Original Research Article

Adeno and Herpes Simplex Viral Infection in Keratoconjunctivitis A Comparison between Two Diagnostic Laboratory Methods

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A B S T R A C T

Adenovirus and Herpes simplex virus (HSV) are common viral causes of keratoconjunctivitis (KC). A rapid laboratory diagnosis is often very beneficial. The aim of this study was to show the prevalence of Adenovirus and HSV in patients diagnosed as KC and to evaluate the efficacy of rapid direct antigen detection of Adenovirus and HSV-1 by direct immunofluoresence assay (DIF) compared with Vero cell line culture for diagnosis of viral KC. Fifty eight patients clinically diagnosed as viral KC were included in this study; forty patients with follicular conjunctivitis and eighteen patients with keratitis. Conjunctival and corneal scraps were tested by DIF for Adenovirus and HSV compared to viral isolation which was performed on Vero cell line. Adenovirus was detected in 45% and 5.6% of conjunctivitis and keratitis patients respectively while HSV was detected in 27.5% and 38.9% respectively. Adenovirus was detected in 32.8% of patients by DIF while 13.8% by cell culture. Positive predictive value (PPV), Negative predictive value (NPV),Sensitivity, specificity, and efficiency of DIF for Adenovirus were 42.1%, 100% 100%, 78%, and 81% respectively as compared to cell culture .HSV was detected in 27.6% by DIF while 8.6% by cell culture. Sensitivity, specificity, PPV, NPV and efficiency of DIF for HSV were 60%, 75.5%, 18.8%, 95.2% and 74.1% respectively as compared to cell culture. From this study we conclude that Adenovirus was a common pathogen in conjunctivitis and HSV was a common pathogen of KC. IFA is rapid and simple test and can be used as primary screening tool. The combination of cell culture and DIF establishes the best set of methods of diagnosis of clinically suspected infected cases of viral KC.

Introduction

Conjunctivitis and keratitis are the most two common forms of ocular morbidity seen in general practice and at eye units (Postema et al., 1996). The etiology of conjunctivitis and keratitis includes viral such as adenovirus and Herpes simplex virus (HSV), bacterial, Chlamydial or parasitic in addition to trauma, allergy, and dietary deficiency (Asbell et al., 1996).

Disease severity can be mild to strictly disabling. Ocular adenovirus infections occur all over the world in sporadic and epidemic forms. It is spread via droplet, direct contact (Cheung et al., 2003), fecal-oral transmission, and contact with non-chlorinated or insufficiently chlorinated water (Melendez et al., 2009).

Herpes simplex keratitis is a vision threatening ocular infection frequently caused by HSV-1. It is animportant cause of blindness and occurs worldwide (Athmanathan et al., 2001), causing several complications and increasing the risk of severe corneal thinning and perforation (Butler et al., 2005).

Cell culture isolation needs viable organisms requiring specific transport media and quick transport of specimens between patient and laboratory. It is expensive and time-consuming but remains the gold standard, as isolation of the organism is definitive and allows advance characterization (Saitoh-Inagawa et al., 1999). In addition, cell culture provides a sensitive method for diagnosis (Harrington et al., 2004).

Immunodiagnostic assays as immunofluorescence using direct antigen detection are more rapid than culture, but with less sensitivity (Harrington et al., 2004).Antigen detection techniques avoid the necessity for preservation of infectivity obligatory for culture but occasionally can produce false negative and false positive test results. In addition, the wide range of sensitivity and specificity values obtained by the antigen detection tests is influenced by many factors as; the quality of the test itself, the quality of the gold standard test used (culture or other comparative test); the "cut off" values for positive and negative results; the experience of the observer to differentiate between specific and non-

specific staining in the case of the direct immunofluorescence test; and false positive results caused by cross reactivity with other organisms in the case of enzyme immunoassays (Elnifro et al., 1999).

During the last decade, however, it has been concluded that polymerase chain reaction (PCR)–based laboratory investigation is a valuable approach for achieving reliable diagnosis of viral and chlamydial KC (Elnifro et al., 1999).

