Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Special Second International Scientific Conference for the Life Sciences
Faculty of Education for Women / University of Kufa / 2016 Issue

Isolation and Molecular study of Human Adenovirus

Dr. Fadyia Mahdi Muslim Alameedy

Department of Biology, Faculty of Science, Kufa University, Najaf, Iraq

Corresponding Author: Email:fadyiam.alameedy@uokufa.edu.iq; fadyiamahdi@yahoo.com

Abstract

Human samples of adenovirus were isolated from the period of March, 1st, 2015 to 28th of May 2015 from eye secretions of patients were diagnosed through rapid device test and real time pcr. Positive cases of human adenovirus were sixty nine represent (83.13%). Isolation process of adenovirus was successfully performed on chicken embryo fibroblast cell culture for 4 th passage for suspected samples obtained from (eye swab) as well as isolate from chick embryo [specific pathogen free (SPF)],who were diagnosed . The study groups were divided into three groups with different age and gender. The group of 16-30 years old , the highest percent of infection (55.04%) in comparison with other aged group followed by the group of 31-45 years old (31.88%) ; then, aged 5- 15 years old (13.04%). The males groups represented the highest number of infection with adenovirus in comparison with females groups represented by 44 males (63.76%).

Introduction

Adenoviruses is considered the biggest no enveloped viruses, as they have the maximum size capable of being communicated through the endosome. The virion as well is of a exclusive "spike" or fiber linked to every base of pennon of the capsid helping in linking the host cell by means of the receptor of coxsackie-adenovirus on the host cell surface. [1]

It is a fact that genome of adenovirus is characterized as linear, has no segments, has a pair of strands of DNA about (30 to 38 Kbp). This permits the virus to hold (30 to 40) genes in theory. At all events related of the contagion unexpectedly through (2-4) weeks. [3]

Signs and symptoms of viral conjunctivitis may guaranty the following (itchy eyes, tearing, redness, discharge and light sensitivity). For conjunctivitis associated with plain adenovirus with closely depending on the cell of the host to permanence as well as duplication. The virus is genome it has an end of (55 kDa protein) Tied for whole of the five ends of the line (dsDNA). They were employed as (primers) at replication and make sure that the ends of the viral genome are sufficiently doubled. [1]

Adenovirus widespread 1cz2uses prevail a genome of linear dsDNA and are capable of spreading in mammalian nucleus cells using the host's machinery of replication.[1]

Viral conjunctivitis including : (picornavirus , Enterovirus 70 , poxvirus , HSV, Coxsackie A 24 , molluscum contagiosum, vaccinia ,VZV , HIV are a widespread self-limiting position that is particularly breed by Human Adenovirus.[2]

Viral conjunctivitis be greatly catching, habitually for 10 to 12 days from onset as protracted as the eyes are scarlet. Patients should circumvent emotive their eyes trembling hands, and allotment towels, amongst other actions. Conduction may occur through molluscum contagiosum, illness will persist until the skin lesion is treated. Elimination of the central core of the lesion or enticement of hemorrhage within the laceration usually is adequate to cure the disease.[4]

Preventing transmission of viral conjunctivitis is substantial. Both patient and provider should wash hands thoroughly and often, retain hands away from the infected eye, and avoid sharing towels, linens, and cosmetics. Infected patients should be advised to stay home from school and work.[5]

The most mutual viruses associated with conjunctivitis are adenovirus and herpesvirus. Adenoviral 47 and 13 conjunctivitis among several can reason conjunctivitis. Adenoviral infections happen around the world and maybe considered the most ordinary outward optical contagion. Adenovirus cause a range of medical visual malady. Mainly strains secluded are serotypes three and four outbreaks of potentially other grave infection may is caused by adenovirus type "8, 19 and 37". [6]

2. Material and Methods

2.1 Samples Collection

This search was isolated of adenovirus during a period unlimited beginning 1 March 2015 until 28 from May 2015. Eighty three (44 (63.76%) males and 25(36.23%) females) with non normal patients in age ranged ranged five to forty five years of specimens were arbitrarily. All cases were arranged accept to [12]

2.2.Patients and clinical specimens:

Sixty nine conjunctival swabs were collected from 83 randomly selected. The material collected of conjunctival in sterile absorbable cotton swab was transported in viral transport MEM with 3% FCS and antibiotics. The samples were together from the infected among two upto five days onset of the Signs and were treatment here 20 - 30 min in the laboratory.

