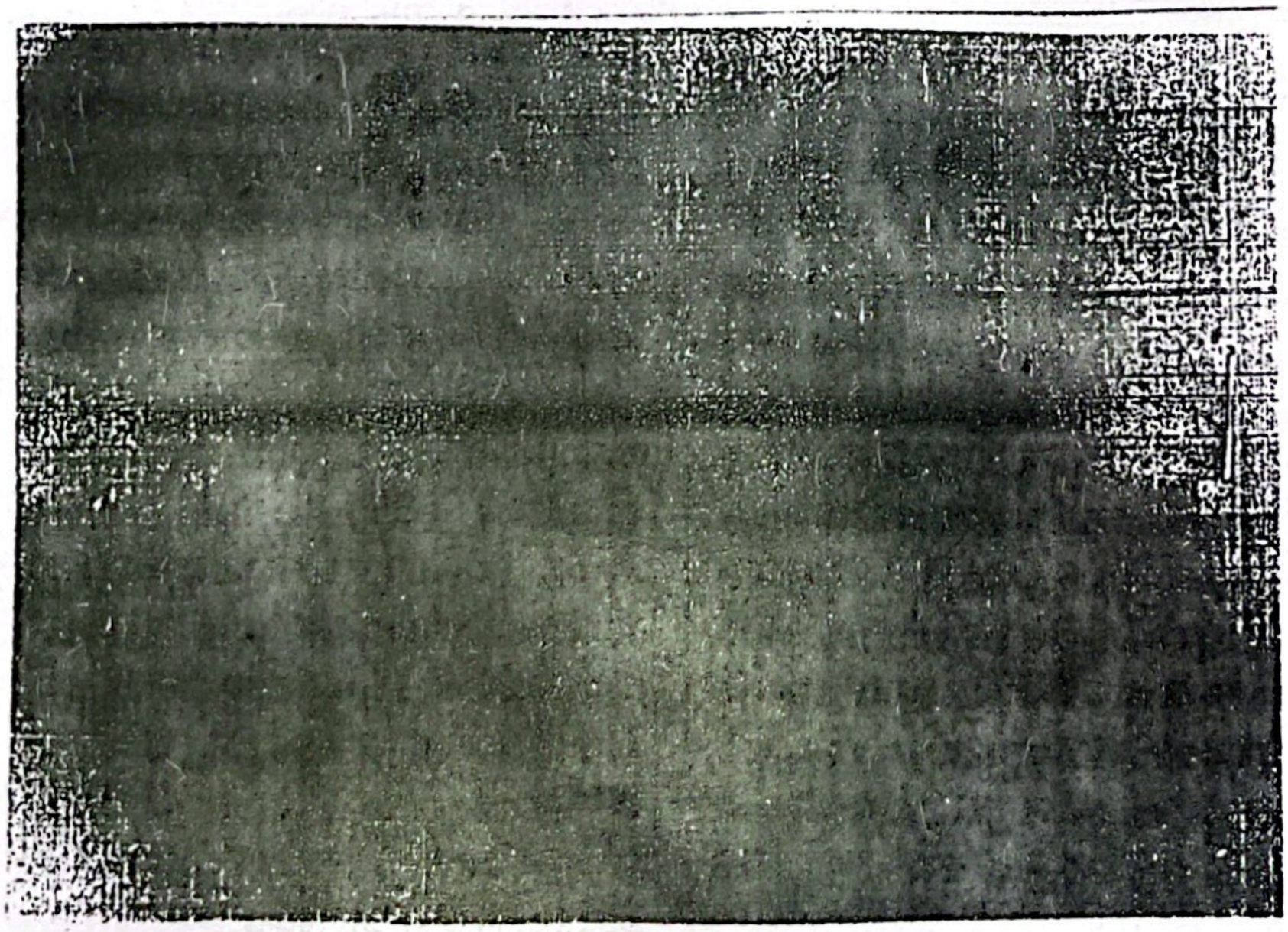

Fig. (2): The effect of herpes virus in infected cell cultures showing giant and multinucleated cells.

Table (1) : Laboratory findings of clinically diagnosed cases of follicular conjunctivitis .

Causative agent	Laboratory positive cases cell culture (CPE)		confirmatory test	
	No.	%	No	%
Herpes simplex 26	20	76.9	20	76.9
Epidemic Kerato-conj . (Adenovirus)6	4	66.6	4	66.6
hemorrhagic conj.(Enterovirus)10	8	80.0	8	80.0
varicella zoster 3	-	-	-	-
Newcastle disease 8	6	75.0	2	25.0

Table (2) : Frequency distribution of virus isolation rate (n= 53)

clinically diagnosed cases	No.	Laboratary confirmed cases	rate of viral isolatio
Herpes simplex	26	20	37.7 %
Epidemic kerato-conj - (adenovirus)	6	4	7.5 %
Hemorrhagic conj. (Enterovirus)	10	8	15.0 %
Varicella zoster	3	-	-
Newcastle disease	8	2	3.8 %
Total No	53		

farm and 2 technicians in a veterinary research laboratory. All cases were characterized by sudden onset of follicular conjunctivitis, edema of the eyelid, watery ocular discharge and periauricular lymphadenopathy. The affection was unilateral encroaching over the other eye in two patients. None of the cases showed corneal affections, and the conjuctival follicles disappeared within a week.

