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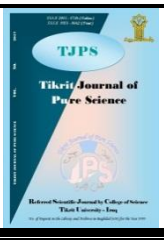

Laboratory Diagnosis of Human Adenovirus associated with Respiratory Tract Infection in children

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ABSTRACT

Human Adenovirus (HADV) is classified in Adenoviridae family, it is important viral pathogen of respiratory tract infection (RTIs) among children. This study aimed at detecting HADV infections among hospitalized children under 10 years old with respiratory tract infections in Salahaldin Governorate, in addition to compare different diagnostic methods for the detection of this virus.

Nasopharyngel Swabs (NPS) and whole blood samples were taken from 400 hospitalized children between end of August 2013, to the end of May 2014, each sample was analyzed for HADV by real time –PCR, IgM and IgG specific to HADV determining from blood specimens in serum using Enzyme Immuno sorbent assay (ELISA) technique.

Nasopharyngel Swab was more sensitive than the whole blood specimens for the detection of HADV and was identified in 3% by RT-PCR, in 72.2% by IgM – HADV antibodies, 83.1% by IgG-HADV antibodies.

Autumn – Spring periods were considered as the most common time for HADV infection with peak in September and February.

Introduction

Respiratory Tract infection (RTIs) is one of the important causes of deaths among children especially in developing countries [1]. It is major health problem worldwide, with high morbidity, hospitalization and mortality rates among children [2]. HADV belongs to the family Adenoviridae [3]. And it is important cause of (RTIs) in children and represent a high percentage of the pediatric medical admissions due to Pneumonia and bronchiolitis [4]. Adenovirus infections are widely distributed and common. Infections occur most frequently during childhood, where they tend to be self- limited and induce type-specific immunity following recovery. Approximately half of the human Adenovirus serotypes are known to cause illness in human beings. Adenovirus serotypes have different cell tropisms and cause distinct clinical manifestations. Some genotypes are more virulent than others, causing unusually severe manifestations and higher mortality rates [5]. Adenovirus infections were traditionally associated with respiratory, ocular and gastrointestinal disease, many other clinical manifestations have been associated with Adv replication, especially in immunocompromised patients.

Clinical manifestations depend on the host and the serotype involved and include pharyngitis, conjunctivitis, pertussis- like syndrome, keratoconjuctivitis, bronchiolitis, pneumonia, acute haemorrhagic and gastroenteritis. Less frequent clinical syndromes are hepatitis, meningitis, encephalitis, myocarditis, pericarditis, genital infections, pancreatitis and fatal disseminated disease [6]. Most children become infected with one or more Adv types early in life. These infections are usually selflimited and mild. When symptomatic, the usual signs and symptoms are fever, nasal congestion, coryza, pharyngitis and cough, with or without otitis media.

Adenovirus may cause an exudative tonsillitis that is clinically indistinguishable from group A Streptococcus. If conjunctivitis accompanies the syndrome, the disease is called pharyngoconjunctival fever. The most frequent serotypes associated with paediatric URTIs are types 1-3 and 5-7 [7].

Adenovirus can also cause ALRIs in children less than 4 years old that may require hospitalization. The syndromes include bronchitis, bronchiolitis, croup, and pneumonia. Severe necrotizing pneumonia in which all or part of a lung has increased translucency radiographically, which is called swyer - james syndrome.

Severe adenovirus pneumonia may also result in bronchiolitis obliterans, asubacute inflammatory process in which the small airways are replaced by scar tissue resulting in a reduction in lung volume and lung compliance [8].

In cold seasons from Autumn to Spring, most frequently recent reports are associated with human respiratory diseases in newborn and children have clinical diagnosis of bronchiolitis and Pneumonia investigated HADV [9,10,11]. Which is also can be recovered throughout the year, and although higher frequency is observed in winter and spring [3]. HADV genome is linear non-segmental and double stranded (ds) DNA that is between 26 and 48 kilo base pairs (Kbp) [12]. The laboratory diagnosis of HADV infection is mostly done by the detection of viral antigens in respiratory samples using the confirmatory test with (ELISA) for HADV [13]. The diagnosis can be also done by real time –PCR [11]. The use of rapid test for the diagnosis of HADV infections is useful for the consideration of timely treatment with antiviral agents also reducing nosocomial spread [14].

HADV has not been studied in Salahaldin Governorate of Iraq and no laboratory method have been used to detect this virus among patients in Salahaldin Governorate. The frequency of this virus as causative agent among hospitalized children with respiratory tract infections have not been clarified.

The laboratory diagnostic methods that are routinely done in Iraqi medical laboratories and hospitals, do not include the diagnosis of HADV in their schedule investigations, and generally no regular diagnostic methods are done for respiratory viruses in Iraqi hospitals. Where as (RTIs) is so common and as RTIs in Iraq is a real problem, this study was prepared and its aim was to detect HADV in respiratory specimens of children with (RTIs)

Materials and Methods:

This study was carried out on patients attended the general hospital in Baiji in the central of Baiji city and to the teaching hospital of Tikrit city. The patients were the children less than 10 years old and they were admitted to these hospitals with respiratory tract infections. A total of 400 children who required medical treatment were included both sexes were chosen. One hundred children that have no RTIs were chosen as a control group. The period of the study was started from the end of August 2013 to the end of May 2014.

Collection of Specimens: Four milliliters (mls) of venous blood were collected from each child. The blood then was divided into two parts:

The first part (3ml) was kept into a test tube without anticoagulant and centrifuged for 15 minutes at 3000 revolution per minute (RPM), the serum was then putted into one aliquot stored in one microcentrifuged (eppendrof) tube labeled with patient's code number and frozen upon reaching the deep freeze at -70℃ till tested. Once thawed refreezing was avoided.

The second part (1ml) was put into a test tube with anticoagulant, ethelyne diamine tetramide (EDTA), and then mixed on a roller, labeled with patient's code number and frozen upon reaching the deep freeze at -70℃ till tested. Once thawed refreezing was avoided. One nasopharyngel Swabs (NPSs) were taken from each child by flocked swab applicators, thus one applicator was applied for one nostril. The (NPS) was placed immediately after collection in 3mls viral transport Medium (VTM) (Micro rheologics company/ Italy). ELISA test kit for IgM anti – HADV detection and IgG anti –HADV detection were imported from (Cusabio company/ China) and Real Time – PCR kit for qualification of human adenovirus, was imported from (Primer design TM genesig company/ United Kingdom).

