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Research Article

Single Nucleotide Polymorphism in the IFNG rs1861494 Gene among a Subset of Iraqi Pediatric Patients with Gastroenteritis Co-infected with Cryptosporidium and Adenovirus

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Abstract

Background: Research identifies enteric adenoviruses as the third most common cause of infantile gastroenteritis, while Cryptosporidium causes parasitic gastroenteritis. Many studies have revealed the role of IFN-γ in inflammation and autoimmune diseases. ***Objective:*** To investigate the IFNG rs1861494 gene polymorphism among Iraqi pediatric patients with gastroenteritis co-infected with cryptosporidium and adenovirus. ***Method***: This case-control study enrolled 75 pediatric patients with severe gastroenteritis, whose ages ranged from 3–120 months and had a mean age of 30.64 months. The apparently healthy control (AHC) in this study included 25 pediatric individuals with a mean age of 27.64 months. We extracted DNA from stool specimens to further extract total genomic DNA, extract the human adenovirus (HADV) viral genome via PCR, and detect the IFNG rs1861494 polymorphism using the ARMS PCR technique. ***Results***: The male gender percentage in patients and AHC groups was 54%, while the female counterpart was 46%. The positive PCR result for HADV7 was 28%, whereas the positive result for Cryptosporidium was 6%. In the studied groups, the GG genotype increased at a rate OR=2.67 as compared to the AG and AA genotypes. HADV7 and Cryptosporidium showed a strong correlation with the SNP IFNG rs1861494 results in gastroenteritis (r=0.968, p=0.007 and r=0.984, *p*=0.008). ***Conclusions***: The identified enteric co-infection of *Cryptosporidium and* HADV-7, *as well as the IFNG* rs1861494 polymorphism, may shed light on possible pathogenic roles in gastroenteritis.

Keywords: Cryptosporidium, Gastroenteritis, HADV-7, IFNG rs1861494 Polymorphism, Pediatric patients.

تعدد أشكال النوكليوتيدات المفردة في جين IFNG rs1861494 بين مجموعة فرعية من مرضى الأطفال العراقيين المصابين بالتهاب المعدة والأمعاء المصابين بالكريبتوسبوريديوم والفيروس الغدي

الخلاصة

الخلفية: تحدد الأبحاث الفيروسات الغدية المعوية باعتبارها السبب الثالث الأكثر شيوعا لالتهاب المعدة والأمعاء عند الأطفال ، بينما تسبب الكريبتوسبوريديوم التهاب المعدة والأمعاء الطفيلي. كشفت العديد من الدراسات عن دور IFN-γ في الالتهابات وأمراض المناعة الذاتية. **الهدف**: التحقيق في تعدد الأشكال الجيني IFNG rs1861494 بين الأطفال العراقيين المصابين بالتهاب المعدة والأمعاء والمصابين بالكريبتوسبوريديوم والفيروس الغدي. **الطريقة**: سجلت دراسة الحالات والشواهد هذه 75 مريضا من الأطفال المصابين بالتهاب المعدة والأمعاء الحاد، تراوحت أعمارهم بين 3-120 شهرا وكان متوسط أعمارهم 30.64 شهرا. شملت السيطرة الصحية على ما يبدو في هذه الدراسة 25 فردا من الأطفال بمتوسط عمر 27.64 شهرا. قمنا باستخراج الحمض النووي من عينات البراز لمزيد من استخراج الحمض النووي الجينومي الكلي، واستخراج الجينوم الفيروسي للفيروس الغدي البشري (HADV) عبر تفاعل البوليميراز المتسلسل، والكشف عن تعدد الأشكال IFNG rs1861494 باستخدام تقنية ARMS PCR. **النتائج**: كانت نسبة الذكور في المرضى ومجموعات AHC 28%، بينما كانت نسبة الإناث 46٪. كانت نتيجة تفاعل البوليميراز المتسلسل الإيجابية ل HADV7 28%، بينما كانت النتيجة الإيجابية للكربتوسبوريديوم 6% في المجموعات المدروسة، زاد النمط الجيني GG بمعدل OR = 2.67 مقارنة بالأنماط الجينية AG و AA. أظهر HADV7 وكربتوسبوريديوم ارتباطا قويا مع نتائج SNP IFNG rs1861494 في التهاب المعدة و الأمعاء. **الاستنتاجات**: العدوى المعوية المشتركة المحددة للكربتوسبوريديوم و HADV7، بالإضافة إلى تعدد الأشكالIFNG rs1861494 ، قد تلقي الضوء على الأدوار المسببة للأمراض المحتملة في التهاب المعدة والأمعاء.

