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Adenovirus Infection in Children with Acute Viral Gastroenteritis: Fecal Leukocyte Frequency and Evaluation the level of Cytokines [IL-6 and IL-12] as Mediators Effectors against Disease in Hillah City, Iraq

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Abstract: Many different diseases are caused by adenoviruses that are members of the adenoviridae family and belong to the mast adenovirus genus. Plague, cystitis, conjunctivitis, diarrhoea, hepatitis, myocarditis, intussusception, and encephalitis are common clinical symptoms that can arise from an adenovirus infection. More and more, adenoviruses are being acknowledged as potentially fatal infections in immunocompromised patients, especially those who are HIV positive and have received an allogeneic bone marrow transplant, where the death rate is typically extremely high. This research was carried out in four districts of Babylon Governorate in Iraq: Al-Muhawil, Al-Musayyab, Al-Hashimiyah, and Al-Kifl. Fifty stool samples that had already been tested for adenoviruses using commercially available EIA were included in the study population. Distribution of gender-specific traits according to viral antigen positive 42.86±5.00 and 57.14±6.81 for male and female respectively. Distribution of characteristics by adenovirus antigen positivity according to Age group recorded 22.86±3.00, 42.86±5.03 and 34. ±4.19 in (0-12, 13-24, and 25-72 Months respectively). Cytokine (IL-6) concentrations in blood samples taken from children with adenovirus infection and healthy controls 181.45±32.00 and 109.30±21.94 respectively. Concentrations of cytokine (IL-10) in sera of adenovirus-infected patients and control children recorded 14.18±2.09 and 5.23±0.12 respectively. These results have the potential to inform the development of novel vaccines and treatments for paediatric viral illness.

Keywords: Adenovirus, Children, Gastroenteritis, Leukocyte Frequency, Cytokines, Disease.

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

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Introduction

One of the most common causes of gastroenteritis in kids is adenovirus. It is responsible for around 2-15% of cases of acute diarrhoea in children. There are more than a hundred distinct kinds of adenoviruses, each with its own set of unique genetic and biological traits. In many cases, gastroenteritis is caused by subgroups 40 and 41 of the Adenovirus. In nations with low or medium income levels, the virus primarily affects children under the age of two. Based on the cause and symptoms, infectious diarrhoeas are categorised as inflammatory or non-inflammatory. When inflammatory diarrhoea is present, one telltale sign is the presence of white blood cells in the stool. It follows that they are agents of diarrhoea that do not cause inflammation [1, 2]. However, they can communicate with the host's enteric cells to dampen an inflammatory response, allowing for the detection of moderate faecal leukocytes. Nevertheless, there is a lack of information regarding the quantity of faecal leukocytes, which are indicators of the inflammatory response, in children who have contracted these infections. Viruses are the most common culprits. More than 200,000 children die each year from viral gastroenteritis. There are 51 known serotypes of human adenovirus, which are further classified into 6 species (A–F) according to their capacity to agglutinate different kinds of red blood cells. Adenoviruses typically cause respiratory infections, but there are serotypes called enteric adenoviruses (EAds) that can cause diarrhoea. Serotypes 40 and 41 of the adenovirus species F and serotypes 12, 18, and 31 of the adenovirus species A are the most frequently linked to gastroenteritis and diarrhoea in young children [3-5]. Nevertheless, Ad40 and Ad41 mainly impact the gastrointestinal tract and account for 5–20% of paediatric diarrhea-related hospitalisations. Nevertheless, there are signs that the identification of adenovirus in diarrhoeal stools is likely under-reported since commercially available EIA can not specifically detect all types of circulating adenovirus. Fetal leukocytes have the potential to heighten gastrointestinal infections that invade the lining of the inside of the body. Nevertheless, in rare instances of viral diarrhoea, faecal leucocytes may be seen. Rates of faecal leukocytes in children with rotavirus, adenovirus, and a combination of the two viruses were measured in our study. Faecal leukocyte rates were significantly elevated in cases of rotavirus and adenovirus co-infections [6, 7]. The study found that among children younger than 10 years old, the prevalence of rotavirus was 10.7%, adenovirus was 5%, and the incidence of rota-adenovirus co-infections was 1.4%. In the same research, faecal leukocyte frequency was 3.3% in rotavirus-only patients, 22% in adenovirus-only patients, and 48.4% in rotavirus-adenovirus co-infected individuals [8]. In rota-adenovirus coinfections, faecal leukocytes were found most often, but in rotavirus infections, they were found least frequently. To this day, we still don't know how viruses can infect one another. Based on the increased frequency of coinfections during times of high rotavirus prevalence, we hypothesise that rotavirus infections may have particular mechanisms that make secondary infections more likely. According to our analysis of the causative agent's seasonal variability, the spring was the most common time for rotavirus positivity, the autumn for adenovirus positivity, and the spring for rota-adenovirus co-infection. The winter season was the most common for rotavirus positivity, summer for adenovirus, and winter for rotavirus and adenovirus co-infection [9-11]. The season when rotavirus was most commonly found also happened to be the season when rota-adenovirus coinfection was most common. Our study set out to assess the IL-6 and IL-12 levels in Hillah city, Iraq, as potential disease-fighting mediators and effectors.

