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# **Molecular detection of adenovirus among patients with common cold symptoms in Fallujah by real time PCR**

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**Abstract**---Introduction: The confirmation of adenovirus infection using Real time PCR (qPCR) afford a greater accuracy, with a specific, sensitive, quantitative technique as well as time consuming. Adenovirus infections can be detected using a variety of techniques, although the most accurate ones are molecular ones. Method: This study includes 90 patient who suffering from respiratory symptoms (common colds) and/or diarrhea with high temperature, their ages range from newborn to elderly, who are inpatients and outpatients resident in Fallujah Maternity and Children's Hospital and teaching hospital in Fallujah / Anbar governorate, from 1<sup>st</sup> November 2021 to 1<sup>st</sup> May 2022. All samples subjected to Adenovirus identification using Real Time PCR Test. Results: Out of total 90 blood sample there were 12(13.3%), 78(86.7%) Negative and positive results respectively, from Real Time PCR results.

**Keywords**---molecular detection, RT-PCR, adenovirus, common cold.

## **Introduction**

Adenovirus is thought to be the cause of many illnesses in adults. were first documented in afflicted military personnel during World War II (Gray *et al.*, 2000). the human adenovirus (HAdV) is a significant contributor to the common cold and epidemic keratoconjunctivitis (Onda *et al.*, 2021). addition to respiratory illnesses, adenoviruses can cause diarrhea, mesadenitis, hemorrhagic cystitis, and other pathological disorders, according to further research and the identification of novel serotypes (Yamamoto *et al.*, 2017). The most common cause of respiratory

system disorders was adenovirus (Xie *et al.*,2018). The respiratory ailment caused by adenovirus infection, on the other hand, could range from a cold to pneumonia, croup, or bronchitis. In patients with weakened immune systems, the outcome of adenovirus infection could be complex (Shieh ,2021). Either Each patient had respiratory samples taken either nasopharyngeal swabs or sputum. Real-time quantitative PCR was used to determine the viral load (Huh *et al.*,2019).

The sources of infection for many people are a sick individual in the acute stage of viral infection, a convalescent, or a virus carrier ,there for Adenoviruses are usually passed from one person to another through the air, Shaking hands or touching in close proximity which is one example of close personal contact. moreover sneezing and coughing into the air before washing hands. Some adenoviruses can be disseminated by an infected person's faeces (Khanal *et al.*,2018; Dodge *et al.*,2021). The study aimed to screening of suspected infection with adenovirus (AdvS) in patients with common colds and enteric symptoms to confirm the infection using Real Time PCR technique .

## **Method**

Blood and nasopharyngeal samples were collected aseptically from patient their ages range from children to elderly aged, suffering with acute gastroenteritis from respiratory symptoms , common colds , or diarrhea with high temperature which collected from Fallujah Maternity and Children's Hospital and teaching hospital in Fallujah in Anbar governorate, admitted with to hospitals or outpatient wards, a total of (90) blood samples obtained From 1<sup>st</sup> November 2021 to 1<sup>st</sup> May 2022.

## **Sample collection**

The blood was drawn from the patients and placed in a gel tubs then separated using the centrifuge to obtain serum for 5 minutes (3000 x), the serum was transferred to a plain tube for preservation and the Nasopharyngeal samples were collected using sterile swab in placed in VTM Tube . all the samples were kept in the freezer (-20°C) until the time of the experiment.

## **Detection of Adenoviruses by Real Time PCR Extraction of Adenoviral DNA**

We were extracted DNA of 90 serum samples according to the instructions of the supplied kit (InnuPREP virus DNA / RNA extraction kit) A J Innuscreen GmbH/ Germany Samples were stored in clean Eppendorf tub at deep freeze; kit of extraction was stored at room temperature RT (15-30°C) until the day of the experiment, any lyophilized or dissolved substance must be stored at -20°C. All of the extracted DNA samples were used to confirm its purity and concentration using Nano Drop (Nabi , Korea ). Each DNA sample (2-3µl ) was placed on the pedestal. The cap was closed and a measure clicked, making sure that the concentration and purity were recorded. Purity was measured under a 260/280 column (good purity ranged from 1.80-2.00). This was repeated for each sample.

Moreover, each DNA sample were electrophoresed using Agarose gel for confirming intact DNA and purity. The agarose gel (Conda / USA) was prepared at

a 1.5% concentration and poured in Gel electrophoresis device template (CBS Scientific , USA) then the solid agarose transferred and fixed in the Electrophoresis device tank which filled later with TBE buffer (Conda / USA) and an Electric current of 7 v\c2 applied through the device for 1 hour after loading a mixture of sample and loading dye (Intron / Korea) into the gel wells, the gel scanned later using gel illuminator (Lab net , USA) at 336 nm.