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INTRODUCTION

Human adenoviruses are recognized among the major causes of a variety of diseases, including acute gastroenteritis. Based on PCR and sequencing analysis techniques, more than 100 human adenoviral genotypes (HADV), which belong to the genus Mast adenovirus, have been classified into seven species (HADV-A to HADV-G) [1]. Among them, 40 and 41 serotypes of the HAdV F species are termed enteric adenoviruses since they are reported in young children as the most frequently occurring causes of HADV-associated gastroenteritis, reaching up to 20% in diarrhea cases [2]. However, other serotypes in the species of HADV-A, HADV-B, HADV-C, HADV-D and HADV-G are also recognized in sporadic diarrhea cases. However, most previous studies have failed to recruit healthy controls, making it difficult to definitively determine their role in causing diarrhea [3]. Generally, cryptosporidiosis is a self-limiting infection in immune-competent hosts, but increasing evidence indicates that human cryptosporidiosis may have distant consequences, including a potential link to cancer. Typically, cryptosporidiosis manifests as acute gastroenteritis, with both host and pathogen factors potentially influencing its severity. However, the contamination of public water supplies in developed countries frequently led to large outbreaks of Cryptosporidium infections [4]. *Cryptosporidium parvum*, in particular, causes enteric parasitic diseases worldwide, which are among the few intestinal parasites transmitted via fecal-oral and/or zoonotic routes. These diseases primarily affect children under the age of five, particularly those living in areas with poor sanitation, inadequate water supply, and poor hygiene practices [5]. Although waterborne transmission is well documented, neither the natural reservoir nor the exact route of infection are well known [4]. Molecular studies have demonstrated that at least 15 different species, most commonly human Cryptosporidium hominis species and *Cryptosporidium parvum*, infect a range of mammals, including humans [4,5]. Genome studies found 163 loci that were linked to IBD and had a lot in common with loci in genes that control the innate immune response, T-cell differentiation, and immune cell signaling [6]. Risk regions located upstream of chromosome 12 and downstream of IFNG play a major role in modulating intestinal inflammation and are associated with Crohn's disease (CD) and ulcerative colitis (UC) [7]. A small change (SNP) in the IFNG 3rd intron, rs1861494 T/C, has been found to be out of sync with severe refractory UC development [8]. Researchers found that people who had been exposed to the specific antigen of human cryptosporidial infections made more IFN-γ. IFN-γ-dependent responses are important for both innate and protective immune responses [8.9]. It has been suggested that exposing Cryptosporidium-infected cells to an outside source of IFN-γ and turning on TNF-α expression could stop *Cryptosporidium* from growing and, more importantly, help CD4+ lymphocytes get rid of the infection [10]. Moreover, CD8+ T-cells also respond to the gp15-specific antigen in cryptosporidial infection, and they likely increase IFN-production when stimulated by the cryptosporidium-infected cells [9]. It's also possible that antigen-sensitized CD8+ T cells could lower the number of parasites by destroying infected intestinal cells [11]. The goal of this study is to look into the IFNG rs1861494 gene polymorphism in a group of Iraqi children who have gastroenteritis and are also infected with cryptosporidium and adenovirus.

METHODS

Sample collection

This prospective case-control study enrolled 100 children, which included 75 pediatric gastroenteritis patients admitted to the Babylon Teaching Hospital for Pediatric and Gynecology and Al-Noor Pediatrics Hospital, and 25 apparently healthy children considered controls. This study was conducted from January 2022 to March 2022. Ethical approval for the study was issued by the Biology Department, College of Science and Al-Hilla Health Directorate, Al-Qasim Green University, Babylon, Iraq. A stool sample was collected in sterile screw-capped bottles from each participant and processed for detection of the adenovirus HADV-7 and the intestinal parasite Cryptosporidium. The detection of Cryptosporidium was done by microscopic examination of stool specimens as well as by the acid-fast staining technique. The presence of adenovirus HADV-7 in stool samples was determined by extracting the total DNA from the samples, followed by PCR amplification of a fragment of the viral genome using specific primers.