The purpose of this study was to show the prevalence of Adenovirus and HSV in patients clinically diagnosed as KC and to evaluate the efficacy of rapid direct antigen detection of Adenovirus and HSV-1 by DIF compared with Vero cell line culture for diagnosis of viral KC.

Patients and Methods

Fifty eight patients clinically diagnosed as viral KC were included in the study. Forty patients were suspected as having follicular conjunctivitis in the form of watery discharge, preauricular lymphadenopathy, conjunctival follicles, redness, conjunctival hyperemia, eyelid edema. Eighteen patients had clinically showed typical dendritic epithelial defect in the cornea with underlying subendothelial infiltrate pathognomonic of viral keratitis. An informed written consent was obtained from all participants and the Ethics Committee of Mansoura University Hospital approved the study.

Sample Processing

Using standard techniques under topical anesthesia, two scrapings were collected from the affected conjunctiva or cornea under slit lamp magnification with sterile blade (no.15) on a Bard-Parker handle.

Scraped materials were fixed with acetone into Multitest slide for DIF staining technique for Adenovirus and HSV. Another sample was transported on Dulbeccos minimal essential medium (DMEM) (pH 7.2 – 7.4, 200 IU/ml penicillin G, 200 μg /ml streptomycin sulfate and 5 μg /ml amphotericin B) for viral culture.

Direct IFA

IFA was done by ACHERP kit (Vircell, Granda, Spain). Smears were fixed in cold acetone and reacted with monoclonal fluorescein isothiocyanate-labeled (FITC) anti-HSV specific for glycoprotein of Herpes simplex capside. The staining kit for Adenovirus contains FITC monoclonal antibody for Adeno group specific hexon antigen. The kit was used both for staining of the suspected cell culture and for direct staining of conjunctival and corneal scraps. The results were assessed under a reflected light fluorescence microscope at 250 X with wave length of 450 nm. Positive staining was represented and qualified by presence of ≥1 epithelial cells exhibiting specific bright apple-green fluorescence. A presumptive negative result was indicated by the absence of fluorescence in a minimum sampling of 20 basal cells.

Cell Culture

For cell culture, scrapings were inoculated into monolayer Vero cell line which obtained from Environmental Virology Laboratory at National Research Centre. Then cultivated on 24-well plates and examined under inverted microscope for the presence of cytopathic effect (CPE) typical for HSV which begins as clusters of enlarged, rounded refractive cells and spreads to involve the entire monolayer, usually within 48 hours and the results were confirmed by IFA for HSV 1 antigen. The

characteristic CPE for adenovirus consists of grape like clusters of rounded cells. The results were confirmed by immunofluoresence technique.

Statistical Analysis

Data were analyzed using SPSS (Statistical package for Social Sciences) version 10. Qualitative data was presented as number and percentage. Quantitative data was presented as mean and standard deviation. The chi-square (χ^2) was used to find the association between variables of qualitative data. P value of < 0.05 indicates a significant result. Validity tests were done using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Efficiency.

Results and Discussion

This study included 58 patients; 34 males and 24 females. The mean age was 32.3 ± 21.9(SD) and 49.2± 17.5 (SD) for conjunctivitis and keratitis patients respectively. Most of them were from rural area (85%) and 15% from urban.

Adenovirus was detected in 45% and 5.6% of conjunctivitis and keratitis patients respectively while HSV was detected in 27.5% and 38.9% respectively (table 1).

Adenovirus was detected in 19/58 (32.8%) by DIF while 8/58 (13.8%) by cell culture (table 2). Sensitivity, specificity, PPV, NPV and efficiency of DIF for Adenovirus were 100%, 78%, 42.1%, 100% and 81% respectively as compared to cell culture (table 3).

HSV was detected in 16/58 (27.6%) by DIF while 5/58 (8.6%) by cell culture (table 2). Sensitivity, specificity, PPV, NPV and efficiency of DIF HSV were 60%, 75.5%,

18.8%, 95.2% and 74.1% respectively as compared to cell culture (table 4).