2.3. Isolation of human adenovirus: Fifty microlitres of samples were injected with virus going on near a 24 h old monolayer of chicken embryonic fibroblast (CEF) cell culture grown over tissue culture plate once aspirating out the growth medium. The inoculated culture was reserved in a rocker for thirty min. At the end of 30 min Dulbecco's minimal essential medium supplemented with 1 per cent foetal calf serum was added. The cultures were incubated at 37^{o}C. The cultures were observed daily under phase contrast using inverted microscope for the presence of cytopathic effect.

2.4. Detection of adenovirus

Two method diagnostic procedure had been second-hand for Specifying with adenovirus encompass, device rapid test (SAS™ Adeno Test), [7] and real time PCR using the primer design viral DNA kit (P.D. UK) agreement with the manufacturer's education successfully amplified. (Table.1).

Table(1): Detection primers of human adenovirus A to F.[8]

HAdV forward primer (5′-**CATCATCAATAATATACCTTAAAACTGG**-3′),
HAdV reverse primer (5′-**TCGCTSGCACTCAAGAGTGG** -3′)

2.5. Preparation of cell culture

It was performed according to Moresco *et al* ., (2010).

2.6. Virus isolation

It was performed according to OIE,(2009).

2.7. Virus titration on CEF cell culture:

This test was performed according to Frey and Liess, (1971).

2.8. Virus titration on embryonated egg

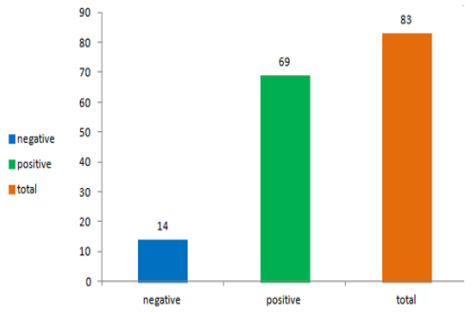
This test was performed according to Frey and Liess, (1971). Was calculated according to Reed and Muench, (1938), Virulent calculated according to the equation derived according to Hanson,(1975).

3 Results

3.1 Device Rapid Test

A total (83) different clinical case collected, only 69 cases were positive while (14) negative as detected by D.R.T. in shape (1). The become old between five to forty five year old spited keen on three collections. Thirty eight case of (16-30) years. Twenty two case of (31-45) years, while only nine cases of (5-15) years in figure (2). Anyhow infected female the lowest group of infection compared of male in figure (3).

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Special Second International Scientific Conference for the Life Sciences
Faculty of Education for Women / University of Kufa / 2016 Issue



Figure(1): Detection of Adenovirus by Rapid device test

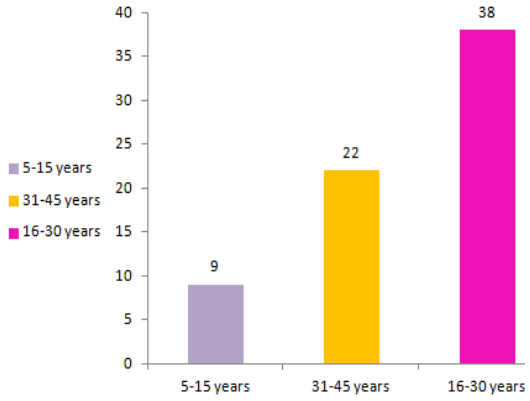


Figure (2): The distribution of patients according to age groups.

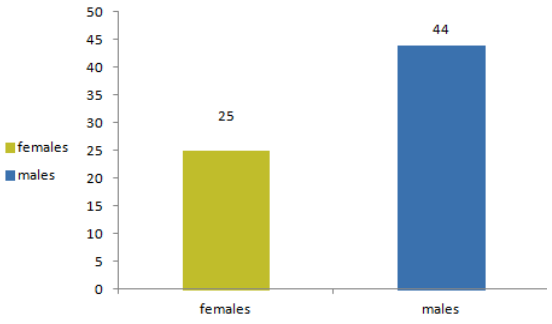
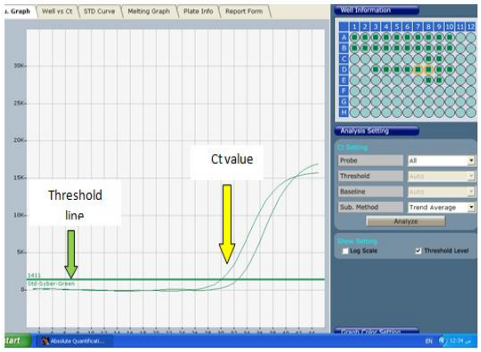


Figure (3): Total patients of Adenovirus according to gender.