Materials and Methods

Blood Sample Collecting

Al-Muhawil, Al-Musayyab, Al-Hashimiyah, and Al-Kifl are the four districts of Babylon Governorate, Iraq, where this research was carried out. Fifty stool samples, chosen at random, that had already been tested for adenoviruses using commercially available EIA made up the study population. Children under the age of five who were admitted or presented to clinics or hospitals in any of the four states with acute diarrhoeal disease had their stool samples taken, along with 20 control samples that did not have diarrhoea. Parents reported cases of diarrhoea if their children passed loose, liquid, watery, or bloody stool three times in a 24-hour period.

- Ten millilitres of each subject's venous blood was carefully drawn into a sterile test tube and sealed with a screw cap. Two hundred units of heparin without preservatives (20 units/ml) were already present in this tube. Next, divide into the following parts:
- A 2.5 ml blood sample was utilised to isolate serum for the purpose of assessing levels of immunoglobulins and complement components.
- The morphological assessment for the lymphocyte transformation and phagocytosis assays was performed on 0.5 ml of blood using the stain technique.
- To conduct the lymphocyte transformation assay, which detects the presence of 3H* -thymidine, use either "separated lymphocyte" or "whole blood" in a 7-milliliter vial of blood.

Before transit, faeces samples were preserved at -20°C in clean, labelled screw-capped tubes in the Department of Microbiology. For each child, only one stool sample was taken. The specimens were brought to the Babylon Hospital for Women and Children in iceboxes, and before analysis, a balanced salt solution was used to prepare a 10% faecal suspension of each specimen. Adenovirus AdVAs were examined in recently collected stool samples by means of a commercially available immunochromatographic assay (AV-RV Combo test, Rapid Diagnostic Test). The scanning element of the test kit was a nitrocellulose membrane that had been coated with monoclonal antibodies against rotavirus and adenovirus. According to the manufacturer's recommendations, the test method is a quick and qualitative approach that relies on the formation of antigen-antibody complexes on the test region (T1-T2) of the test card. After a 10-15 minute incubation period [12-14], RVA and/or AdVA bind to the antibody-coated membrane, causing a visible pink line to appear. Inflammatory diarrhoea was diagnosed when there were 5 or more white blood cells per millilitre in stool samples.

Cytokines and Biochemical assays

Sandwich ELISA kits (R&D Systems, Minneapolis, MN, USA) were used to detect IL-6 and IL-10 levels in the blood.

Table 1. Reagents used for measurement of cytokine levels with the Luminex Flowmetrix system

Assay	Standard		Capture antibody			Detector antibody		
	Cytokine	Source	Clone	Type	Bead set	Clone	Type	Concn (g/ml)
4-Plex	IL-10	R&D Systems	23738.111	IgG2	8047	Polyclonal	Goat IgG	120
5-Plex	IL-6	Pharmingen	MQZ-13A5	IgG1	6964	MQZ-39C3	Rat IgG1	120

Statistical analysis

The chi-square test was performed to compare the two groups for age, gender, and seasonal distribution based on the categorical data provided by the rotavirus and AdVA tests. All analysis activities were carried out using IBM SPSS Statistics Version 20.0, a statistical software tool. All of the statistical tests were two-sided and had a significance level of 0.05.