During the course of this study, cases of conjunctivitis due to causes other than viral agents were met with. They comprised three children receiving topical atropine medication for different indication. The salient signs included folicles at the lower fornix., edema of the conjunctiva and eyelid, together with unilateral eczyma of the latter. Moreover, three children (6-10 years old) were suffering from suppurative tonsillitis. Opthalmic examination revealed conjunctival follicles arranged in parallel rows in the lower palpebral part.

Results of the laboratory investigations are presented in Table (1).

DISCUSSION

Almost all ocular tissues are susceptible to viral affections, the conjunctiva being the most commonly affected structure. Its affections are manifested in different forms such as catarrhal inflammation , follicles, papillae, pseudomembrane, chemosis, hemorrhages, etc. Follicular conjunctivitis is found to be practically associated with most cases of viral affections, besides allergy due to medication or as a sequal to septic foci in the body (Pavan - Langston, 1979; Langston and Gary, 1991). Nevertheless, serious follicular conjunctivitis was reported as a result of prmary infection with viruses, viz herpes simplex, varicella zoster and others (Kaski, 1994).

Since conjunctival follicles were seen in all cas of active trachoma (Thygeson and Dawsc 1966), it was necessary to exclude trachoma bc clinically and by appropriate laboratory approac Trachoma is always associated with characteris yellowish small follicles affecting thc upp palpebral conjunctiva and fornix. The follicles a surrounded by dilated capillaries and do n protrude from the surface except in advanc stages. The cornea also contains trachomato follicles or pannus at the upper limbal area Moreover trachoma cases usually end b cicatrization, wheaeas viral cases impro spontaneously without such sequal. Giemsa stained preparations excluded *Chlamyd trachomatis* from conjunctival scrapings.

Clinical differential diagnosis of viral follicul conjunctivitis is difficult (Kaski, 1994). Relatir the laboratory confirmed cases to the clinicall diagnosed ones (Table 1), it was found th herpes simplex, epidemic , kerato conjunctiviti hemorrhagic conjunctivitis and Newcastle disea conjunctivitis were confirmed by appropria laboratory tests in 76.9%, 66.6%, 80% and 25% of the cases respectively . Most of thc case showed small elevated reddish follicles at th lower palpebral conjunctiva and lower. forni Therefore, associated conjunctival and othe ocular changes may be of value in the differenti diagnosis.

In hemorrhagic conjunctivitis, the presence of fine petechiae on the conjunctiva (Fig.1). I herpes simplex, the presence of centra widespread brightly fluorescent ulcers in th cornea; in Varicella zoster, the ulcers are smal few and found at the upper limbal area , i addition to the presence of vesicular eruption along the branches of the trigeminal nerve; i epidemic keratoconjunctivitis the presence o small, rounded foci of subepithelial infiltration i the center of the cornea.

Case history is considered to be a valuable toal in establishing a clinical diagnosis of follicular conjunctivitis. For example, history of contact with birds is suggestive of Newcastle disease virus infection. The associated symptoms may also be helpful in the clinical diagnosis , viz: neurological signs in hemorrhagic conjunctivitis (Bahgat and Koka Wahab, 1989), pharyngitis and submaxillary lymphadenopathy in pharyngo-conjunctival fever.

Accordingly, to confirm the clinical diagnosis of viral follicular conjunctivitis, appropriate laboratory investigations should be carried out. (Nahmias et al., 1974).

From a total of 53 clinically diagnosis cases, the frequencuy distribution of the laboratory confirmed viral follicular conjunctivitis (Table 2) figured up to 37.7% (herpes simplex), 15% (hemorrhagic conjunctivitis due to adenovirus), 7.5% (epidemic keratoconjunctivitis due to enterovirus) and 3.8% (Newcastle disease virus). These findings indicate that herpes simplex virus is the most common etiologic viral agent of conjunctivitis, thus supporting the results of most investigators (Nahmais, 1974; darouger et al., 1978; Pavan-langston, 1979).

Although varicella zoster infection was clinically diagnosed in three patients , yet the virus could neither be isolated nor demonstrated by any of the appropriate laboratory tests. This may be ascribed to the difficulty in growing this virus to high titers in the conventional tissue cultures and no experimental animal is suscptibel to it, Even in specific cell lines, CPE is focal, develops slowly and the virus replicates in the cell nucleus remaining cell-associated (Juel - Jensen and MacCallum, 1972; Kaplan, 1973).

The relatively low isolation rate of Newcastle disease virus from cases of follicular conjunctivitis (3.8%) may be explained by the fact that this infections does not elict good antibody response in the human subject (Calnek et al., 1994).

On the basis of the results achieved in this study, it can be concluded that appropriat laboratory tests are imperative in the differential diagnosis of viral follicular conjunctivitis . When the clinical sample contains a very low viral titer, this affection can be diagnosed by cell culture inoculation where the CPE appears 3 days p.i.

(Fig.2) , where when the virus titer is high rapid diagnosis can be established within few hourse by direct detection of the virus by immunofluorescence.

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