

## Human Adenoviruses 40/41 and Cytokines Response in Children with Diarrhoea

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### ABSTRACT

**Background:** One of the most significant etiologic factors in acute gastroenteritis in children is adenovirus types 40/41.

**Objective:** The aim of this research was to diagnosis of adenovirus type 40/41 in children with diarrhoea in three Cities in Iraq; Babylon, AL-Najaf and Karbala, and evaluation of serum concentration of Interferon gamma (IFN- $\gamma$ ), Interleukin (IL) 6 and 10. **Patients and methods:** A total of 450 stool samples were collected from children with diarrhoea, age range from 1 to 36 month. Adenovirus 40/41 has been detected in stool using Polymerase Chain Reaction (PCR) method. Serum concentration of IFN- $\gamma$ , IL-6 and 10 have been measurement using Enzyme-linked Immunosorbent Assay (ELISA) technique.

**Results:** Out of 450 stool samples, there were 150 Children (33.3%) infected with adenovirus 40/41, the highest rate of infection was in the Karbala City with 73 infections (16.2%) followed by Najaf and Babylon were recorded 55 (12.2%) and 22 (4.8%) respectively. Cytokines profile revealed a significantly substantial rise in viral patients compared to controls. IFN- $\gamma$ , IL-6 and IL-10 levels in the age range of 1 to 36 months were higher than those in the control group at 69.5, 61.3 and 112.3 pg/ml respectively.

**Conclusions:** There were high infections with adenovirus type 40/41 in Karbala City. Interferon- $\gamma$ , IL-6 and IL-10 play an important role in infection which can be used in immunotherapy.

**Keywords:** Adenovirus, 40/41, PCR, IFN- $\gamma$ , IL-6, IL-10, case control study, University of Kufa.

### INTRODUCTION

Adenovirus types 40 and 41, which cause 3.2 to 12.5% of all cases of acute diarrheal illnesses in children and infants worldwide and have a higher detection rate in underdeveloped nations, have been linked to acute diarrheal diseases<sup>(1)</sup>. Children under the age of two are most commonly affected by adenovirus types 40 and 41, which specifically infect the enterocytes of the small intestine and produce chronic watery diarrhea that, if ignored, can result in serious dehydration<sup>(2)</sup>. In cell culture, these viruses do not or do not grow well. By virtue of their pathogen-associated molecular patterns (PAMPs), such as capsid or DNA, adenovirus is well known to activate innate immunity, which results in the generation of IFN- $\gamma$  and other cytokines such as IL-6 and IL-10<sup>(3)</sup>.

A cytokine with a variety of functions, IL-6 has been demonstrated to be essential for the immunological and host defense systems. In addition, endogenous IL-6 has been demonstrated to work in concert with other cytokines and growth factors to prevent programmed cell death brought on by cytotoxic drugs in viral infections<sup>(4,5)</sup>.

One of the most powerful and promising anticancer cytokines is IL-10. It is a heterodimeric cytokine that is mostly generated by activated macrophages and dendritic cells and is made up of two distinct disulfide-linked subunits known as p35 and p40. The production of interferon by natural killer cells, T cells, dendritic cells, and macrophages is stimulated by IL-12<sup>(6,7)</sup>. In the same way, it promotes T helper type 1 development and increases the cytotoxic activity of Natural killer (NK) cells and cytotoxic T lymphocytes

(CTLs)<sup>(8)</sup>. Children and infants who live in areas with poor healthcare access are particularly vulnerable to diarrheal disease and the growth faltering, impaired neurocognitive development, and increased mortality are linked to repeated episodes of moderate-to-severe diarrhoea and high rates of enteric pathogen infection lead to defect in immune system due to increase cytokines production<sup>(9,10)</sup>.

Therefore, the aim of this research was to diagnosis of adenovirus type 40/41 in children with diarrhoea in three Cities in Iraq; Babylon, AL-Najaf and Karbala, and evaluation of serum concentration of Interferon- $\gamma$  (IFN- $\gamma$ ), Interleukin (IL) 6 and IL-10.