3.2. Real Time PCR

Eighty three simple were suspected of virus infected .Positive cases just sixty nine by" RT-PCR "of diagnosis in figure(4).



Figure(4): The Graphic Results that obtained of Adenovirus by the Real Time PCR Thermocycler : Exicycler ™ Quantitive Thermal Block .

3.3. Isolation and propagation of adenovirus in chicken embryo fibroblast cell culture

Isolation process of adenovirus was successfully performed on chicken embryo fibroblast cell culture for 4 th passage for suspected samples obtained from (eye swab) in (Table 1).

On the primary isolation process, cytopathic effect was observed on fibroblast cell cultures after 24 hours post inoculation (P.I.), which appeared early in few infected round and refractile cells. After 48 hours P.I the CPE appeared much more and represented by more rounding, swelling, granulation of the cells, after 72 hours P.I the CPF increased much more characterized by enlarged rounded cells that were aggregated into grape-like clusters, forming giant cells.

Cytopathic variations have been observed in three days of inoculation. The variations consist of : (an enlarged cells , oval foci and a few small) that were more multiple, in converse to non infect fibroblast (Figure 5).

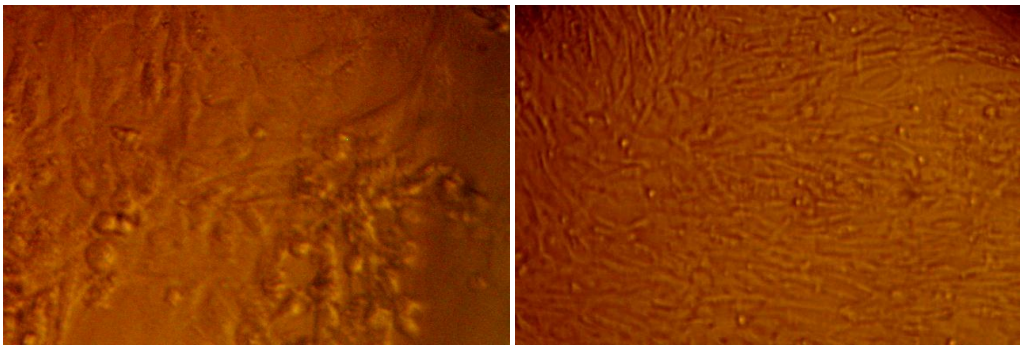
3.4.Isolation and propagation of Adenovirus in chicken embryonated eggs

Chick embryo [specific pathogen free (SPF)] was inoculated with eye swab that was collected from infected patients, who was diagnosed as positive adenovirus. The virus

Al-Kufa University Journal for Biology
Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Special Second International Scientific Conference for the Life Sciences
Faculty of Education for Women / University of Kufa / 2016 Issue

was inoculated at different regions of 11 days old chicken embryos. These inoculated embryonated eggs were daily inspected till 5 th days P.I or when embryos death. Allantoic and amniotic fluid was collected separately , then infectious dosage 50 was calculated according to Reed and Mench ,(1938) methods; five embryos were inoculated for each detection and control embryos were inoculated with sterile PBS pH 7.2 , $TCID_{50}$/0.5 ml was found equal to $10^{7.6}$ / 0.1 ml (Table 2).

Macroscopic appearance of chicken embryos inoculated with isolated adenovirus reveled clear dwarfism and severe congestion (B) in comparison with control embryos which received only sterile PBS (A) which appeared normal, (Figure 6) that was appeared without change of ECEs.



A **B**

Figure (5): The cytopathic effect of Chicken Embryo Fibroblast Cell Culture.

(A): CPE of CEF of Adenovirus.
(B): Normal (CEF) cell culture

Table(1): Demonstrating the result of virus isolation by inoculation of Adenovirus in SPF chicken embryos.