Materials

ELISA kit for IgG HADV antibodies detection(Cusabio Company/ China Cat .No. CSB-E05005h)

This ELISA test kit is used for the qualitative determination of IgG HADV antibodies concentration in serum. The kit compounds are:

1- Microplate wells: containing 96 test well coated with ADV antigen.
2- Negative control: one vial containing 0.5 ml.
3- Positive control: one vial containing 0.5 ml.
4- Sample diluent: one bottle containing 12 ml.
5- HRP-conjugate: one bottle containing 12 ml.
6- Wash buffer (20x concentrate): one bottle containing 15 ml.
7- Substrate A: one bottle containing 7 ml.
8- Substrate B: one bottle containing 7 ml.
9- Stop solution: one bottle containing 7 ml.

ELISA kit for IgM HADV antibodies detection (Cusabio Company/ China.Cat.NO.CSB-E05006h).

This ELISA test kit is used for the qualitative determination of IgM HADV antibodies concentration in serum.

The kit compounds are the following:

1- Microplate wells: containing 96 test well coated with ADV antigen.
2- Negative control: one vial containing 0.5 ml.
3- positive control: one vial containing 0.5 ml.
4- Sample diluent: one bottle containing 12 ml.
5- HRP-conjugate: one bottle containing 12 ml.
6- Wash buffer (20x concentrate): one bottle containing 15 ml.
7- Substrate A: one bottle containing 7 ml.
8- Substrate B: one bottle containing 7 ml.
9- Stop solution: one bottle containing 7 ml.

Quantification of Adenovirus type B (Hexon gene) by RT-PCR kit: This kit was designed and manufactured by primer design ^{TM} genesig company/ United Kingdom.

The kit contents are:

- AdvB specific primer/ probe mix (150 reactions BROWN) FAM labeled, BHQ quenched.
- AdvB positive control template (for standard curve RED).
- Internal extraction control DNA (150 reactions BLUE).
- Internal extraction control primer/ probe mix (150 reactions BROWN) choice of VIC channel or CY5 channel.
- Endogenous ACTB primer/ probe mix (150 reactions BROWN) FAM labeled, BHQ quenched.
- RNAse/ DNAse free water.

Reagents to be supplied by the user:

- DNA extraction kit.
- Oasig^{TM} lyophilised 2xqPCR Mastermix.

This kit is designed to work well with all commercially avialable mastermix.

In this study, the mastermix was ordered from GeNet Bio company/ Korea. The contents of the kit are:

1. prime Q- master mix (2x), Q-9200, (1.0 ml) consists of:

1- HS prime Taq DNA polymerase.
2- Reaction buffer.
3- Enzyme stabilizer.
4- dNTPs mixture and PCR enhancer.

2. prime Q- master mix (2x, with ROX dye), Q-9210, (1.0 ml) consist of:

1- HS prime Taq polymerase.
2- Reaction buffer.
3- Enzyme stabilizer.
4- dNTPs mixture and PCR enhancer.
5- 50X ROX dye (50µl X1).

Methods

ELISA test kit for IgG HADV antibodies qualitative determination concentrations in serum. - Test procedure according to supplied company kit:

1. All reagents and samples were prepared and brought to room temperature (18-25°C).
2. The assay layout sheet was used to determine the number of wells to be used and put any remaining wells and the desiccant back in to the pouch and stored unused wells at 4 °C.
3. A blank well was setted without any solution.
4. One hundred µl of negative control, positive control were added, controls need test in duplicate.
5. One hundred µl of sample diluent were added to rest wells, then 10 µl of sample per well were added then coverd with the adhesive strip provided and incubated for 30 minutes at 37 °C.
6. A spirating and washing each well was done for 5 times. Washing by filling each well with wash buffer (200µl) by autowasher, and standing for 20 seconds and completed removal of liquid at each step for good performance after the last washing, any remaining wash buffer was removed by a spirating or, decanting with inverted and bloted the plate against clean paper towels.
7. Then 100 µl of HRP-conjucate was added to each well (not to blank well), and coverd the microtiter plate with a new adhesive strip and incubated for 20 minutes at 37 °C.
8. The aspiration/ wash process for 5 times as in step 6 was repeated.
9. Fifty µl of substrate A and 50 µl substrate B were added to each well and incubated for 10 minutes at 37 °C with protected from light.
10. Then 50 µl of stop solution was added to each well, with gently tap the plate to ensure thorough mixing.
11. Blank well was taken as zero and the optical density (OD) of each well were determined within 10 minutes by using a microplate reader set to 450 nm.

Calculation of results:

For calculation the valence of human ADV antibody (IgG) the sample well was compared with control, according to following cut – off supplied company kit:

If optical density (OD.) negative <0.05, calculate it as 0.05.

while OD. Sample/ OD. ≥ 2.1: positive.

While OD. Sample/ OD. < 2.1: negative.

ELISA test kit for IgM HADV antibodies qualitative determination concentrations in serum. - Test procedure, according to supplied company kit:

1. All reagents and samples were prepared and brought to room temperature (18-25°C).
2. The assay layout sheet was used to determine the number of wells to be used and put any remaining wells and the desiccant back in to the pouch and stored unused wells at 4 °C.
3. A blank well was set without any solution.
4. One hundred µl of negative control, positive control were added, controls need test in duplicate.
5. One hundred µl of sample diluent were added to rest wells, then 10 µl of sample per well were added then coverd with the adhesive strip provided and incubated for 30 minutes at 37 °C.
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7. Then 100 µl of HRP-conjugate was added to each well (not to blank well), and coverd the microtiter plate with a new adhesive strip and incubated for 20 minutes at 37 °C.
8. The aspiration/ wash process for 5 times as in step 6 was repeated.

9. Fifty µl of substrate A and 50 µl substrate B were added to each well and incubated for 10 minutes at 37 °C with protected from light.
10. Then 50 µl of stop solution was added to each well, with gently tap the plate to ensure thorough mixing.
11. Blank well was taken as zero and the optical density (OD) of each well was determined within 10 minutes by using a microplate reader set to 450 nm.

Calculation of results :

For calculation the valence of human RSV antibody (IgM) the sample well was compared with control, according to following cut – off value supplied company kit:
If optical density (OD.) is negative <0.05, calculate it as 0.05.
while OD. Sample/ OD. ≥ 2.1: positive.
While OD. Sample/ OD. < 2.1: negative.

Real time-PCR detection:

1. The reaction mixture was prepared according to the table below:

A- AdVB detection mixture.

Component	Volume
Prime Q- master mix	10µl
AdVB primer/ probe mix (BROWN)	1µl
Internal extraction control primer/ probe mix (BROWN)	1µl
RNase/ DNase free water (WHITE)	2.5µl
Prime Q- master mix with ROX dye	0.5µl
Final volume	15µl

B- Endogenous ACTB detection mixture (optional).