### PATIENTS AND METHODS

This case control study was performed in 3 cities; Babylon, AL-Najaf and Karbala during period from 1<sup>st</sup> March 2021 to the end of August 2021. A total of 450 stool samples were collected from children with diarrhoea age range 1 to 36 month attending to the Al-Jerahi Hospital (Babylon City), Al-Furat Al-Awsat Hospital (AL-Najaf City) and AL-Zahra teaching Hospital (Karbala City). In contrast, 60 healthy children age range 1 to 36 month have been considered as control group<sup>(11,12)</sup>.

### Diagnosis of adenovirus:

Five gram of stool has been collected from each children suffering from diarrhoea in disposable containers<sup>(13,14)</sup>. Monoplex PCR was done to detect number of genes that encode for adenovirus type 40/41 to detect Penton base and Fiber genes<sup>(15,16)</sup> using primers provided from (Bioneer Company /Korea)

which are listed in **Table 1** and amplification conditions of genes were used by PCR reactions (**Table 2**).

**Table (1):** Types of primers that were used in PCR for detected adenovirus type 40/41.

PCR product	Sequence (5'→ 3')	Position	Gene	Primer
1,253bp	CTATCAGAACGACCACAGC	14,152–	Penton base	Penton-F
	AACTT	14,175		
	TCCCGTGATCTGTGAGAGC	15,384–		
	RG	15,404		
1,153bp	TAATGTTTGTGTTACTCCG	31,180–	Fiber	Fiber-F
	CTC	31,199		
	GGGGAGGCAAATAACTA	32,311–		
	CTCG	32,332		

**Table (2):** Hexon gene and amplification conditions by PCR reactions.

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
Hexon	94 C° for 5 min.	35	94 C° for 1 min.	56C° for 50 sec.	72 C° for 50 sec.	72C° for 1 min.

**Cytokines measurement:**

Five ml of blood were collected from infected and healthy children and left to clot at room temperature for 30 minutes to allow the clot to form, the clotted sample were centrifuged at 1500 rpm for 20 minutes to obtain serum and kept at deep freeze -20 °C till used to evaluation of concentration of IFN-γ, IL-6 and 10 using ELISA technique <sup>(16,17)</sup>. All kits provided from Elabscience® ELISA Kit Germany.

**Ethical considerations:**

The study concept for human studies was approved from Kufa University's College of Science and AL-Kufa General Hospital by The Institutional Ethics Committee. Additionally, before taking part in the study, each individual gave written, informed consent. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

**Statistical analysis**

Data was collected and analysed by using SPSS (Statistical Package for Social Sciences, version 20, IBM, and Armonk, New York). Quantitative data was summarized as mean and standard deviation. Qualitative data were given as number (n) and percentage (%). Correlations were determined by Spearman correlation. P-value ≤0.05 was considered significant.