### Quantitative detection of Adenovirus (Adv) DNA by q-PCR

Pre-extracted DNA specimen from adenoviruses were used for q-PCR reaction according to the instructions of the supplied kit ( Euro Clone/ Italy).

### Reaction steps

1. 30  $\mu$ l of MasterMix was added into PCR tubes.
2. 10  $\mu$ l of the isolated nucleic acid sample or 10  $\mu$ l of Calibrator/Positive Control was added into the individual PCR tubes. The final reaction volume will be 40  $\mu$ l. It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation.
3. The tubes were closed, centrifuged shortly, inserted into the device and left them amplify according to the following PCR profile.

Amplification profile:

Step	Temperature	Time	Data collection	Cycles
Hold	37 °C	2 min.		1
Hold	37 °C	10 min.		1
PCR	95 °C	5 s	FAM+HEX	45
	60 °C	40 s		
	72 °C	20 s		

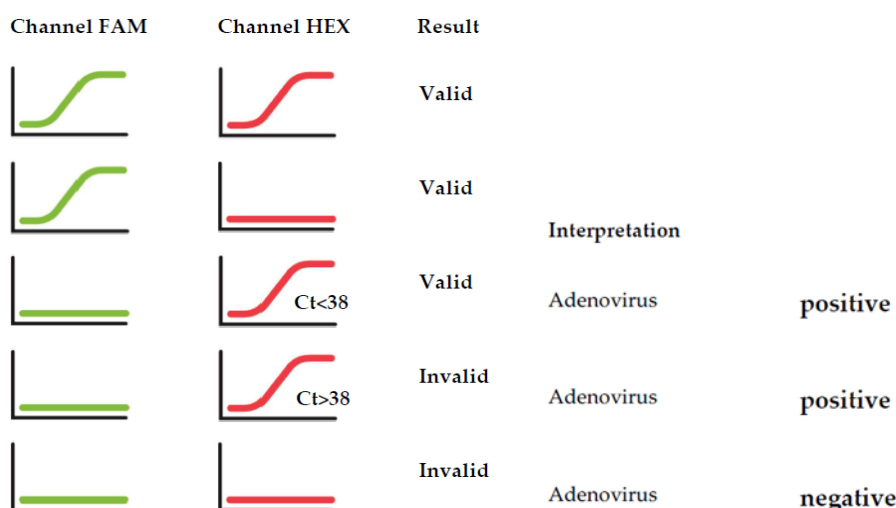


Figure (1 ): Interpretation of q-PCR result obtained by FAM and HEX

Use the following formula to calculate the virus concentration in copies/ml while taking into account the volume of material entering the isolation:

$$\text{cp /ml} = \frac{\text{SC} \times \text{EV}}{\text{IV}}$$

SC - Sample concentration (cp/ $\mu$ l)

EV - Elution volume ( $\mu$ l)

IV - Isolation volume (ml)

### Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T- test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

### Results and Discussion

Adenoviruses can cause illnesses ranging from minor respiratory viral infections, diarrhea and conjunctivitis, to severe disseminated disease.(Hiwarkar *et al.*,2018). The study included 90 samples from children and elderly which suffering from respiratory symptoms , common colds , or diarrhea with high temperature, the results showed that 2 (13.3%), 78 (86.7%) Negative and positive result respectively in the Real Time PCR test. The concentration and purity of the extracted DNA from the samples were detected using gel electrophoresis technique as appear in Figure (2). The results of the study demonstrate that out of total 90 DNA samples extracted from blood which subjected for Real-time PCR 78(86.7%) sample were positive for adenovirus and 12(13.3%) samples give no result, as in (Table 1) and Figure (3). the curves of Ct Values of the results presented in Figures (4), and (5). These results agree with the conclusion of Sharti *et al.* (2021) where Real time PCR is the most reliable test for detection of Adenoviruses in respiratory infection. Because there was a considerable delay between the time the stool samples were collected and when the experiment was carried out, it was thought that the virus might be lost, and also because the test kit was expensive, stool samples were not included in this study.



Figure (2) : Gel electrophoresis of genomic DNA extraction, 1% agarose gel at 5 vol /cm for 30 min.

Real time considered as the most sensitive and specific method for viral detection especially adenovirus, which improved by the high percentage of viral detection in our study compared with other diagnostic ways.

Table (1) : Showed the results of Real time PCR

Type of diagnostic test	Positive		Negative		Total
	No	Percentage %	No	Percentage %	
Real time	78	86.7	12	13.3	90
Chi-Square - $\chi^2$ (P-value)	48.41 **	(0.0001)			--
** (P≤0.01).					