Component	Volume
Prime Q- master mixture	10µl
Endogenous ACTB primer/ probe mixture (BROWN)	1µl
RNase/ DNase free water (WHITE)	3.5µl
Prime Q- master mix with ROX dye	0.5µl
Final volume	15µl

2. Fifteen µl of this mixture was pipetted into each well according to real-time PCR experimental plate set up was pipetted.
3. The sample DNA template for each sample in RNase/DNase free water was prepared (the concentration was suggested 5 ng/µl) but if the concentration of DNA is not known, then the DNA sample reactions will diluted 1: 20 (10µl of sample DNA and 190µl of water).
4. Then 5µl of diluted DNA template into each well was pipetted according to experimental plate setup.
For negative control wells, 5µl of RNase/ Dnase free water was used. The final volume in each well is 20 µl.

Amplification protocol

Amplification protocol included the following:

	Step	Time	Temp
	UNG treatment (if required)*	15 mins	37 °C
	Enzyme activation (if required)	15 mins	95 °C
50 cycles	Denaturation	10 s	95 °C
	DATA COLLECTION**	60 s	60 °C

* Required if your master mix includes UNG to prevent PCR carryover contamination.
** Fluorogenic data for the control DNA should be collected during this step through the FAM and VIC channels.

Interpretation of results

Target	Internal control	Negative control	Positive control	Interpretation
+ve	+ve	-ve	+ve	+ve
+ve	-ve	-ve	+ve	+ve
-ve	+ve	-ve	+ve	-ve
-ve	-ve	-ve	-ve	Experiment fail
+ve	+ve	+ve	+ve	Experiment fail

Statical Analysis:

The statical package for social science (SPSS, U.S.A., version 17, 2014), two approaches are used namely descriptive and analytic. Chi – square text (X2) and fisher's exact test are used to test the association between categorical variables, probabilities of 0.05 ($p \leq 0.05$) was considered as statistically significant.

Results and discussion

Detection of HADV infection:

Detection of HADV from nasopharyngeal swab and whole blood samples usingreal-time PCR technique:

The real time - PCR technique was used for detection the DNA of HADV from NPS and whole blood samples from 23 (5.75%) patients, the results showed that 8 (34.7%) out of 23 were positive for HADV while 5 (21.7%) out of 23 patients, were negative for HADV from NPS samples. On the other hand, the results showed that 5 (21.7%) out of 23 patients were positive for the HADV, while 5(21.7%) out of 23 patients were negative from whole blood samples (Fig. 1).

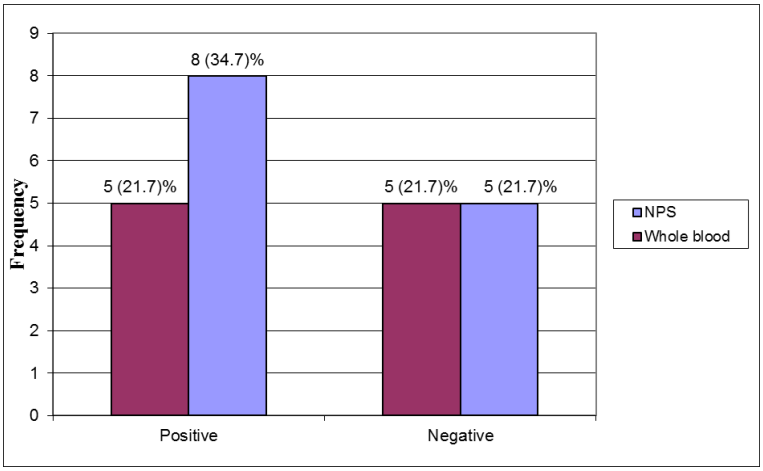

Fig. 1: Human adenovirus detection by the real time PCR technique in nasopharyngeal swab (NPS) and whole blood specimens.

The plastic tip flocked swab applicator was designed for the collection of respiratory samples by NPSs from children, thus flocked swabs were superior to conventional swabs for cell recovery. The vertical nylon shaft synthetic fibers act a soft brush to allow the improved collection and release of patient's samples [15]. For whole blood samples, the virus had been recovered from peripheral blood and multiple organs from children with disseminated adenovirus disease [16]. Also there are reports that the detection of viral DNA in peripheral blood provides an excellent marker for viral disseminated in the course of adenovirus infection with ARTIs in immunosuppressed patients [17]. And firmed in immunocompetent children with an acute adenovirus viremia infection of the respiratory tract, by using nested polymerase chain reaction (PCR) [18].

To our knowledge, the NPS and whole blood samples were not practiced before by medical staff in health authorities and self training was the only way to begin sample collection in the study.

The NPS when compared to whole blood samples and TS was more annoying for children and more difficult to be performed. But the frequency of NPS was more than that for whole blood samples and therefor NPS was better than whole blood samples.

In the present study, there was the use of only NPS, and whole blood samples were used. Many other studies found that nasopharyngeal aspirate (NPA) is an optimal specimen for the diagnosis of respiratory infections by RT-PCR [19]. For the Nasopharyngeal aspirate or Nasopharyngeal wash these two specimens could not be performed and no trained medical personnel have previous training about this sample collection protocol, with not available instrumentations.

Detection of HADV using real time-PCR:

Real time technique was used to detect DNA of HADV from NPS and whole blood samples. The results showed that 13(3.25%) out of 23(5.75)% children were infected with HADV by RT-PCR, (Fig. 2).

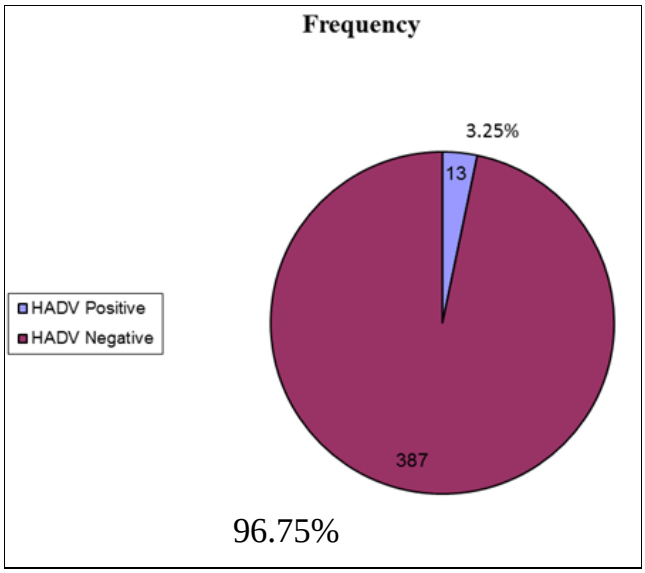

Fig. 2: Frequency of HADV infections among patients in the study group using real time-PCR technique.