**RESULTS**

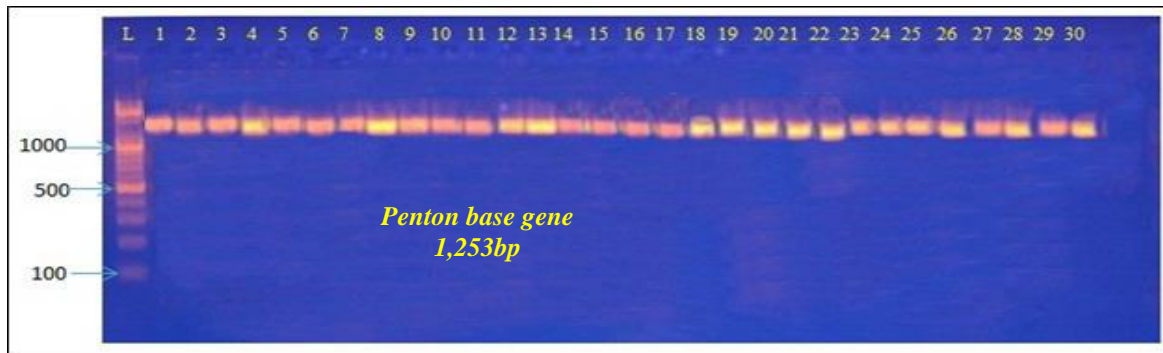
**Total diarrheal infections:**

Table 3 showed there were 450 children infected with diarrhoea during period from 1<sup>st</sup> March 2021 to the last August 2021. Age group 29-36 month was the highest infected with 159 cases (35.3%) followed by age group 1-8 month with 139 cases (30.9%). Most infections were in Karbala City with 180 cases (40%) followed by AL-Najaf City with 170 cases (37.7%) and Babylon City with 100 (22.3%).

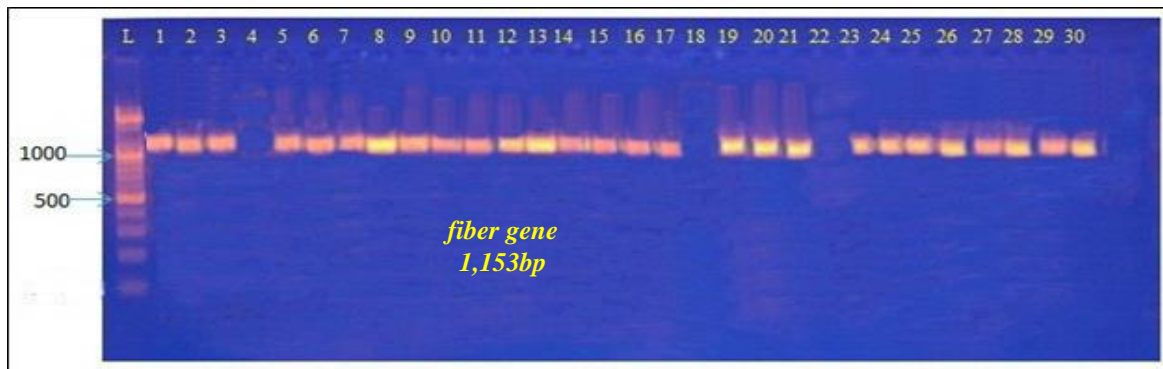
**Table (3):** Total diarrheal infections in children from 1<sup>st</sup> March 2021 to the last August 2021.

Age City	1-8		9-18		19-28		29-36		Total (%)
	Male	Female	Male	Female	Male	Female	Male	Female	
Babylon	18	12	13	9	11	7	22	8	100 (22.3)
AL-Najaf	28	24	15	11	14	11	49	18	170 (37.7)
Karbala	33	24	17	16	16	12	39	23	180 (40)
Total (%)	139 (30.9)		81 (18)		71 (15.8)		159 (35.3)		450 (100)

**Total adenovirus infections:** Out of 450 stool samples have been tested with PCR technique, the results indicated that there were 150 cases (33.33%) gave positive result with Penton base and fiber where were the sizes 1,253bp and 1,153bp, respectively (**Figures 1 and 2**). Karbala City recorded 55 cases followed by AL-Najaf with 37 cases while, the lowest infections were in Babylon City with 22 cases (**Table 4**).



**Figure (1):** PCR amplified products of *Penton base gene* of the adenovirus (Ad40 and Ad41) using specific primers with expected size 1,253bp, DNA marker (100bp ladder).