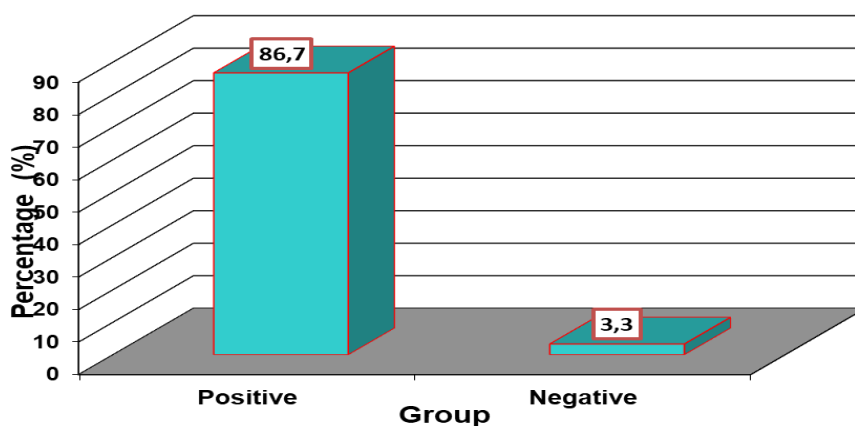


Figure 3. Showed the results of Real time PCR

CT

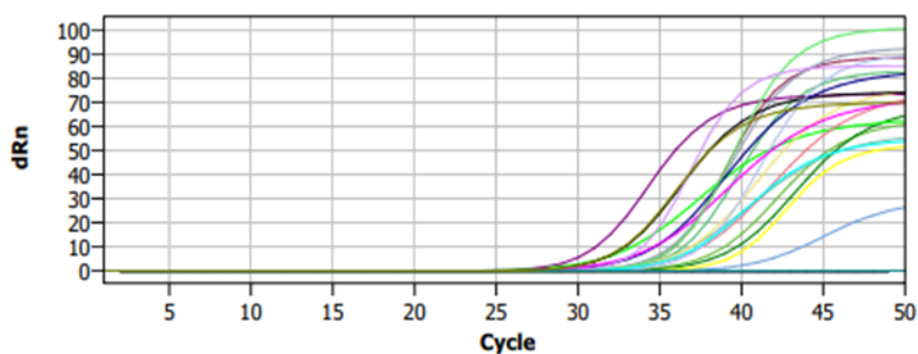


Figure (4) :Showed FAM Adenovirus detection gene

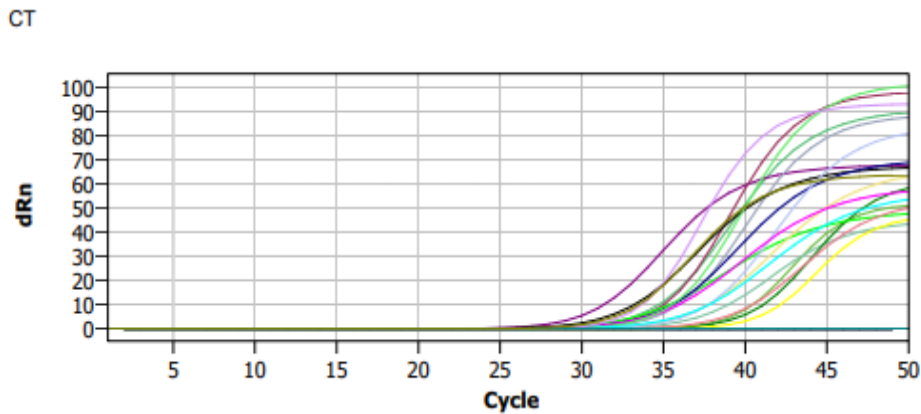


Figure (5) : Showed Internal Control IC Adenovirus Detection gene

The high prevalence of adenovirus noticed in diarrheic align with common cold cases in our study's result ,which agrees with the results of Thewainy and Hasony (2019) study where Viruses are the most frequent cause of acute intestinal infections in children (Agawam *et al.*,2021).this would be attributed to many causes, which would be probability of infection etiology from water sources especially in children who deals in a careless manner, as Nguyen *et al.* (2021) reported an existence of adenovirus in some water supplies which may represent probable source of those viruses in some patients. This is further supported by a study done in Iraq, where Jaff *et al.* (2015) discovered that children who had access to tab water at home had a greater rate of viral infections than children who did not, and that infants who were exclusively breastfed had lower rates of adenovirus gastroenteritis. Moreover, the study conducted mostly during winter season which is known for spreading of many infectious agents and respiratory ,diarrheic cases which is improved by Zhou *et al.* (2021) study, who revealed that adenovirus is one of the most likely causes of viral infection with respiratory since and diarrhea, and it has been shown that it spreads more readily during the colder months. The results of a study in Iraq in Samawah by Thewainy *et al.* (2022) conducted to study seasonal variations throughout the year, there can be variations in the frequency of mono viral diarrhea according to the season. Adenovirus infections increased in the winter-spring season (January to May) and from September to February (autumn-winter) , Throughout the majority of the months for which data was gathered, human adenovirus was found (autumn-winter). the peak of adenovirus infection in November, Adenoviruses were the cause of infection in the winter (45.6%), and they were the cause of infection in the spring (7%). While, in the autumn a 42.8% increase in viral infections, peaking in December.