The study showed that for a period of one year (end of August 2013 to the end of May 2014), 13(3.25%) out of 400 hospitalized children aged less than 10 years admitted to hospital suffering from RTIs were positive for HADV by RT-PCR technique.

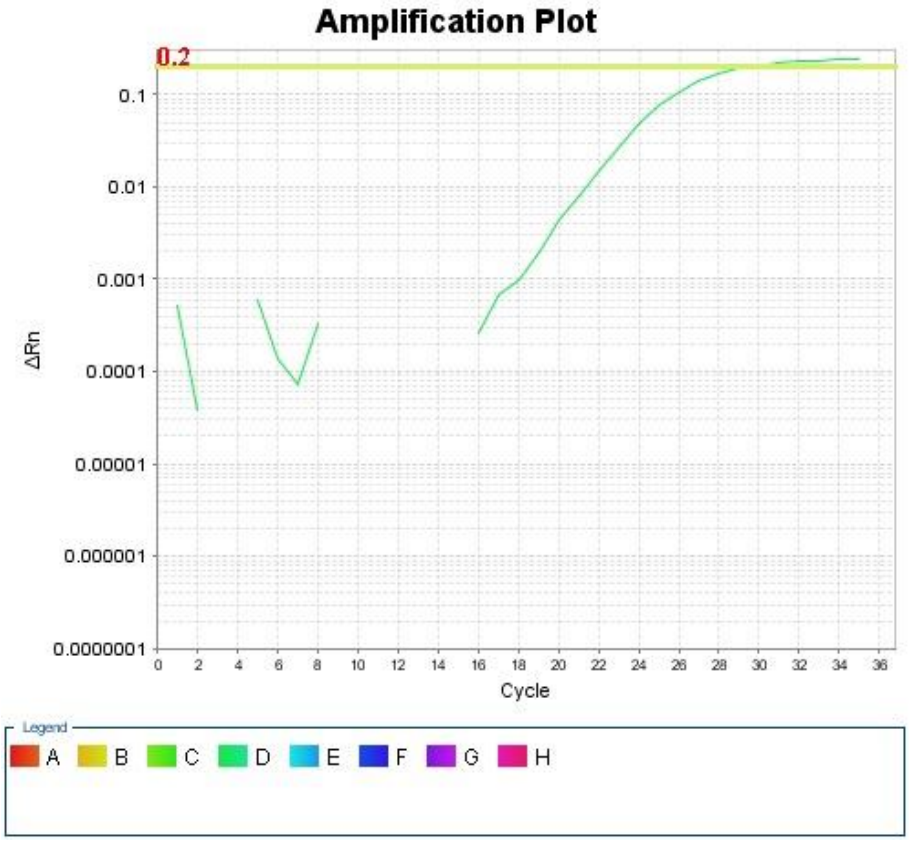

Fig. 3: Detection of HADV and positive control (PC) by RT-PCR

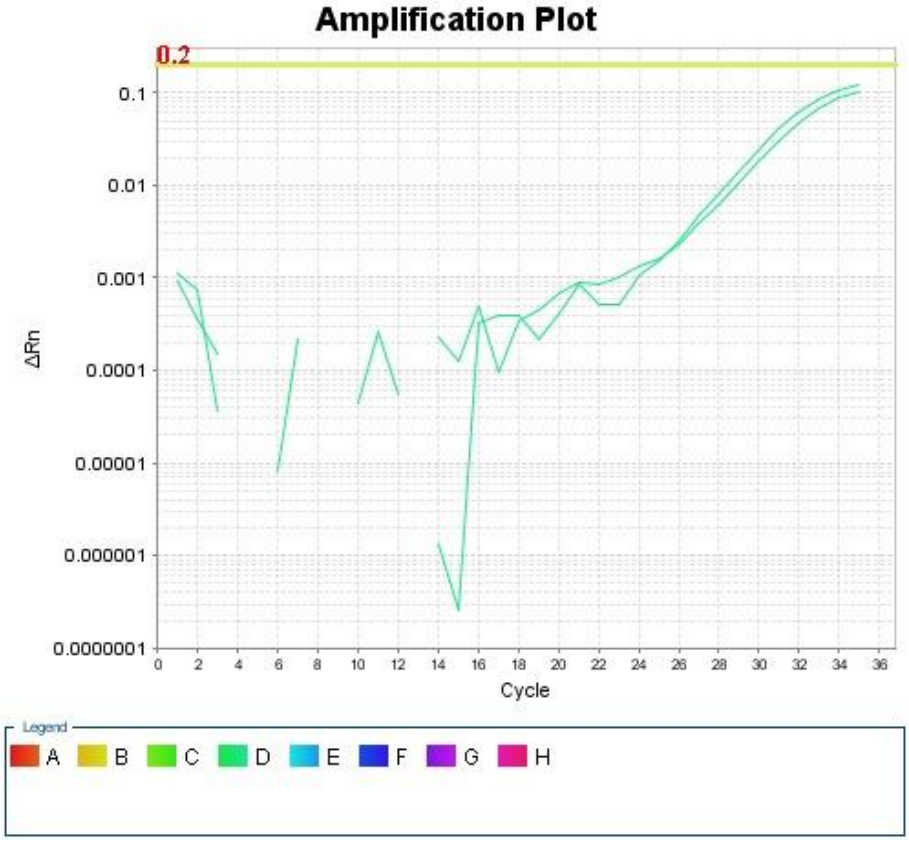

Fig. 4: Detection of HADV by RT-PCR

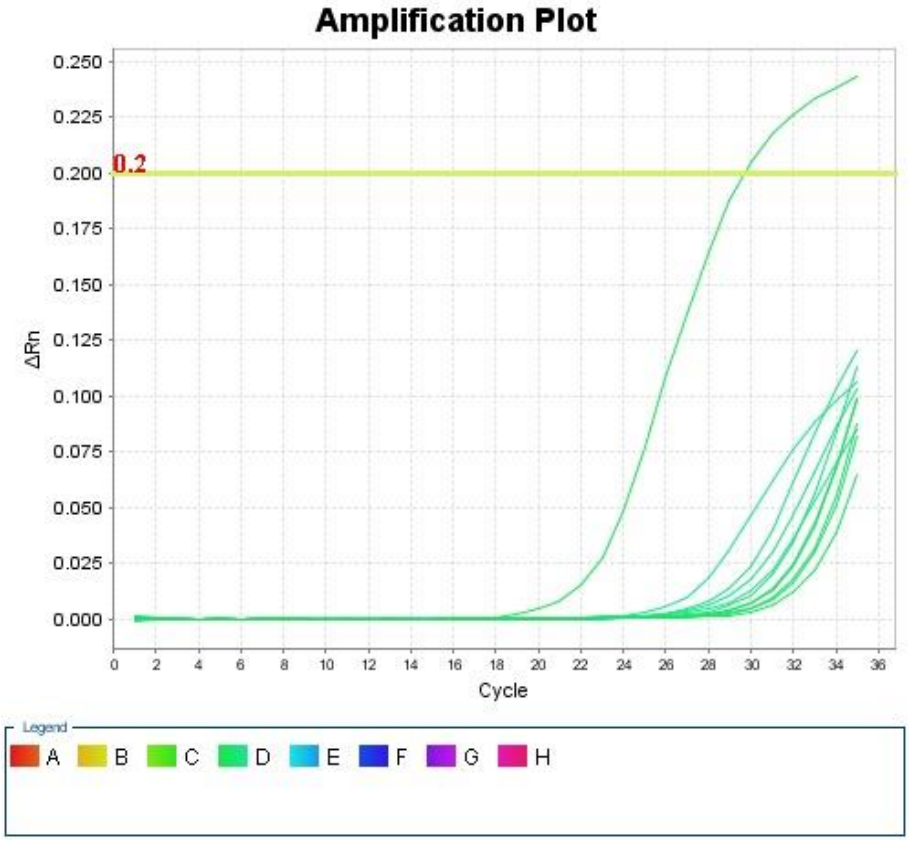

Fig. 5: Detection of HADV by RT-PCR

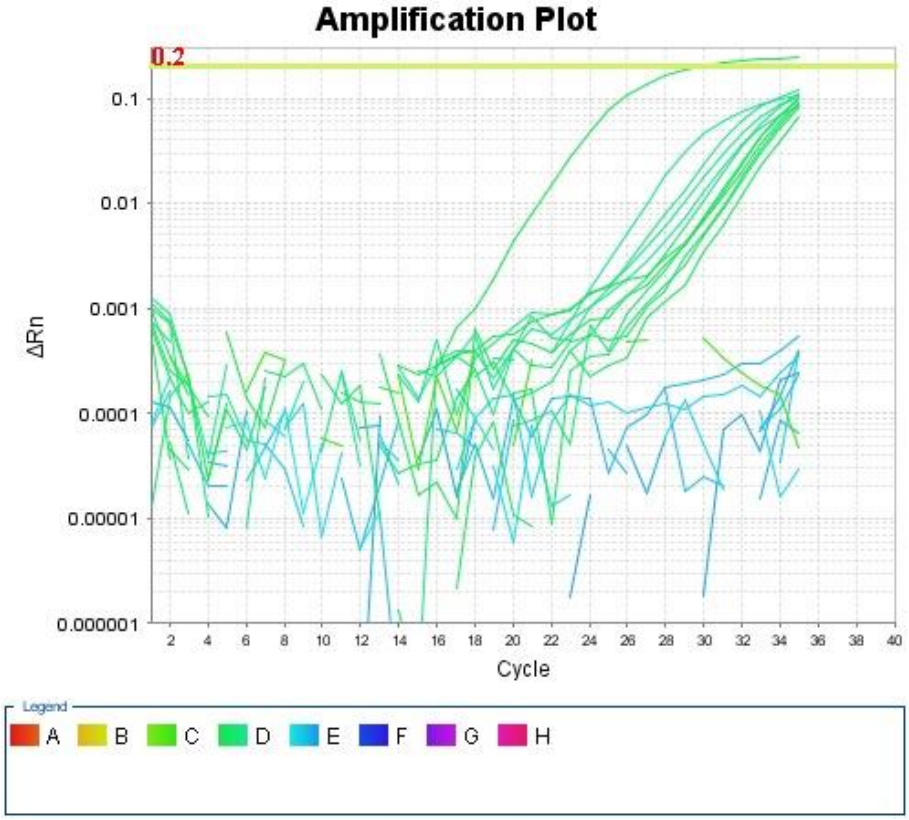

Fig. 6: Detection of HADV and internal control by RT-PCR

To our knowledge, it is the first study recorded that HADV infections were in Salahalden Governorate.

The result in the current study is similar or lower than those reported in different countries using RT-PCR.

In Turkey the infections percentages were (71.4%); [20]; in China (6.33%), [2]; in Roma (29.6%), [11] in Spain (77.9%) [21]; in USA 9(12%) [22]; in southern Brazil (6%) [23]; and in another study also in south of Brazil (0.8%) [24]. These variations in incidence among studies might reflect different epidemiological patterns of HADV infections in different countries which, in turn, might be related to environmental factors, geographical factors, or related to type of sample as some studies chose only children with bronchiolitis and pneumonia but not all respiratory infections as whole, or differences in host genetic susceptibility, sampling techniques, detection methods, and/ or different viral strains circulating in different places.

Seropositivity of anti-HADV IgM antibodies among patients with RTI.

The seropositivity of IgM antibodies specific to HADV in the study group showed that 60(72.2%) out of 83 patients were infected with HDAV positive antibody titers, while only 3(0.9%) out of 317 patients, who were negative for HADV (False positive), were having positive antibody titers by ELISA. The results were statistically highly significant (P < 0.000) seropositivity of IgM antibodies among 83 HADV infected children revealed that positivity was found in 7 out of 10 patients aged <1 year (70%), 10 (71.4%) out of 14 patients aged 1 to < 2years, 7 (63.6%) out of 11 patients aged 2 to <3, years, 8 (72.7%) out of 11 patients aged 3 to <4 years, 5(71.4%) out of 7 patients aged 4 to <5 years, 5(71.4%) out of 7 patients aged 5 to <6 years; 6(85.7%) out of 7 patients aged 6 to <7 years, 4(80%) out of 5 patients aged 7 to <8 years, 5(71.4%) out of 7 patients aged 8 to <9 years, 3 (75%) out of 4 patients aged 9 to <10 years.

The highest seropositivity rate was found in the age group 6 to <7 years, while the lowest was found in the age group 2 to <3 years. The results were highly statistically significant (P =0.000)(Figure 7).