DNA extraction and PCR for adenovirus

The extraction of DNA from stool specimens was done according to protocol mentioned in the extraction kit procured from G-Spin company (Intron, Korea cat.no=14001). The DNA extracted was subjected to PCR amplification using specific HADV-7 Primers: F-5-AGTTCCAGCACTGCAATCG-3 and R-5-CACAAAAGCGTCGCTATCAA-3. Gel electrophoresis was carried out using 2% agarose at 75V, 20 mA for 120 min and stained with ethidium bromide.

Genotyping of IFNG SNP rs1861494

Genotyping of *IFNG* SNP rs1861494 was performed using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). The primers used in the specific amplification of each allelic variant were as follows: Allele A specific reverse 5′-AAGTAGGTGAGGAAGAAGCA-3′; Allele G specific reverse 5′-AAGTAGGTGAGGAAGAAGC A-3′; Common forward 5′-CCTTGGTGGCTGAGT TGG-3′. The ARMS-PCR was carried out in a total volume of 25 µl in premix PCR tubes to which template DNA (2 µl), Forward and reverse primers (1 µl) and distilled water were added. The amplified products were

separated by gel electrophoresis on 1.5% agarose gel and stained. The rs1861494 genotypes were assessed by the presence/absence the bands of size 272bp (G allele) and 163 bp (A allele).

Statistical analysis

To detect the significance between the studied variables, Chi–square test was applied, where all these statistical analyses were done by using the Version– 25 SPSS program where the p <0.05 value was considered for significant differences.

RESULTS

Table 1 shows the mean age of the pediatric patients with gastroenteritis (30.64±9.31 months) was more than that of the apparently healthy pediatric control subjects (AHPCS) (27.64±11.96 months).

Table 1: Distribution of pediatric patients with gastroenteritis and their apparently healthy pediatric control subjects according to the age

Group	n	Age (month)	Range (month)	*p*
Gastroenteritis patients	25	30.64±9.31	3-120	0.74
AHPCS	75	27.64±11.96	10-120	
Total	100			

There was a non-significant statistical difference (p=0.74) between gastroenteritis patients and apparently healthy pediatric control groups (AHPCS). The male gender in this study constituted 54% of the pediatric patients with gastroenteritis and AHPCS, while 46% were female patients with AHPCS (Figure 1).

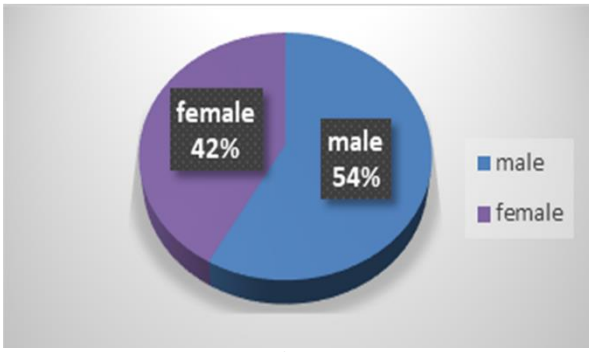

Figure 1: Gender Distribution of the Study Population.

The amplification detection of HADV 7 by PCR technique in samples from pediatric patients with gastroenteritis revealed 28% positive results (14 of 50 cases), while 72% (36 of 50 cases) had negative results, as shown in Table 2 and Figure 2. The age group of children with gastroenteritis most often infected with HADV7 was 3–36 months, making up 16% of the group (eight out of fifty patients). The next most common age group was 37–72 months, making up 8% (4 out of fifty patients), and the last most common age group was 73–120 months, making up 4% (2 out of fifty patients), and significant differences were detected in this regard (p< 0.05) (Table 3).