Results and Discussion

Viral gastroenteritis in under-three-year-old children predominantly stems from Enteric Adenovirus infections while Rotavirus stands as the second leading viral trigger of this disorder. The survey showed 42.86 ± 5.00 and 57.14 ± 6.81 positive adenovirus antigens between male and female subjects (Figure 1). Distribution of characteristics by adenovirus antigen positivity according to Age group recorded 22.86±3.00, 42.86±5.03 and 34. ±4.19 in (0-12, 13-24, and 25-72 Months respectively) (Figure 2). In order to determine the best course of therapy and provide an accurate prognosis prediction, it is essential to identify the agents that cause gastroenteritis. One in three children die from acute gastroenteritis each year, with adenovirus infections accounting for the vast majority of these cases. In children with a severe clinical course of gastroenteritis, it is especially important to identify the agents that cause the disease so that the right treatment can be chosen and antibiotics are not used needlessly.

People of all ages are susceptible to developing acute gastroenteritis; however, the disease's intensity differs according to age and season as a result of several etiological causes. According to the agents that cause infectious gastroenteritis, viral infections are the most common, accounting for 30–70% of cases. In children less than five years old, the most common causes of gastroenteritis, according to studies, are rotavirus and enteric adenovirus [15-19]. Because of the severe complications it can bring, viral gastroenteritis is a leading cause of death and disability worldwide, particularly among children [20, 21]. Our results corroborated those from the literature regarding the prevalence of enteric adenovirus. In gastroenteritis, many agents can coexist. Although bacterial and viral cohabitation is possible, viral coexistence is the more prevalent occurrence.

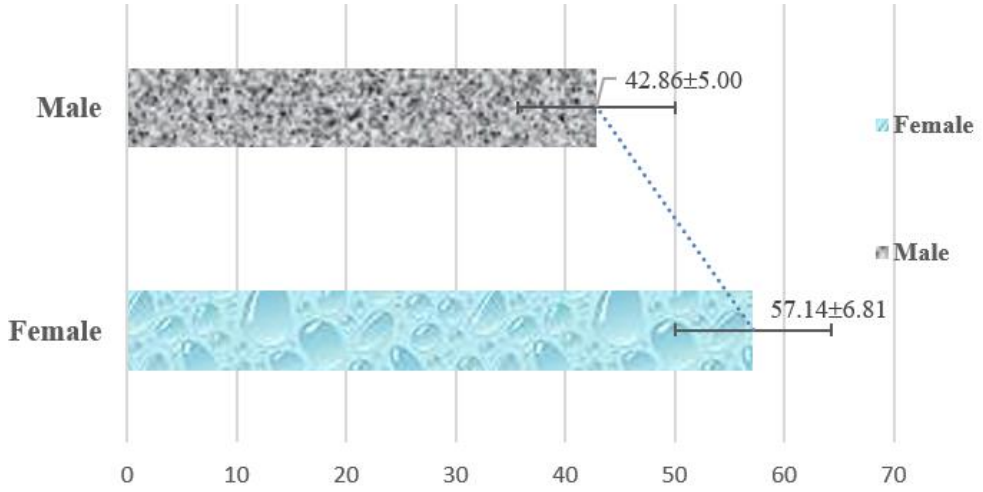

Figure 1. Distribution of characteristics by adenovirus antigen positivity according to gender