Males are infected with adenoviruses non-significantly higher than females in our study group, where the infection rate was (48.9% and 37.8%) in males and females respectively as showed in (Table 2) which corresponds to a study conducted in Lebanon, and the percentage of males infected with adenovirus was (51.3%), while females were 38 (48.7%). Our findings were consistent with the results obtained by Jaff *et al.* (2015) study who revealed higher proportion of enteric adenoviruses infection (2%) in males than (1%) in female, this may

attributed to the nature of work of many males that obligate them to deal with many sources of infection including infected people or by environmental sources in addition to their hygiene habits.

Table 2. Showed the Percentage of infections between Male and female

Gender	Positive		Negative		Total	P-value
	No	Percentage %	No	Percentage %		
Male	44	48.9	8	8.9	52	0.0008 **
Female	34	37.8	4	4.4	38	0.0001 **
Total	78	86.7	12	13.3	90	0.0001 **
P-value	--	0.581 NS	--	0.581 NS	--	--

\*\* ( $P \leq 0.01$ ), NS: Non-Significant.

The highest percentage of adenovirus infection among age groups appeared neonates under 6 months of age (33.3 %) significantly, followed by the older ages between 6 months and 1 year (15.6 %), A little distribution of adenovirus infection in older patients observed in our study, where the lowest distribution observed in the age group (11-30 years) with 6.7 %. The reason is that the cases collected are in the children's hospital, and that most of the patients in the hospital are children and young ages. as indicated in Table (3).

Table 3 Showed the Percentage of Adenovirus infection among study sample according to age groups

Age groups	Positive		Negative		Total	P-value
	No	Percentage %	No	Percentage %		
< 6 months	30	33.3	4	4.4	34	0.0026 **
6 months - 1 year	14	15.6	6	6.7	20	0.237 NS
1 - 10 years	8	8.9	1	1.1	9	0.0361 *
11- 30 years	6	6.7	0	0.0	6	0.194 NS
31 - 50 years	11	12.2	0	0.0	11	0.0251 *
> 50 years	9	10.0	1	1.1	10	0.472 *
Total	78	86.6	12	13.3	90	0.0001 **
P-value	--	0.0087 **	--	0.0861 NS	--	---

\* ( $P \leq 0.05$ ), \*\* ( $P \leq 0.01$ ).

The high distribution in neonates in our study is consistent with other studies conducted on adenovirus prevalence, as mostly known, HAdV infections were discovered in people of various ages affecting people of all ages, particularly young children and the elderly (Zhang *et al.*, 2020). Indeed, a convergent result obtained by Zaraket *et al.* (2020) where distribution of age revealed neonates under 11 months showed a high percentage (26.0%). Same results obtained by According to Jaff *et al.* (2015), there were more positive instances in children aged 1 to 2 years

than in infants younger than 1 year old in their study Al-Sayidi *et al.* (2014) study who revealed the highest incidence in children less than 3 years moreover indicating that the younger group was more susceptible to viral infection than the older group. This could be explained by the possibility that older kids have acquired protective immunity as a result of prior exposures that protects them from infection with this agent, as demonstrated by (Jaff *et al.*, 2015). In other side, Neonatal infections may manifest as silent or moderate cases, most likely as a result of maternal antibody defense. According to Zhang *et al.*, children between the ages of 4 and 23 months have the highest prevalence of clinical disease and the highest risk of contracting a serious illness that requires hospitalization (2020). Children also wanted to eat on their own more and were more active, especially outside, but they did not know how to practice good hygiene. The risk of infection may rise as a result of these factors. Early childhood HAdV infection resulted in an acquired immunity against the virus, as seen by the lowest HAdV-positive rate in older groups that may be attributable to a recent HAdV infection. More than 90% of youngsters have acquired multiple infectious diseases by the time they become 3 years old. Older children and adults who could be the family's source of infection for young children may show signs of subclinical infection. Another significant source of infection in children hospitalized with illnesses, particularly respiratory ones, is hospital infection. The maximal excretion of adenovirus in patients with gastroenteritis occurs 3 to 13 days after the symptoms have arisen, which means that many cases in older individuals may not be identified or missed out due to virus shedding during a symptomatic stage (Jaff *et al.*, 2015).

## Conclusion

Real time PCR technology is the good technique for identification of infection with adenovirus. A high incidence of adenovirus infection in people with a common cold symptoms. The incidence of adenovirus is high in children under one year, more than in older children or adults.

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