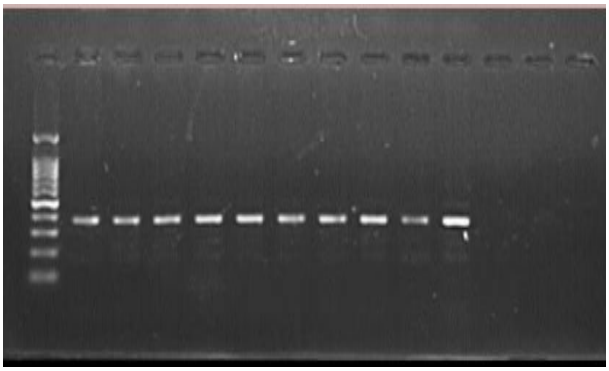

Figure 2: Detection of HADV7 gene using PCR; Showed band (380pb) molecular size in pediatric patients with gastroenteritis. M: DNA ladder 100-1100 bp. The PCR amplified products migrated into 2% agarose, 75V, 20 mA for 120 min; 15 µl in each well; stained with ethidium bromide.

Table 2: Percentage of PCR positive results for HADV7 infection in pediatric patients with gastroenteritis.

Viral genome	Value	*p*-value
Positive	14(28)	0.04
Negative	36(72)	
Total	50(100)	

Table 3: HADV 7-DNA PCR results of pediatric patients with gastroenteritis according to their age

Age (Months)	HADV7-DNA PCR Results			*p*
	Total	+ve	-ve	
3-36	37(74)	8(16)	29(58)	0.00
37-72	8(16)	4(8)	4(8)	
73-120	5(10)	2(4)	3(6)	
Total	50(100)	14(28)	36(72)	

Table 4 illustrates the results of HADV-7 DNA detection in pediatric patients with gastroenteritis according to their gender. Among the pediatric patients with gastroenteritis, 14.8% (8 out of 54 patients) were males and 13.04% (6 out of 46 patients) were females.

Table 4: Percentage of HADV 7 infection in pediatric patients with gastroenteritis in relation to the sex

Patients with gastroenteritis	Value	HADV7 Infection	
		+ve	-ve
Male	54(54)	8(14.8)	46(88.8)
Female	46(46)	6(13.04)	40(89.1)
p-value		0.07	

The PCR results of HADV-7 DNA detection in pediatric patients with gastroenteritis showed non-significant sex differences (p= 0.07). We sent stool samples for culture, searching for any other cause of diarrhea. The results showed that 8% (6 patients out of 75) had diarrhea due to cryptosporidiosis (Table 5).

Table 5: Percentage of *Cryptosporidiosis* infection- positive stool samples in pediatric patients with gastroenteritis by using PCR technique

Total *Cryptosporidium*	Stool from patients with gastroenteritis	*p*
Positive	6(8)	0.04
Negative	69(92)	
Total	75(100)	

Values were expressed as *n*(%).

Table 6 and Figure 3 both display the amplified IFNG rs1861494 target sequences in the studied pediatric gastroenteritis patients using the ARMS PCR technique. A test called ARMS PCR on the IFNG SNP rs1861494 showed three bands, one 163 bp (the G allele), one 109 bp (the A allele), and one 272 bp (the wild-type allele) in size (Figure 3).

Table 6: Genotype distribution and odd ratio of *IFNG* rs1861494 gene polymorphisms between the pediatric patients with gastroenteritis and AHPCS

Genotyping of *IFNG* rs1861494	Study Groups			*p*	OR	95% CI
	Gastroenteritis and +ve for HADV7 (n=28)	Gastroenteritis -ve for HADV7 (n=22)	AHPCS			
AA	8(28.5)	8(36)	9(36)	0.02	0.545	0.368-0.817
GG	13(46.5)	9(41)	8(32)	0.03	2.679	1.232-1.453
AG	7(25)	5(23)	8(32)	0.02	0.266	0.487-0.423
Total	28	22	25			

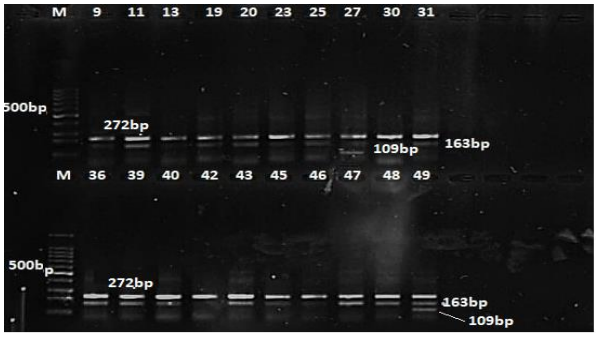

Figure 3: SNPs *IFNG* rs1861494 gene polymorphism by PCR; Agarose gel electrophoresis, 1% agarose gel electrophoresis, TBE 1X, at Voltage 75 volt for 60 min, lane M represent DNA marker size (100bps).