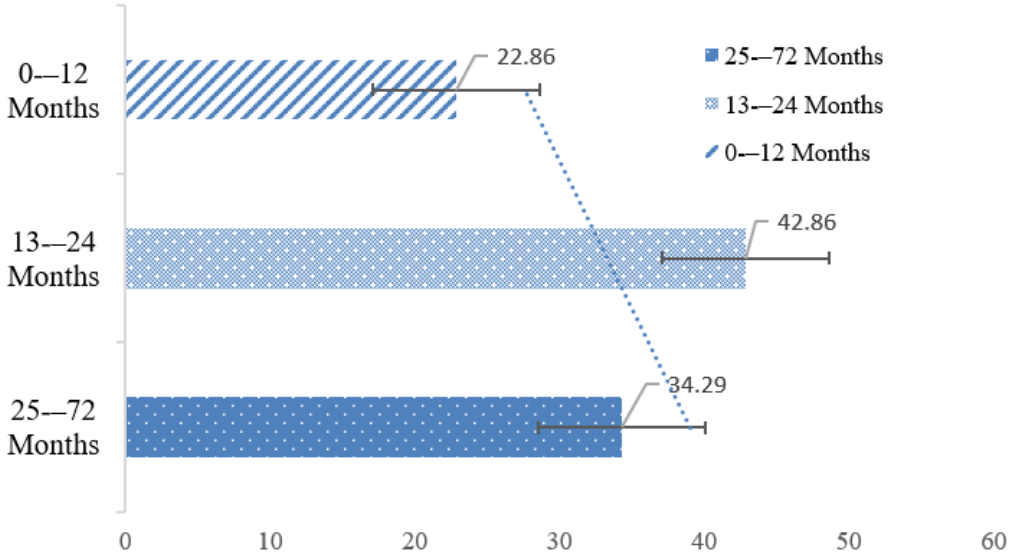

Figure 2. Distribution of characteristics by adenovirus antigen positivity according to Age group

The distribution of white blood cells was substantially lower (P<0.05) in the acute-phase serum group compared to the control group when stool microscopy examination was performed (5.65 ± 1.24) than those of control group (13.07 ± 2.25) Figure 3. Lymphocytes count findings which are displayed in Figure 4, showed that the count of lymphocytes were significantly less P<0.05 in Acute-phase (17.95±3.13) than those of Control group (29.81 ± 4.00). Neutrophils counts illustrated in Figure 5 showed a significant less P<0.05 in Acute-phase (45.01 ± 5.00) than those of Control group (38.00 ± 3.96). A decrease in white blood cell count can be caused by a number of different things, one of which being viral infections. As a haematological abnormality seen in liver illnesses, portal hypertension and hypersplenism can occasionally cause or worsen a mild leukopenia. The lowest neutrophil concentrations are attained when viral nucleic acid detection in PMNs, possible viral production in, or neutrophil phagocytic activity is detected. From asymptomatic infections to severe dehydration, viral gastroenteritis can cause a broad variety of clinical symptoms. In certain cases, doctors may not be able to tell the difference between gastroenteritis caused by bacteria and viruses. But whether it's a bacterial or viral cause, the treatment approach changes [21-27]. For instance, it is essential to administer the right antibiotics to reduce mortality and morbidity in cases of bacterial invasive gastroenteritis infections. Identifying bacterial agents is a typical practice when working with stool samples.

There is some disagreement about whether or not the presence of faecal leukocytes indicates intestinal inflammation in cases of infectious gastroenteritis. Based on the increased frequency of coinfections during times of high rotavirus prevalence, we hypothesise that rotavirus infections may have particular mechanisms that make secondary infections more likely. According to our analysis of the causative agent's seasonal variability, the spring was the most common time for rotavirus positivity, the autumn for adenovirus positivity, and the spring for rota-adenovirus co-infection [28-31]. We discovered that 17.5% of cases involved rotavirus, 6.5% involved adenovirus, and 2.1% involved a combination of the two viruses. More so than in the rotavirus or adenovirus alone groups, the co-infection group showed higher levels of inflammatory markers including C-reactive protein, absolute neutrophil count, and white

blood cell count [32-36]. In a related study, researchers discovered that faecal leukocyte detection rates in gastroenteritis were 19% for bacterial pathogens and 7.9% for viral agents. However, this difference was not deemed significant when it came to distinguishing between the aetiology of gastroenteritis caused by bacteria and viruses.

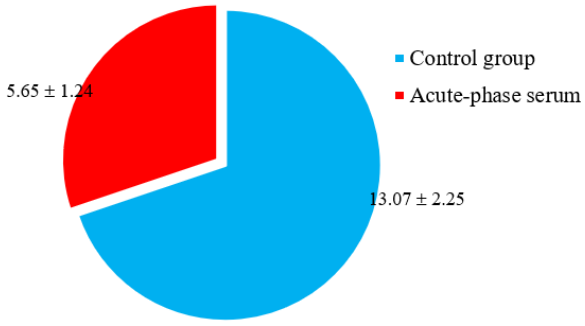

Figure 3. White Blood Cell count distribution in acute-phase patients and control group with stool microscopy evaluation

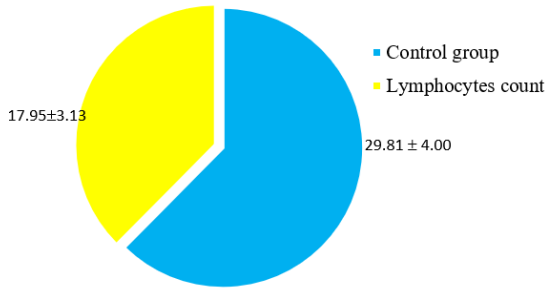

Figure 4. Lymphocytes count distribution in acute-phase patients and control group with stool microscopy evaluation