Human adenovirus is responsible for causing a wide variety of diseases affecting all organ systems. Highly contagious keratoconjunctivitis is primarily caused by adenovirus types belonging to species D (serotypes 8, 19, 37) (Harrington et al., 2004). HSV 1 ocular infection occurs in many countries and is the most common infective agent of blindness. Rapid and accurate diagnosis is essential for prompt and proper treatment (Pramod et al., 2000).

In this study, adenovirus was detected in 45% and 5.6% in conjunctivitis and keratitis patients respectively and it was detected in 32.8% by DIF while 13.8% by cell culture. Most of the published studies (Weitgasser et al., 2002, Percivalle et al., 2003,) reported much higher incidence (50-100 %) of adenovirus detection by DIF. Our rate of detection of adenovirus by cell culture is going parallel to that found by Madhaan1999 and Torres et al.1998 but higher rates (50%-95 %) were reported by other authors(Loseva 1998, Elnifroet al., 2000, Percivalle et al., 2003,Tamizi et al., 2005,Melendez et al., 2009). This discrepancy in isolation rates among different studies could be attributed to differences in the time of studies, as adenoviral eye infections are more common in hot weather, many studies done during an epidemic yield high rate of adenovirus infection. In addition, the different culture cell lines used yield different rates of isolation.

Adenovirus was more common than HSV as a causative agent of follicular conjunctivitis; a result documented before in many studies (Cooper et al., 1999, Uchio et al., 2000, Percivalle et al., 2003,).Direct antigen detection by DIF was the most sensitive, specific and accurate method (100%, 78%, 81% respectively). This was in agreement with many published reports (Barone et al., 2000, Weitgasser et al., 2002, andPercivalle et al., 2003) but in Contrast to Aoki and Tagawa2002 who reported lower sensitivity of DIF (67%).

In our study, HSV was detected in 27.5% and 38.9% in conjunctivitis and keratitis patients respectively and it was detected in 27.6 % by DIF while 8.6 % by culture. This indicates that HSV keratitis was more common than adenovirus (38.9 % versus 5.6 %). The frequency of HSV keratitis was lower than that reported by some publications [70% - 80 %] (Athmanathan et al., 2001, Farhatullah et al., 2004) but higher than reported by other reports [15 % - 23 %] (Kodama et al., 1992, AbouTouk 1998, Kaye et al., 2000). The result of HSV culture was nearly parallel to Kaye et al. 2000 [10%] and Khodadoost et al. 2004 [12%] , higher than reported by Kaye et al. 2000 and Tamizi et al. 2005 [2%] but lower than reported by Athmanthan et al., 2002 [23.5%].

The false positive values of DIF in adenovirus and HSV diagnosis were 22% and 24.5% while the false negative values were zero and 40% respectively. The false positive results may be caused by non specific sticking of the antibody to the cellular components or arise from binding of protein A of many S. aureus strains to the FC piece of monoclonal antibody or by cross reaction with certain Gm –ve bacteria (Cles et al., 1988). Also, the use of antiviral agent may have a negative viral culture but still provide the antigen that detected by DIF (Bordin et al., 1992).However, the false negative proportion can be obtained because some structures may fluorescence such as lymphocyte, PMNs, folded epithelial cells and bacteria(Stamm 1990).

Table.1 Frequency of Viral Infection in Keratoconjunctivitis (n = 58)

Type of virus	Follicular conjunctivitis (n=40)			Keratitis (n=18)			Total samples (n=58)	
	No.	% from ocular disease	% from total	No.	% from ocular disease	% from total	No.	%
- Positive:	29	72.5	50	8	44.5	13.8	37	63.8
Adenovirus	18	45	31.0	1	5.6	1.7	19	32.7
HSV	11	27.5	19	7	38.9	12.1	18	31.1
- Negative:	11	27.5	19	10	55.6	17.2	21	36.2

HSV= Herpes simplex virus

Table.2 Evaluation of Diagnostic Tests for Adenovirus and HSV (n=58)

Diagnostic method	Adenovirus		HSV	
	Number of samples	%	Number of samples	%
Direct Ag. detection by immunofluorescence: - Positive - Negative	19 39	32.8 67.2	16 42	27.6 72.4
Identification of culture by Immunofluorescence : - Positive - Negative	8 50	13.8 86.2	5 53	8.6 91.4