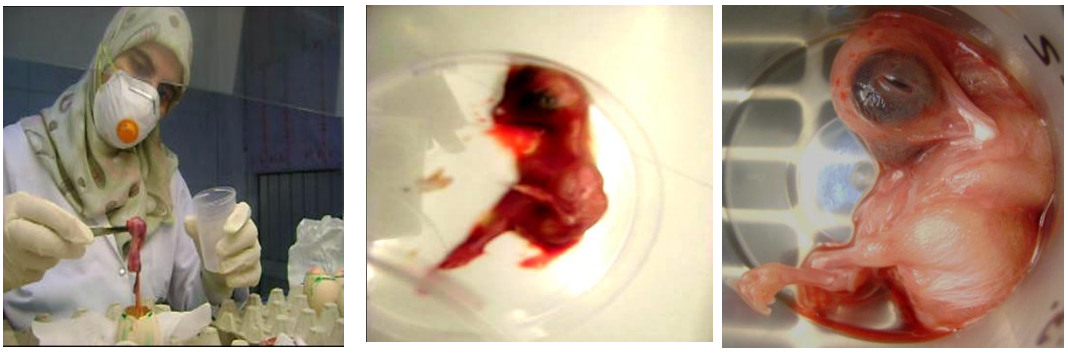
Clinical sample used	total number inoculated	Number of dead embryos after 72 hours P.I./ total numbers inoculated	Percent of infection %	Number of dead embryos after 96 hours P.I. / total numbers inoculated	Percent of infection %
Eye swab	5	4	80	1	20
	5	4	80	1	20
	5	3	60	2	40
	5	5	100	0	0
	5	5	100	0	0
	5	5	100	0	0
Phosphate buffer solution	5	0	0	0	0

Table (2): Determination of TCID50 for Adenovirus by Reed and Meunch,(1938) method.

Virus Dilution	Infected Cell (CPE) +ve	Normal cell (non infected) wells	Total infected wells	Total non infected cell (normal wells)	Infected cell ratio	Infected cells %

10^{-1}	5	0	14	0	41/41	100%
10^{-2}	5	0	36	0	36/36	100%
10^{-3}	5	0	31	0	31/31	100%
10^{-4}	5	0	26	0	26/26	100%
10^{-5}	5	0	21	0	21/21	100%
10^{-6}	1	4	16	2	18/16	88.88%
10^{-7}	1	4	11	6	17/11	64.7%
10^{-8}	2	3	6	9	/615	40%
10^{-9}	1	4	3	13	/316	18.75%
10^{-10}	1	4	1	17	18/1	5.55%

$TCID_{50}$/0.5 ml =$10^{7.6}$



A B

Figure.(6): Macroscope appearance of isolated Adenovirus inoculated chicken embryos(A) in comparison with control PBS inoculated embryos (B).

3.5. Hemagglutination test (HA)

The result of HA reflected the ability of the isolated virus to haemagglutinate chicken RBCs . The haemagglutination titer varies with passage number. The HA titer of the isolated virus in first passage was (16) HA unit, whereas its titer was (32) HA unit for 2nd passage and reached a titer of (64) HA unit in the 4th from ECEs ,while CEEFCC titer 0f (128) HA unit in the 4th . Whereas its titer was (0) HA unit for control group (Table 2).

3.6. Heamagglutination inhibition test (HI): The haemaggutination activity of sera of recovered patients and sera of experimental animal was assay by HI test. Results appeared that all convalescent sera of infected and recovered patients gave HI titer range from 32 up to 256 HI unit, 4th inoculated of embryonated chicken eggs ,while chicken embryo fibroblast cell culture titer of (128) HA unit in the 4th passage. Whereas its titer was (0) HI unit for control group (Table 3).

Table .3: HA and HI tests of isolation Adenovirus in embryonated chicken eggs and chicken embryo fibroblast cell culture .

Isolation Virus / Passage number	ECEs		CE FCC	
	HA	HI	HA	HI
1st	16	32	4	8
2nd	32	64	8	16
3rd	64	128	32	64
4th	128	256	64	128

4. Discussion

The samples of Adenovirus had been gathered of eye excretion of infected groups, this results complied by way of [6, 16] Most ages 16–30 years, further exposed to infection during the study showed to be reliance of age. A study showed (63.76%) of males were more influenced than females (36.23%), outcomes as well had correspond with study [17] in London (The majority of ocular adenovirus isolates (66%) were from the age groups 20-40; only 15% were from patients below 20 years, and 19% from patients over the age of 40. Infection was commoner in males than in females (ratio1-4 to1).

The results obtained are in agreement with those of a previous virology and biochemistry, National institute for medical research in Britain study (Graham and Smiley, 1977). Wei *et al*., (2005) was showed through the study of similarity of change in cytopathic effect of cell culture also. In this study, the presence of human adenovirus in a collection of eye specimens from conjunctivitis was confirmed by using rapid device test and real time PCR assays with tissue culture for isolation the virus. In the present study, isolated a cytopathogenic virus from eye secretion by using chicken embryo fibroblast cell and chick embryo [specific pathogen free (SPF). The success of virus isolation depends on the concentration of virus in the inoculums.