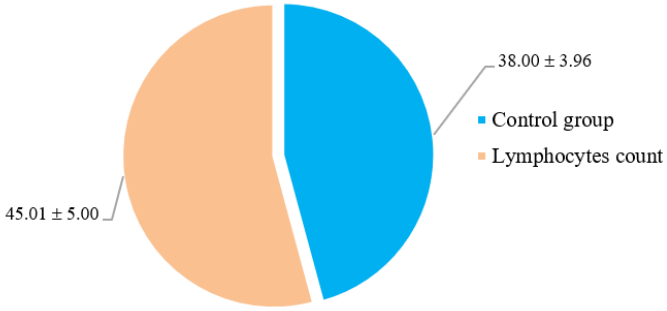

Figure 5. Neutrophils count distribution in acute-phase patients and control group with stool microscopy evaluation

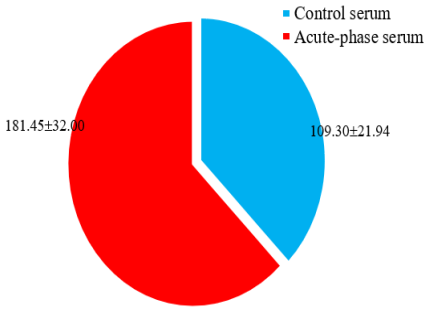

Figure 6. Concentrations of cytokine (IL-6) in sera of adenovirus-infected patients and control children

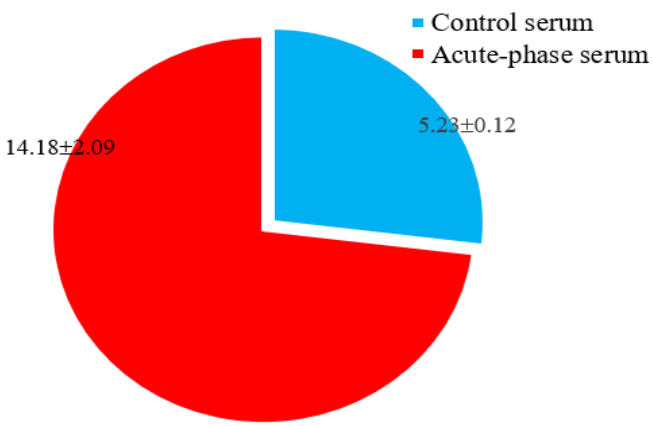

Figure 7. Concentrations of cytokine (IL-10) in sera of adenovirus-infected patients and control children

Next, we looked for correlations between the 35 patients' symptom scores and the cytokine levels (IL-6 and IL-10) in their acute-phase sera. Concentrations of cytokine (IL-6) in sera of adenovirus-infected patients and control children recorded 181.45±32.00 and 109.30±21.94 respectively Figure 6. Concentrations of cytokine (IL-10) in sera of adenovirus-infected patients and control children recorded 14.18±2.09 and 5.23±0.12 respectively Figure 7. An expanding body of evidence points to cytokines—a broad collection of tiny proteins with pro- and anti-inflammatory characteristics—as key players in the development and defence against viral illnesses. We measured cytokine concentrations in serum using a recently developed bead-based immunoassay to investigate cytokine patterns and possible functions in children with spontaneous adenovirus infections [37-41]. The cytokine levels in the sera of children with rotavirus diarrhoea can be easily determined using this test since it is quick, sensitive, and accurate. Traditional EIAs and bioassays measure the levels of a single cytokine and require twenty times more specimen volume than this assay, which is already attractive due to its ability to measure multiple cytokine levels in a single reaction using a substantially smaller volume of serum.

The cytokine response in children with acute rotavirus diarrhoea exceeded levels measured in control children. The acute phase sera contained significantly elevated levels of the IL-6 and IL-10 cytokines as well as IL-6 and IL-10. These data show that young children who contract natural adenovirus infection generate Th1 and Th2 cytokines that mainly produce IL-2 and IFN between the late stages of acute infection. Professor Detection determined that children with acute or persistent diarrhoea presented elevated serum levels of three cytokines in comparison to control children without adenovirus diarrhoea: IL-10, TNF and IFN.