HSV= Herpes simplex virus

Table.3 Comparison between Direct Antigen Detection by DIF and Culture in Diagnosis of Adenovirus Infection (n = 58)

DIF for adenovirus	Culture						Sensitivity	Specificity	PPV	NPV	Efficiency	P-value
	+ve		-ve		Total							
	No.	%	No.	%	No.	%	%	%	%	%	%	
+ve	8	100	11	22.0	19	32.8	100	78	42.1	100	81	< 0.001
-ve	0	0	39	78.0	39	67.2						
Total	8	13.8	50	86.2	58	100						

DIF = Direct immunofluorescence NPV = Negative predictive value

PPV = Positive predictive value

Table.4 Comparison between Direct Antigen Detection by DIF and Culture in
Diagnosis of HSV (n=58)

DIF for HSV	Culture						Sensitivity	Specificity	PPV	NPV	Efficiency	P-value
	+ve		-ve		Total							
	No.	%	No.	%	No.	%	%	%	%	%	%	
+ve	3	60	13	24.5	16	27.6						
-ve	2	40	40	75.5	42	72.4	60	75.5	18.8	95.2	74.1	0.07
Total	5	8.6	53	91.4	58	100						

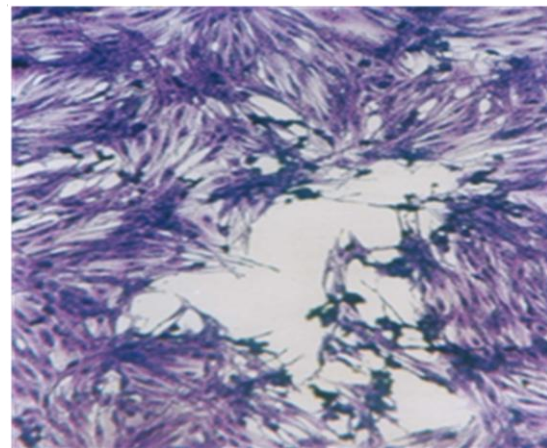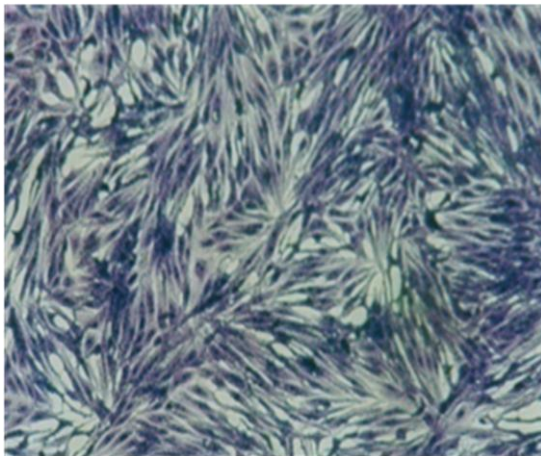DIF = Direct immunofluorescence   HSV = Herpes simplex virus

PPV = Positive predictive value.   NPV = Negative predictive value

(A)   Normal Vero Cell Line.   (B) Cytopathic Effect on Vero Cell Line

Stained by Crystal Violet

HSV had been detected in 27.6% by DIF, a result which is similar to Pramod et al., 1999) but in contrast to Farhatullah et al. 2004 who had detected higher incidence (78.6%). This may be due to the differences in locality and population. In the present study, DIF for diagnosis of HSV had showed 60%, 75.5% sensitivity and specificity respectively. Elnifro et al. 1999 recorded a wide range of sensitivity and specificity values obtained bythe antigen detection tests aren't only influenced by the quality of the test itself, but also by the quality of the used gold standard test.

From this study, we can conclude that adenovirus was a common pathogen in conjunctivitis and HSV was a common pathogen of keratoconjunctivitis. IFA is rapid and simple test and can be used as primary screening tool. The combination of cell culture and DIF constitutes the best set

of tests used for diagnosis of clinically suspected cases of viral keratoconjunctivitis.

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