The frequency of the AG genotype was higher in patients with gastroenteritis who were positive for HADV7 and AHPCS groups (7 out of 28, 25%) and (8 out of 25, 32%), respectively. Compared to the AG and AA genotypes in the studied groups, the GG genotype increased at a rate of OR=2.67, whereas the AHPCS group (8 out of 28, 28.5%) and (9 out of 25, 36%) showed a decrease in the frequencies of the AA genotype in patients with gastroenteritis who were positive for HADV7. Patients showed a strong positive relationship (with a highly significant correlation) between HADV7 and SNP IFNG rs1861494 (r= 0.968, p= 0.007). Similarly, there is a strong positive relationship (with a highly significant correlation) between *Cryptosporidium* and SNP *IFNG* rs1861494 in gastroenteritis patients (r= 0.984, p= 0.008). Furthermore, the ages of patients with gastroenteritis revealed a strong positive relationship (with a highly significant correlation) between HADV7 and IFNG rs1861494 (r= 0.855, p= 0.001), (r= 0.788, p= 0.009), and (r= 0.739, p= 0.004), respectively. However, the age of patients with gastroenteritis revealed a non-significant relationship between *Cryptosporidium* and HADV7, as well as *Cryptosporidium* and IFNG rs1861494 (r= 0.945, p= 0.07) and (r= 0.732, p= 0.06), respectively. Finally, Table 7 revealed a non-significant relationship (r= 0.663, p= 0.08) between *Cryptosporidium* and sex of the patients. Pediatric patients with gastroenteritis who tested positive for HADV7 had a significantly higher frequency of GG genotypes (46.5%; 13 out of 28) compared to those in the AHPCS groups, which reached 32% (8 out of 25).

Table 7: Spearman's Rho statistical testing of age, gender, *Cryptosporidium*; HADV7 and SNPs *IFNG* rs1861494 to evaluate the studied markers in the studied group of infants and children with gastroenteritis

Spearman's rho		Age group (Months)	IFNG rs1861494	*Cryptosporidium*	HADV7
HADV7	*r*	0.855	0.986	0.947	
	p	0.001	0.007	0.07	
IFNG rs1861494	*r*	0.788		0.732	0.855
	p	0.009		0.06	0.001
*Cryptosporidiu*m	*r*		0.732		0.947
	p		0.06		0.07
Sex	*r*	0.166	-0.149	0.663	0.145
	p	0.249	0.477	0.08	0.034

DISCUSSION

It is not feasible to conduct controlled studies for sex-based differences in the pathophysiology of acute pediatric diarrhea in relation to transmissibility, infectivity, and immunity [12]. Researchers proposed that gender and age-related environmental exposures to clinical infections could explain the regional differences in disease incidence. Alternatively, cohort and case-control studies of socioeconomic and demographic data may further elucidate the role of the social determinants of disease [13]. In the current study, it was found that pediatric patients with severe gastroenteritis, whose ages ranged from 3-120 months, had a mean age of