Our research confirms that elevated TNF and IL-6 levels directly correlate with disease-induced fever manifestations during child adenovirus infection acute phase results. TNF and IL-6 serve as endogenous pyrogens because these multi-functional cytokines have both this capability. High blood levels of specific cytokines [42-45] can lead to triggering of both acute-phase protein response and hypothalamus inflammation with sensory nerve inflammation in addition to other mechanisms that result in fever development. During adaptive immunity's second wave of infection response IL-2 and IFN- release exclusively from antigen-activated T cells while macrophages and T cells and NK cells make the most IFN- and IL-2 respectively. Unfavorable relationships emerged between IL-6 and IL-12 and symptoms of diarrhea and vomiting on the first blood testing day which indicates these cytokines work against viruses to help patients recover from severe adenovirus infection and potentially safeguard against future infections. Past research has shown that healthy individuals' peripheral blood mononuclear cells release IFN in response to rotavirus stimulation, and that this secretion inhibits the entry of rotavirus into human intestinal epithelial cells. The results indicate that there is a clear correlation between certain symptom scores and increased levels of certain cytokines in individuals suffering from acute diarrhoea [46-49]. This study also found that children with adenovirus diarrhoea had significantly higher levels of interleukin-6 and interleukin-12 in their serum. Patients with infectious disorders face the potential benefit of IL-6's anti-inflammatory action as well as the serious risk of its relationship with mortality. This is in line with most cytokines, which have various effects on modulating immune responses and ion transport in the small intestine.

Nonetheless, IL-10 did not correlate with either immunity to rotavirus infection or the presence or intensity of diarrhoea, vomiting, or fever. As has been observed in both humans and animals with meningococcal or pneumococcal disease, it is unclear if IL-10 is linked to additional systemic symptoms or potentially deadly results. Infants' less severe vomiting and dehydration in the first few months of life show that there is a positive correlation between age and illness severity, while we did not find clear age-related changes in cytokine responses to rotavirus infection. Previous research has shown that younger infants had more severe diarrhoea, but our discovery that older children [50] have less stools than younger infants supports the opposite. Research comparing the reactions of younger and older children to cytokines and other host factors is necessary.

We have a number of caveats with this study. To begin, antibodies were generated against recombinant cytokines produced in insect cells or Escherichia coli, which served as the standard antigens in our Luminex bead-based assay. Although these antibodies can identify most isoforms of recombinant cytokines and attach to them with high affinity, it is unclear whether they bind to all isoforms of the natural cytokines. Therefore, it is possible that the levels of certain cytokines may be underestimated due to the use of these recombinant cytokines and the inclusion of non-labeled detection antibodies in the experiment.

Secondly, it is possible that our results were inaccurate because to the small sample size and the wide range of cytokine concentrations. In order to confirm links between cytokine levels and illness severity, investigate potential differences in cytokine profiles in children infected with different adenovirus serotypes, and ultimately find possible links between serotypes and disease severity, future research should involve larger numbers of age- and sex-matched control and patient populations [51, 52]. To further investigate the cytokine profiles' kinetics, a battery of serum samples taken at different times would be required, as the majority of cytokines are thought to have short half-lives. Although we attempted to collect serum samples from young children with adenovirus diarrhoea during the acute phase of infection, we were only successful in collecting a single specimen. Lastly, the serum cytokines found in our study have unclear sources. They may be released into the bloodstream or intestines by NK cells, lymphocytes, monocytes, or macrophages [53]. Additional research is required to analyse the cytokine profiles in small intestine lymphoid or epithelial cells from children with adenovirus diarrhoea, as the majority of cytokines work locally in a paracrine or even autocrine manner.

Conclusion

Finally, we found a number of cytokines that may mediate or act upon the manifestation of clinical symptoms or disease severity in children with diarrhoea after studying their cytokine responses to adenovirus. Our study should be refocused in light of these new results to investigate the role of host variables in disease beginning and progression, an underexplored possibility. Following recent reports for other virus infections, it is possible to use early cytokine profiles to forecast illness outcome or vaccine effectiveness in hosts infected or immunised against adenovirus. Lastly, novel vaccinations and treatments for childhood adenovirus illness may be possible thanks to these results.

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