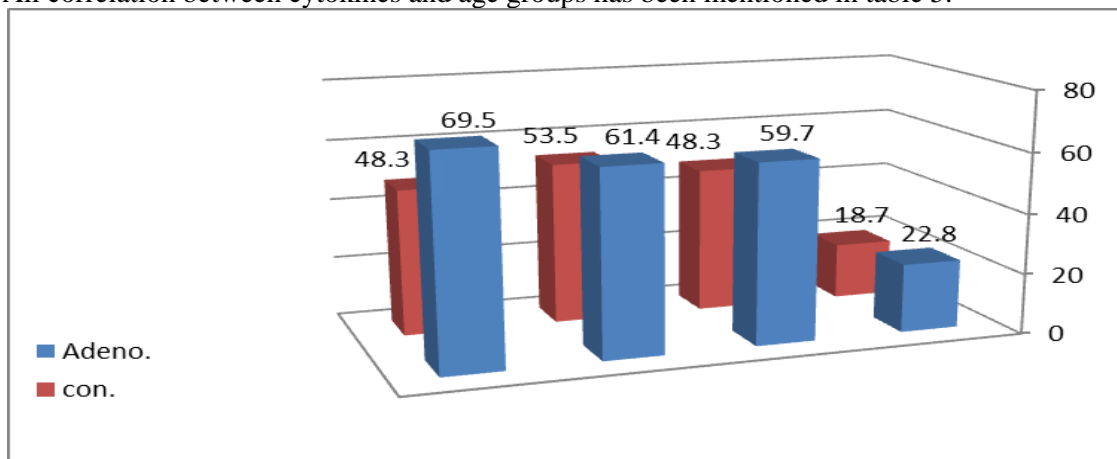


**Figure (2):** PCR amplified products of *fiber gene* of the adenovirus (Ad40 and Ad41) using specific primers with expected size 1,153bp, DNA marker (100bp ladder).

**Table (4): Positive and negative infections in children with adenovirus from 1<sup>st</sup> March 2021 to the last August 2021**

City	Total (%)		Total (%)
	Positive infections	Negative infections	
Babylon	22	78	100 (22.3)
AL-Najaf	37	97	170 (37.7)
Karbala	55	125	180 (40)
Total (%)	150 (33.33)	300 (66.67)	450 (100)

**Serum concentration of cytokines:** The results showed there was significant increase ( $P < 0.05$ ) in serum concentration of IFN- $\gamma$ , IL-6 and IL-10 as compared with control. All age groups of children infected with adenovirus showed significant increase ( $P < 0.05$ ) as compared with control, and the high concentration detected in (29-36) age group (69.5pg/ml) as compared with control (48.3pg/ml) in IFN- $\gamma$  (Figure 3) and (61.3 pg/ml) compared to healthy control group (35.3 pg/ml) in IL-6 (Figure 4) and (112.3 pg/ml) compared with healthy control group (70.5 pg/ml) in IL-10 (Figure 5). All correlation between cytokines and age groups has been mentioned in table 5.