30.64±9.31 months, while AHC had a mean age of 27.64±11.96 months. In this study, the male gender distribution was 54% for pediatric patients with gastroenteritis and AHPCS, while 46% were female patients and AHPCS. However, the prevalence issue of acute pediatric diarrheal illness will necessarily need additional data to further understand the ways that problem contributes to morbidity and mortality in association with gender. According to epidemiologic studies conducted in the USA by Esposito et al. and Khoury *et al*., diarrheal illness disproportionately affects boys, increasing their likelihood of hospitalization or death [12,14]. In recent years, both developing and developed countries have recognized infantile diarrhea as a major public health disease and ranked it as the third leading cause of death globally [15]. Many countries report that viral agents are the most common causes of pediatric diarrheal conditions. Many countries have reported that enteric adenovirus ranks second, after rotavirus, as the most important viral agent that causes pediatric diarrhea and leads to fatalities [2]. Among children less than two years of age with acute gastroenteritis, human adenoviruses, and specifically, types 40 and 41, are the most common causative agents [16]. Recently, other viruses recognized as playing a major role in sporadic cases of gastrointestinal diseases are noroviruses. However, the causative role of this virus is questionable due to its rare detection in fecal specimens of these illnesses [17]. Enteric HADVs have emerged as viral contaminants of human origin, transmitted via the fecal-oral route, and both contaminated water and food are possible vectors. Throughout the year, numerous reports have documented their presence in various types of water, demonstrating their increased tolerance to sewage treatment processes [18,19]. In the current study, we discovered adenovirus infection in 28% of the pediatric gastroenteritis patients. Similar investigations in Australia, Brazil, Indonesia, Saudi Arabia, Korea, Iran, the UK, Turkey, Hungary and Sweden reported enteric adenovirus detection rates among diarrheal patients to range from 1% to 96.3% [20]. In the study by Zaghloul *et al*. (2013), which examined 638 stool diarrheal samples of Tunisian children, the results revealed that rotaviruses, astroviruses, and adenoviruses types 40 and 41 were present in 30% of diarrheal specimens, and the frequency of adenovirus strains was 6% [21]. In 2018, another study on 9439 children in Africa and South Asia found that rotavirus and adenovirus 40/41 were the most common pathogens in those with moderate to severe diarrhea [2]. Zaghloul *et al*. (2013) found 28.33% of HADV in stool samples in their study. [21]. Moyo *et al*. (2014) reported higher rates of adenovirus in infants below one-year-old [22]. However, the clinical state of diarrhea in the examined children did not correlate with the adenoviral frequency, showing a similar prevalence [23]. In recent studies (during 2017 and 2019), acute diarrheal patients in China reported HADV prevalence ranging from 3.1% to 4.44% [1.24]. African countries such as Nigeria and Gabon (19.6%) reported HADV infections in pediatric diarrheal patients to be 19.3% and 19.6%, respectively [25]. However, it was reported that HADV-B or HADV-C had higher stool detection rates than HADV-F in pediatric patients with diarrhea, but it is unclear whether this was caused directly by HADV-B or HADV-C [15]. Different diagnostic techniques, varying economic levels, age differences in the studied children, and variations in the geographical regions of the studied areas may account for the reported differences in these frequencies across different studies. The limited number of previously reported studies hinders the comparison to the previous reports in children. Worldwide, cryptosporidiosis is underdiagnosed since most people probably did not have a proper consultation or laboratory testing for *Cryptosporidium* spp. [20]. Worldwide, the epidemiologically evidenced characteristics of previous cases showed transmission of the waterborne parasite during rainy seasons [26]. Chalmers et al. (2019) detected C. hominis in 64% of patients, indicating either direct human contact or water-borne epidemic transmission of these agents. Investigations of water supplies revealed enteropathogenic bacterial contamination in some samples, which was consistent with fecal contamination [27]. For the current study of Cryptosporidium, the positive result was 6% (6 of 75 cases). Although many practical limitations in this study did not reveal the origin of the *Cryptosporidium*, the current findings are in line with previous studies [28]. Researchers proposed that Cryptosporidium spp. infections increase the risk of severe infectious diseases in children. It was discovered that IFN-γ and other pro-inflammatory cytokines expressed in the mucosa are important for both starting and keeping inflammation going, as well as controlling how bad the CD is [28]. Some researchers, like Gonsky *et al*. (2014), looked into IBD and found that having the IFNG rs1861494 T allele made more IFN-γ and was linked to the disease getting worse and having more problems [6]. The current study found that the GG genotype increased at an OR of 2.67 as compared to the AG and AA genotypes in the studied groups. Conversely, patients with gastroenteritis who tested positive for HADV7 and AHPCS groups had an AA genotype frequency of 8 out of 28, 28.5%, and 9 out of 25, 36%, respectively, indicating a decrease when compared to the AHPCS group. The previous studies on tuberculosis revealed that rs1861494 T allele carriage is associated with susceptibility and a more severe form of the disease. They also exhibit resistance to anti-tuberculosis therapeutics and have a poorer prognosis. Likewise, in hepatic schistosomiasis, T allele carriage causes poor disease control and severe hepatic fibrosis [29]. T allele carriage in chronic myeloid leukemia is associated with a poor response to monoclonal antibody therapy. Cryptosporidium and HADV-7 were found to co-infect the intestines of these Iraqi children, along with the IFNG rs1861494 polymorphism. This may help us understand how these two viruses might cause gastroenteritis.