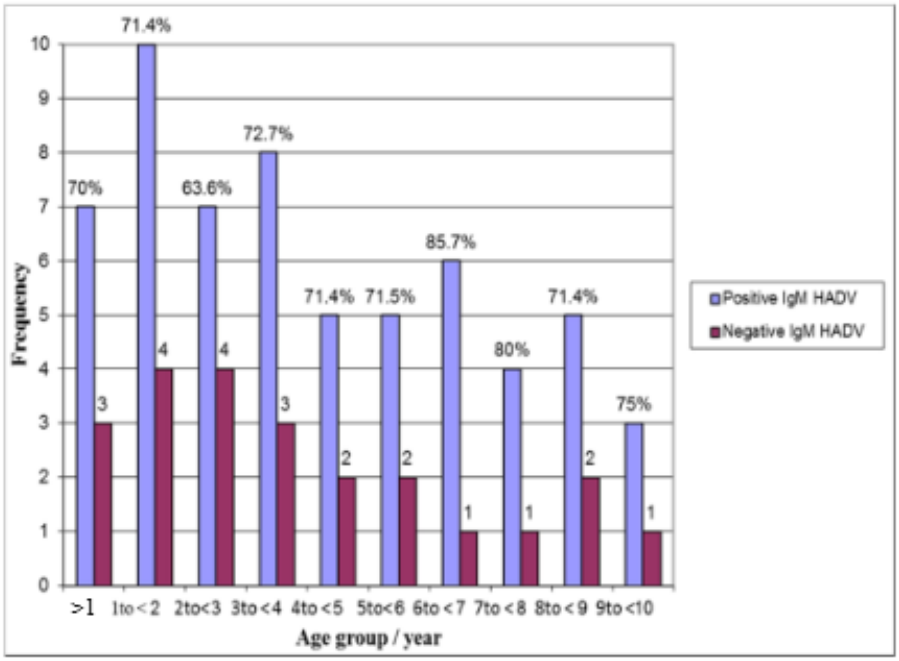

Fig. 7: Seropositivity of anti – HADV IgM antibodies among patients infected with RTI.

Seropositivity of anti – HADV IgG antibodies among patients with RTI.

Seropositivity of IgG antibodies specific to HADV in the study group showed that 69(83.1%) out of 83 HADV infected patients were having positive titers, while only 248(78.2%) out of 317 HADV-negative patients, were IgG seropositivity. The differences in IgG seropositivity between the HADV infected patients and those who were not infected with HADV were statistically not significant (p= 0.3242). The results are shown in Table (1).

The total IgG HADV antibodies was 317 (79.2%) in all hospitalized children. IgG HADV antibodies among the 83 HADV infected children was found in 7(77.8%) out of 9 patients aged <1 year, 10 (71.4%) out of 14 patients aged 1 to < 2years, 8 (80%) out of 10 patients aged 2 to <3 years, 9 (81.81%) out of 11 patients aged 3 to <4 years, 6(85.7%) out of 7 patients aged 4 to <5 years, 6(85.7%) out of 7 patients aged 5 to <6 years; 7(87.5%) out of 8 patients aged 6 to <7 years, 6(100%) out of 6 patients aged 7 to <8 years, 5(83.3%) out of 6 patients aged 8 to <9 years, 5(100%) out of 5 patients aged 9 to <10 years.

The highest seropositivity was found in the age group 7 to <8 years and 9 to10 years, while the lowest in the age group <1 year. The results among age groups were not statistically significant (P <0.3025).

The results showed the presence of wavy increase in percentages of children with positive IgG HADV antibodies in HADV infected group and non-HADV infected group. These results are shown in Figure (8) and Figure (9).

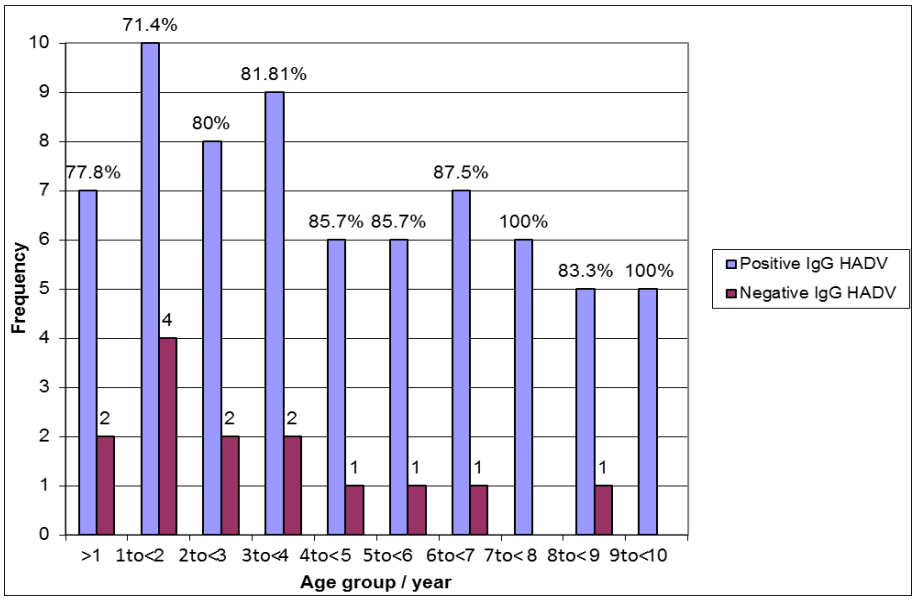

Fig. 8: Seropositivity of anti – HADV IgG antibodies among patients with RTI.

Table 1: Seropositivity of anti – HADV IgG antibodies among patients with RTI.

Patients	Frequency of positive IgG HADV antibodies	%	Frequency of negative IgG HADV antibodies	%
HADV infected group	69	83.2	14	16.8
HADV non-infected group	248	78.2	69	21.7
Total	317	79.2	83	20.7

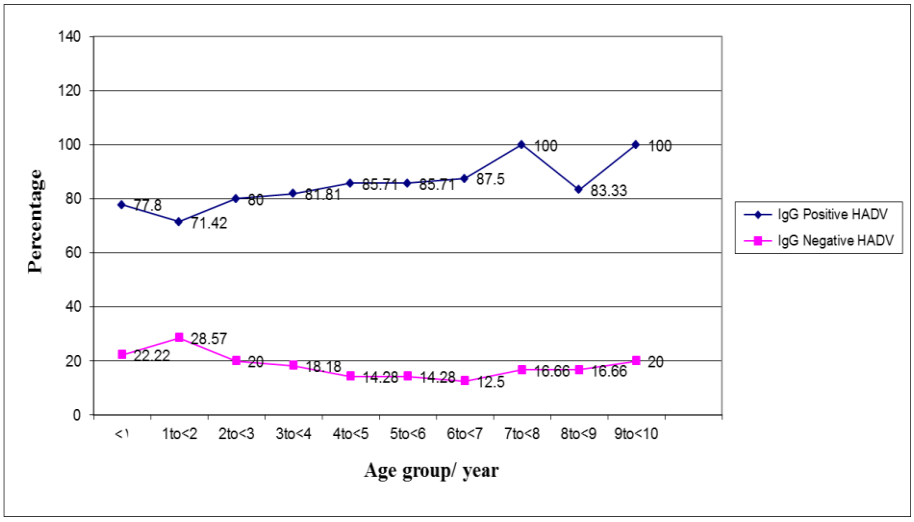

Fig. 9: percentages of anti – HADV IgG antibodies among patients infected with RTI.