**Figure (3):** IFN- $\gamma$  level (pg/ml) in children with adenovirus and control.

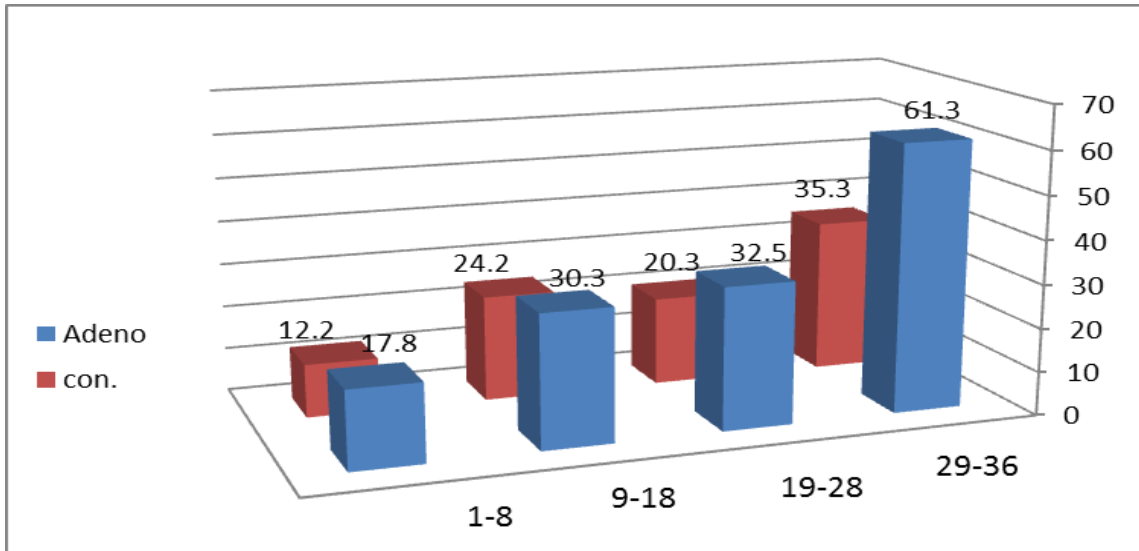


Figure (4): IL-6 level (pg/ml) in children with adenovirus and control

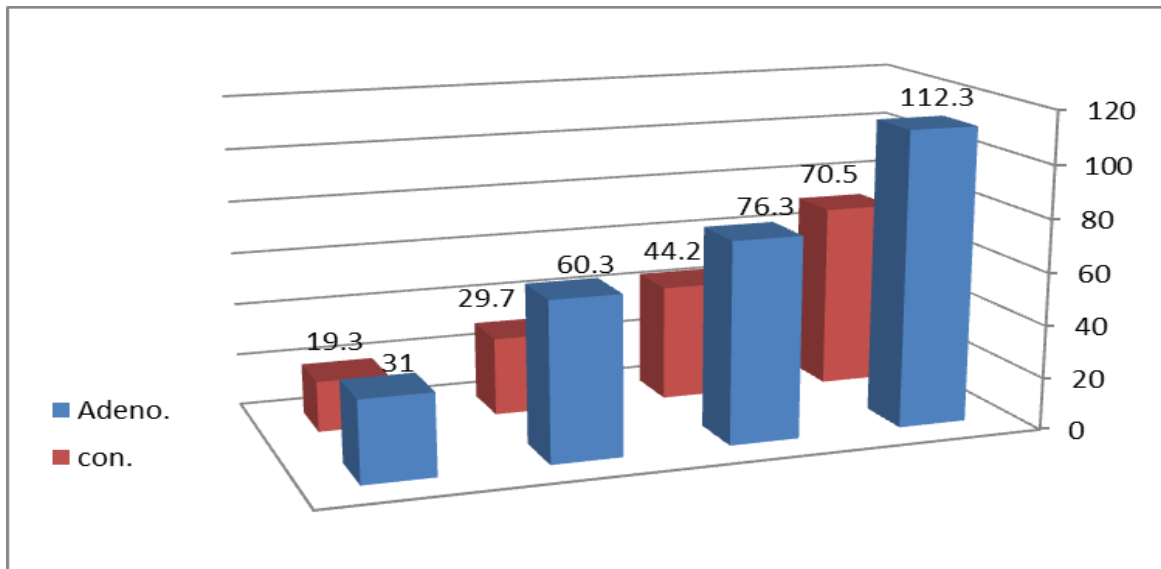


Figure (5): IL-10 level (pg/ml) in children with adenovirus and control.

Table (5): Correlations between cytokines and age groups of children with adenovirus.

	Correlation	Cytokine	Concentration	Adeno.	Age group
Cytokine	Pearson Correlation	1	-.031	.261	.000
	Sig. (2-tailed)		.924	.413	1.000
	N	12	12	12	12
Concentration	Pearson Correlation	-.031	1	.934**	.722**
	Sig. (2-tailed)	.924		.000	.008
	N	12	12	12	12
Adenovirus	Pearson Correlation	.261	.934**	1	.765**
	Sig. (2-tailed)	.413	.000		.004
	N	12	12	12	12
Age group	Pearson Correlation	.000	.722**	.765**	1
	Sig. (2-tailed)	1.000	.008	.004	
	N	12	12	12	12

\*\*Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

In this study, the most children infected with diarrhoea were in Karbala City with 180 cases (40%) followed by AL-Najaf City with 170 cases (37.7%) and Babylon City with 100 cases (22.3%). The risk of diarrheal disease is especially high in children and infants living in places where healthcare is inadequate and growth falters, neurocognitive development is impaired, and mortality is higher if repeated episodes of moderate-to-severe diarrhoea occur. High rates of enteric pathogen infections result in cytokines being produced in excess, leading to immune system defects (18,19).