Conclusion

There is a relationship between the SNP rs1861494 polymorphism and adenovirus-induced diarrhea in pediatric patients in Iraq, and this polymorphism could potentially serve as a biomarker to assess the vulnerability of Iraqi children to contract this virus.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Lu L, Zhong H, Xu M, Su L, Cao L, Jia R, et al. Molecular and epidemiological characterization of human adenovirus and classic human astrovirus in children with acute diarrhea in Shanghai, 2017-2018. *BMC Infect Dis*. 2021;21(1):713. doi: 10.1186/s12879-021-06403-1.
2. Rajaiya J, Saha A, Zhou X, Chodosh J. Human adenovirus species D interactions with corneal stromal cells. *Viruses*. 2021;13(12):2505. doi: 10.3390/v13122505.
3. Carter BL, Chalmers RM, Davies AP. Health sequelae of human cryptosporidiosis in industrialised countries: a systematic review. *Parasit Vectors*. 2020;13(1):443. doi: 10.1186/s13071-020-04308-7.
4. Zahedi A, Paparini A, Jian F, Robertson I, Ryan U. Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management. *Int J Parasitol Parasites Wildl*. 2015;5(1):88-109. doi: 10.1016/j.ijppaw.2015.12.001.
5. Ntunzwenimana JC, Boucher G, Paquette J, Gosselin H, Alikashani A, Morin N, et al. Functional screen of inflammatory bowel disease genes reveals key epithelial functions. *Genome Med*. 2021;13(1):181. doi: 10.1186/s13073-021-00996-7.
6. Gonsky R, Deem RL, Landers CJ, Haritunians T, Yang S, Targan SR. IFNG rs1861494 polymorphism is associated with IBD disease severity and functional changes in both IFNG methylation and protein secretion. *Inflamm Bowel Dis*. 2014;20(10):1794-1801. doi: 10.1097/MIB.0000000000000172.
7. Haritunians T, Taylor KD, Targan SR, Dubinsky M, Ippoliti A, Kwon S, et al. Genetic predictors of medically refractory ulcerative colitis. *Inflamm Bowel Dis*. 2010;16(11):1830-1840. doi: 10.1002/ibd.21293.
8. Ludington JG, Ward HD. Systemic and mucosal immune responses to *Cryptosporidium*-vaccine development. *Curr Trop Med Rep*. 2015;2(3):171-180. doi: 10.1007/s40475-015-0054-y.
9. Kothavade RJ. Challenges in understanding the immunopathogenesis of Cryptosporidium infections in humans. *Eur J Clin Microbiol Infect Dis*. 2011;30(12):1461-1472. doi: 10.1007/s10096-011-1246-6.
10. Costa LB, JohnBull EA, Reeves JT, Sevilleja JE, Freire RS, Hoffman PS, et al. Cryptosporidium-malnutrition interactions: mucosal disruption, cytokines, and TLR signaling in a weaned murine model. *J Parasitol*. 2011;97(6):1113-1120. doi: 10.1645/GE-2848.1.
11. Jarman AF, Long SE, Robertson SE, Nasrin S, Alam NH, McGregor AJ, et al. Sex and Gender Differences in Acute Pediatric Diarrhea: A Secondary Analysis of the DHAKA Study. *J Epidemiol Glob Health*. 2018;8(1-2):42-47. doi: 10.2991/j.jegh.2018.08.102.
12. Esposito DH, Holman RC, Haberling DL, Tate JE, Podewils LJ, Glass RI, et al. Baseline estimates of diarrhea-associated mortality among United States children before rotavirus vaccine introduction. *Pediatr Infect Dis J*. 2011;30(11):942-947. doi: 10.1097/INF.0b013e3182254d19.
13. Flores AR, Szilagyi PG, Auinger P, Fisher SG. Estimated burden of rotavirus-associated diarrhea in ambulatory settings in the United States. *Pediatrics*. 2010;125(2):e191-198. doi: 10.1542/peds.2008-1262.
14. Khoury H, Ogilvie I, El Khoury AC, Duan Y, Goetghebeur MM. Burden of rotavirus gastroenteritis in the Middle Eastern and North African pediatric population. *BMC Infect Dis*. 2011;11:9. doi: 10.1186/1471-2334-11-9.