The frequency of IgG-HADV antibodies in sera of infected patients (83.1%) were close to IgM HADV antibodies seroprevalence (72.2%) in the same group. The rise of IgG may be was due to recent infections or due to previous exposure to HADV. To confirm acute infections, fourfold rise in antibodies in a second serum taken 3 weeks later was needed but this was not tested because of shortage in materials of IgG detection, the availability of IgM conjugate to detect IgM antibodies which was used to detect recent HADV infections, and the hospital leave of patients before a second serum sample could be taken.

There were not statistical significant differences between the IgG seroprevalence HADV- positive children and those who were not infected with HADV (78.2%) and this may be due to the high prevalence of previous unapparent infections in the negative group.

The results revealed that 79.2% of all hospitalized children were positive for IgG HADV antibodies. This percentage was comparable to previous study by Aberle [18,15] and incomparable to study by Lankester [17,25].

The result showed the presence of wavy increase in a percentages of IgG positive children with increasing age, [26] described similar results.

Seasonality of HADV infection:

The highest frequency of HADV infections was shown in September (6 cases [6.74%]), and February (5 cases [5.61%]), while the lowest frequency of infections was shown in December, January, April and May which was 1 case per each of these four months (Figure 10).

This Figure reveales an increase in the frequency of infections which was started from August sharp peaked in September and declined in October and November. Then an increase in the frequency of infections which was started in January, wide peaked in February and March and declined in April. Thus Autumn-Spring period was the most common for HADV infections while Summer was the least. HADV was detected throughout the whole year.

This figure showed increase in the frequency of infections which started in January, peaked in February and declined in March. In this Figure also another peak, started from August, peaked in September and declined in October and November, Thus Autumn-Winter period was the most common for HRSV infections, while Summer was the least.

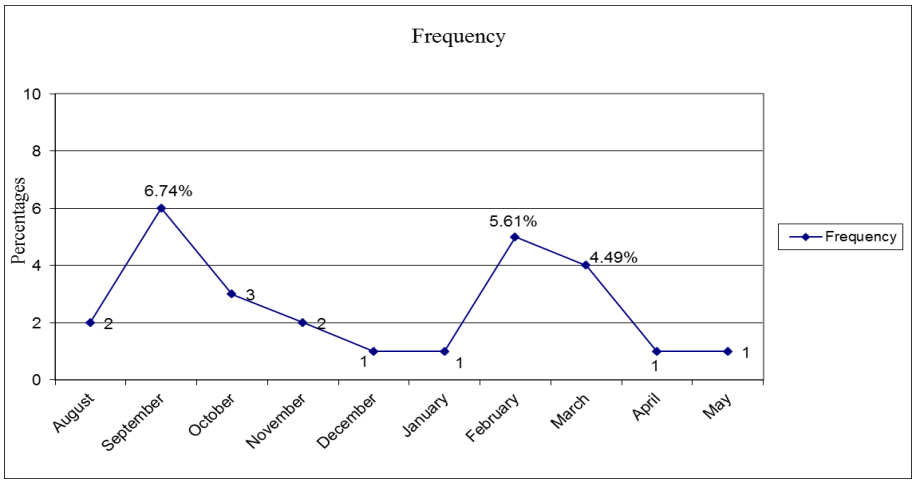

Fig. 10: Seasonal distributions of HADV infections among hospitalized children in the study.

The sample collection was done between August 2013 and May 2014 and the majority of HADV-positive patients were detected during autumn-spring period with sharp peaked in September and wide peaked in February. Other study [23]. From Brazil reported adenovirus circulated in the late winter and early spring in 1991, displayed the highest prevalence in the month of October, while in 1992 adenovirus was detected from May to December, with the highest prevalence also in month of October. These data were close to the present study and to the some annual reports showing the variable circulation of adenovirus from India [27]. And from China [28], narrow peak in December and wide peak from April to May for HADV [11].

In fact, the proportion of highest frequency of HADV positive cases (6.74%) sharp peak declined in October and November then increased from January to reach frequency of (5.61%) wide peak in February declined in April and May. Thus autumn- spring period was the most common for HADV infection while summer was the least, HADV was detected throughout the whole year. The differences in results of seasonal distribution from that in the present study may be due to geographic variation and attributed to climate.

Conclusions

Nasopharyngeal swab sampling have better sensitivity than whole blood samples for detecting HADV. RT-PCR method, more sensitive than antigen or antibody detection methods for the diagnosis of HADV.

References

1- Barati: Mitra; Taher-Mahsid Talebi; Noorbakhsh Samileh; Taj Farideh Ebrahimi. Diagnosis of the adenovirus, RSV, and influenza virus by rapid detection test (immunochromatography) in children with acute respiratory infection. Iranian Journal of pediatric society, (2010); vol.2, No.2, 79-83.

2- Jin Yu; Zhang Rong – Fang; Xie Zhi-Ping; Yan Kun-long; etal; prevalence of adenovirus in children with acute respiratory tract infection in Lan Zhou, China, virology, (2013); 10:271.

3- Zuckerman Arie J. ; Banatvala Jangu E, Pattison John R.; Griffiths Paul D., Schoub Barry D; Principles and practice of clinical virology, 5th ed. John Wiley & sons LTD. England, (2004).

4- Lixy; Chen JY; Kong M; Sux; Yiy P; Zou M. Prevalence of human metapneumovirus in hospitalized children with respiratory tract infections in Tianjin, China. Archives of virology. (2009); 154 (11): 1831-6.

5- Kajon, A.E., X. Lu, D.D. Erdman, J. Louie, D. Schnurr. Molecular Epidemiology and Brief History of Emerging Adenovirus 14-associated Respiratory Disease In The United States. (1996); J infect dis 202: 93-103.

6- La-Rosa G; laconelli M; pcurshaban M. Molecular characterization of Adenovirus from clinical samples through analysis of the hexon and fiber genes. (2011); Journal of general Virology; 92: 412-420.

7- Brandt CD., kim HW, Vargosko A, Infections in 18000 infants and Children in a controlled study of Respiratory Tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. (1969)., Am J epidemiol, 90 (6), 485-500.