Isolated and diagnosis of human adenovirus from eye secretions for the first time the study was conducted in Najaf / Iraq.

Reference

1-Martin M. A., Knipe D. M., Fields B. N., Howley P. M., Griffin D. L. and Robert."Fields' virology". *Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins*. pp. 2395. ISBN 0-7817-6060-7(2007).

2-Gray G.C. Adenovirus transmission worthy of our attention. *J Infect Dis* 194 (7): 871–3(2006).

3-Walsh M. P., Seto J., Liu E. B., Dehghan S., Hudson N. R., Lukashev A. N., Ivanova O., Chodosh J., Dyer D., Jones M. S. and Seto D. Computational analysis of two species C human adenoviruses provides evidence of a novel virus. *Journal of Clinical Microbiology* 49 (10): 3482–3490. (2011).

4-Kolavic G.and Shellie A., "Large Epidemic of Adenovirus Type 4 Infection Among Military Trainees: Epidemiological, Clinical, and Laboratory Studies." *Clinical Infectious Diseases*. 1:808-811. (2002).

5-Kumar R., Kumar V., Asthana M., Shukla S. K. and Chandra R. "Isolation and identification of a fowl adenovirus from wild Black Kites ". *Journal of wildlife diseases* **46** (1): 272–276. (2010).

6- Lenaerts L. D., Clercq E.and Naesens L."Clinical Features and Treatment of Adenovirus Infections". Rev *Med Virol* .18:357-74.(2008)..

7. Ford E., Nelson K..E. and Warren D. "Epidemiology of Epidemic Keratoconjunctivitis". 9: 244-61.(1987).

8. Arrfa R.C."Grayson's diseases of the cornea". 4th ed. St. Louis: Mosby-Year Book.286.(1997).

9.Hierholzer J.C. "Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington DC". Adenoviruses, p. 947-955. (1996)

10. Morris S.J.,Balazs H.,Robert D.G., Mary M.A.,Wilfred P.C., Brian R.,Chao P., Eric L.D. and David P.S."New Adenovirus Species Found in a Patient Presenting with Gastroenteritis", *J. Virol*. vol. 81 no. 11 5978-5984.(2007).

11.Moresco, K. A., Stallknecht, D. E. and Swayne, D. E. "Evaluation and Attempted Optimization of Avian Embryos and Cell Culture Methods for Efficient Isolation and Propagation of Low Pathogenicity Avian Influenza Viruses" *Avian Diseases Digest*, vol. 5, no. s1, p. e142-e143(**2010**)..

12.OIE."Avian influenza. Chapter 2.3.4". *OIE (world Organization for animal health*) Terrestrial Manual.Pp:43-56; 78-89; 143-146.(2009)..

13. Frey, H.R. and Liess, B."Growth Curve Studies and Applicability of Highly Cytopathogenic BVD- MD Virus Strain for Diagnosis Purposes Using Microtiter Method" .*ZBL*.Vet.Med. 18:16-71.(1971)..

14.Reed, L. J. and Muench, H."Simple Method for Estimating Fifty Percent End Points". *Amer. J. Hyg.*; 27: 493-497.(1938).

15. Hanson, R.P. "Newcastle disease .In Hitchner ,S.B., Domermuth , C.H., Purchase , H.G. and Willams , J.E.(ed.) Isolation and Identification of Avian pathogens". *American Association of Avain pathogists, Kennett Squra*, PA.Pp:160-173.(1975).

16. Janani M.K., Malathi J. and Madhavan H.N. "Isolation of a Variant Human Adenovirus Identified Based on Phylogenetic Analysis During an Outbreak of Acute Keratoconjunctivitis in Chennai. *Indian J Med Res* 136, pp 260-264. (2012).

17.Darougar S.., Walpita U., Thaker N. Viswalingam L., Gardner D. and McSwiggan A. "Adenovirus Serotypes Isolated from Ocular Infections in London". 67, 111-114.(1983).

18. Graham F.L. and Smiley J. "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type in Britain" 5. J. gen. Virol. 36, 59-72.(1977).

19.Wei D. G., Ya Z., Jun Y., Jin D., Jun L., Cai-Fang X. "Anti-HBV Activity of TRL Mediated by Recombinant Adenovirus in China". 11(17):2574-2578. (1977).