15. Kumthip K, Khamrin P, Ushijima H, Maneekarn N. Enteric and non-enteric adenoviruses associated with acute gastroenteritis in pediatric patients in Thailand, 2011 to 2017. *PLoS One*. 2019;14(8):e0220263. doi: 10.1371/journal.pone.0220263.
16. Pratte-Santos R, Miagostovich MP, Fumian TM, Maciel EL, Martins SA, Cassini ST, et al. High prevalence of enteric viruses associated with acute gastroenteritis in pediatric patients in a low-income area in Vitória, Southeastern Brazil. *J Med Virol*. 2019;91(5):744-750. doi: 10.1002/jmv.25392.
17. O'Brien E, Munir M, Marsh T, Heran M, Lesage G, Tarabara VV, et al. Diversity of DNA viruses in effluents of membrane bioreactors in Traverse City, MI (USA) and La Grande Motte (France). *Water Res*. 2017;111:338-345 doi: 10.1016/j.watres.2017.01.014.
18. Schlindwein AD, Rigotto C, Simões CMO, Barardi CRM. Detection of enteric viruses in sewage sludge and treated wastewater effluent. *Water Sci Technol*. 2010;61(2):537-544. doi: 10.2166/wst.2010.845.
19. Sanaei Dashti A, Ghahremani P, Hashempoor T, Karimi A. Molecular epidemiology of enteric adenovirus gastroenteritis in under-five-year-old children in Iran. *Gastroenterol Res Pract*. 2016;2016:2045697. doi: 10.1155/2016/2045697.
20. Fodha I, Chouikha A, Peenze I, De Beer M, Dewar J, Geyer A, et al. Identification of viral agents causing diarrhea among children in the Eastern Center of Tunisia. *J Med Virol*. 2006;78(9):1198-203. doi: 10.1002/jmv.20681.
21. Zaghloul MZ, El-Sahn SF, Galal ZA. Confection of rotavirus group A, norovirus and adenovirus in Egyptian children with gastroenteritis. *Life Sci J*. 2013;10(2):848-852.
22. Moyo SJ, Hanevik K, Blomberg B, Kommedal O, Nordbø SA, Maselle S, et al. Prevalence and molecular characterisation of human adenovirus in diarrhoeic children in Tanzania; a case control study. *BMC Infect Dis*. 2014;14:666. doi: 10.1186/s12879-014-0666-1.
23. Li W, Xiang W, Li C, Xu J, Zhou D, Shang S. Molecular epidemiology of rotavirus A and adenovirus among children with acute diarrhea in Hangzhou, China. *Gut Pathog*. 2020;12:19. doi: 10.1186/s13099-020-00359-4.
24. Imade PE, Eghafona NO. Viral agents of diarrhea in young children in two primary health centers in Edo State, Nigeria. *Int J Microbiol*. 2015;2015:685821. doi: 10.1155/2015/685821.
25. Gelaw A, Pietsch C, Liebert UG. Genetic diversity of human adenovirus and human astrovirus in children with acute gastroenteritis in Northwest Ethiopia. Arch Virol. 2019;164(12):2985-2993. doi: 10.1007/s00705-019-04421-8.
26. Xiao L, Feng Y. Molecular epidemiologic tools for waterborne pathogens Cryptosporidium spp. and Giardia duodenalis. *Food Waterborne Parasitol*. (2017);8:14-32. doi: 10.1016/j.fawpar.2017.09.002.
27. Lebbad M, Winiecka-Krusnell J, Stensvold CR, Beser J. High diversity of *Cryptosporidium* species and subtypes identified in cryptosporidiosis acquired in Sweden and abroad. *Pathogens*. 2021 ;10(5):523. doi: 10.3390/pathogens10050523.
28. Naghavi M, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1151-1210. doi: 10.1016/S0140-6736(17)32152-9.
29. Peresi E, Oliveira LR, da Silva WL, da Costa EA, Araujo JP, Ayres JA, et al. Cytokine polymorphisms, their influence and levels in Brazilian patients with pulmonary tuberculosis during antituberculosis treatment. *Tuberc Res Treat*. 2013;2013:285094. doi: 10.1155/2013/285094.