8- Kliegman, Robert; Richard M kliegman. Nelson essentials of pediatrics. St. louis, Mo: Elsevier saunders. (2006).

9- Amy Burkholder, A killer cold? Even the healthy may be Vuleraele. CNN. Retrieved; (2007); 12-13.

10- Oliveira TE; Freitas JR; Ribeiro LZ; Yokosowa J. Prevalence and medical aspect of respiratory syncytial virus A and B groups in children seen at hospital declinicas of Uberlandia, MG, Brazil. Mem Inst Oswaldo cruz. (2008); 103: 417 – 422.

11- Barberi S; Barreto M; Penna F.L. ; Mazzarella B;, respiratory syncytial virus and adenovirus in acute lower respiratory infections in hospitalized infants and children, ojped. (2012); vol.2 .No.1; P.31-37.

12- Wu and Nemerow; Nemerow, GR. Virus yoga: the roll of flexibility in virus host cell recognition. Trends microbial. (2004); 12(4): 162-168.

13- Brooks Gf; Butel JS; Morse SA; Carrol KC; jawetz, Melinick, adelberg's: Medical Microbiology. 24th ed. Lange medical Books/ McGraw-Hill. Newyork, (2007).

14- Kim S, Sung H, Im HJ, Hong SJ, Kim MN. Molecular epidemiological investigation of a nosocomial outbreak of human metapneumovirus infection in a pediatric hemato-oncology patient population. Journal of clinical microbiology. (2009); 47(4): 1221-4

15- Hernes SS, Quarsten H; Hagen E, Lyngroth al, Pripp AH, bjorvant B . Swabbing for respiratory viral infections in older patients: A comparison of rayon and nylon flocked swabs. European Journal of clinical microbiology & infections disease: official publication of the European Society of clinical microbiology (2011); 30(2) 159 – 65.

16- Munoz FM; Piedra PA; Demmler GJ, Disseminated Adenovirus disease in immuno-compromised and immunocompetent Children Clin infect Dis; (1998); 27: 1194-200.

17- Lankester AC; Van tol mj D; Claas ecj; Vossen JM; Kroes A CM; Quantification of Adenovirus DNA in plasma for management of infection in stem cell graft recipients. Clin infect Dis; (2002); 34: 864-7.

18- Aberle-Stephan W; Aberle-Judith H.; Christoph Steininger, Martin-Susanne Matthes; Elisabeth Parcher; Adenovirus DNA in Serum of Children Hospitalized Due to an Acute Respiratory Adenovirus Infection, J infect Dis; (2003) 187 (2): 311-314.

19- Chan KH, Peiris JS, Lim W, Nicholls JM, Chiu SS. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of Respiratory Viruses in Children, J of Clin Virology: the official publication of the pan American society for clinical Virology; (2008); 42 (1): 65-9.

20- Sackesen C, Pinar A, Sekerel BE, Akyon Y, Saraclar Y. Use of polymerase chain reaction for detection of Adenovirus in Children with or without wheezing. Turk. J. pediatr, (2005); 47: 227-231.

21- Gomez, M.P.R.; LopezR.L.; Montez R.G.; Ruiz C.O.; Cuesta S. H.; immunochromatographic test for detection of Adenovirus from Respiratory samples: It is a real solution for pediatric emergency department?, J. viro. Methods, 195 (2014): 236-239.

22- WOO PC; Lau SK; Chu CM; Chan KH. Characterization and complete genome sequence of anovel coronaVirus, coronaVirus HKUI, from patients with pneumonia. Journal virol, (2005), 79(2): 884-895.

23- Stralitto, Selir M. Siqueira, Marilda M. Muller, Rafael L. Fischer, Gilberto B., viral etiology of acute Respiratory Infections among Children in Porto Alegra, RS, Brazil, Rev. Soc. Bras. Med. Trop. (2002), Vol. 35 No.4.

24- Tsuchiya LR lr, Costa lm, Raboni sm, Nogueira mb; Pereira LA. Viral Respiratory infection in curitiba, Southern Brazil. J infect, (2005), 51: 401-407.

25- Sutmoller F, Maia PR. Acute Respiratory Infections in Children living in two low income communities of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz (1995); 90: 665-74.

26- Fox JP.; Hall CE; Cooney MK; The seattle Virus watch. VII. Observation of Adenovirus Infections. Am J Epidemiol (1977); 105: 362-96.

27- John TJ, Cherian T, Steinhaff MC, Simors EAF, John M. Etidogy of acute Respiratory Infections in Children in tropical southern India, (1991) 13: S463-469.

28- Sung RYT, Chan RCK, Tom JS, Cheng AFB, Murray HGS. Epidemiology and etiology of acute bronochiolitis in Hong Kong infants. Epidemiology and infection (1992), 108: 147-154.

التشخيص المختبري للفايروس الغدّاني في الاطفال دون العشر سنوات و المصابين بالتهاب المجاري التنفسية

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الملخص

يصنف الفايروس الغدّاني البشري الادينو HADV ضمن عائلة الفايروسات الغدّانية وهو من الفايروسات المرضية المهمة لالتهاب المجاري التنفسية بين الاطفال. هدفت هذه الدراسة لتحديد الادينوفايروس HADV في الاطفال الراقدين في المستشفى دون العشر سنوات من العمر في محافظة صلاح الدين بالإضافة الى مقارنة عدة طرق تشخيصه لتحديد هذا الفايروس. المسحة الانفية البلعومية و نماذج للدم الكلي أخذت من 400 طفل راقد في المستشفى للفترة بين نهاية آب 2013 الى نهاية ايار 2014، كل عينة تم تحليلها لتشخيص فايروس الادينو بواسطة طريقة تفاعل انزيم البلمرة المتسلسل المتعدد الوقت الحقيقي و الاجسام المضادة نوعIgM و الاجسام المضادة نوعIgG لفايروس الادينو من عينات المصل المأخوذة من الدم باستخدام تقنية الاليزا. أظهرت الدراسة ان المسحة الانفية البلعومية NPS كانت اكثر حساسية من عينات الدم الكامل whole blood للكشف عن الفايروس الغدّاني الادينو فايروس الذي تم تشخيصه في 3% بواسطة تقنية تفاعل انزيم البلمرة المتسلسل المتعدد الوقت الحقيقي و في 72.2% بواسطة الاجسام المضادة نوعIgM و في 83.1% بواسطة الاجسام المضادة نوعIgG. تبينت الدراسة أن فترة الخريف و الربيع كانت أكثر الاوقات أصابه بخمج الفايروس الغدّاني مع اعلى مستوى في شهر أيلول و شهر شباط.