Polymerase Chain Reaction was used in this study to diagnose adenoviruses by considering the many advantages of this method. As we know, PCR is important to diagnose viral DNA for high speed, sensitivity. Adenoviral infections can also be diagnosed more efficiently and effectively using PCR than commercial kits. Only these two genes are detectable by primers (Ad40 and Ad41). According to this study, out of 450 stool samples, it was found that enteric adenoviruses caused only 150 cases (33.33%) of gastroenteritis in children. It is higher than various studies such as **Rezaei et al.** (20) and **Kumthip et al.** (21) where recorded (9% and 7.2%) respectively, it was evident in the present study that types 40 and 41 adenovirus predominated. From 2003 to 2006, **Banyai et al.** (22) investigated diarrheic patients of Hungary by PCR for adenovirus infection; type Ad41 had been circulating all four years, but type Ad40 had only been found in 2003 and 2004. The incidence of adenovirus infection among gastroenteritis patients has been attributable to both types 40 and 41 of adenovirus, but recent investigations indicate that type 40 infection has declined and type Ad41 infection has increased (23,24).

The current study concludes that the serum concentrations of IFN- $\gamma$ , IL-6 and IL-10 were considerably higher in the children infected with adenovirus than in the healthy control group. Strong cellular immune responses, such as proliferative responses and the release of Th1-type cytokines such as IFN- $\gamma$  following activation with certain viral antigens, may be the cause of this adenovirus infections trigger complicated immune reactions that involve both humoral and cellular immune responses (25).

IFN- $\gamma$  is known to increase the expression of MHC class I and II on nucleated cells as well as stimulate several effector actions in mononuclear phagocytes. Its main purpose in living organisms seems to be to stimulate macrophages to fight intracellular infections like norovirus and adenovirus (26). Moreover, several authors suggest that response to adenovirus infections mainly involves IFN- $\gamma$  secretion by CD4 Th1 T cells. **Ponterio et al.** (27) reported that patients generate significant CD4 and CD8 IFN- $\gamma$  responses to specific adenovirus antigens during infection also these

observations suggest that an IFN- $\gamma$  based detection method could be used to diagnose individuals with adenovirus infections.

In adenovirus infection, it has been shown that IL-6 plays a central role in immune host defence, acute phase reactions, and haematopoiesis in addition to blocking programmed cell death induced by cytotoxic agents and acting in cooperation with other growth factors and cytokines, IL-6 plays a pleiotropic role. The effect of IL-6 on cell-cell association, attachment, and migration may also play a role in cell invasion and metastases (5).

Thus, the role of IL-6 and IL-10 in several malignancy pathophysiology has clearly been established and makes IL-6 a rationale target for treatment of cancer explained increased pro inflammatory cytokines IL-6 secretion with increased adenovirus infection. In addition, IL-6 has been shown to play an important role in several types of malignancies (28).

Moreover, IL-10 has been shown to play an important role in tumor-specific immunity. There is no doubt that IL-6 is an essential cytokine for the immune system and the host's defence system. In addition, endogenous IL-6 has been shown to prevent programmed cell death caused by cytotoxic drugs during viral infections in conjunction with other cytokines and growth factors (29). As a result of their pathogen-associated molecular patterns, such as capsids or DNA, adenoviruses activate the innate immune system, which results in the release of IL-6 and IL-10 cytokines as well as IFN- $\gamma$ .

In conclusion, there were high infections with adenovirus type 40/41 in Karbala City. Interferon- $\gamma$ , IL-6 and IL-10 play an important role in infection which can be used in immunotherapy.

**Conflict of interest:** There was no conflict of interest in this study.

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