Research article
SNP IFNG rs1861494 gene polymorphism in pediatric patients with gastroenteritis and its relation to co-infections with Adenovirus and Cryptosporidium

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ABSTRACT

Introduction and Aim: The most common cause of infantile gastroenteritis is enteric adenoviruses, while Cryptosporidiosis is caused by Cryptosporidium. The function of IFN-gamma (IFNG/IFN-γ) in immunological responses, inflammation, and autoimmune disease is increasingly recognized. This study aimed to examine intestinal co-infections of Cryptosporidium and adenovirus in pediatrics and the role of IFNG rs1861494 polymorphism to gastroenteritis among Iraqi children.

Materials and Methods: This case control study included 75 pediatric patients with severe gastroenteritis, aged 3-120 months, with a mean age of (30.64±9.31 months). The apparently healthy control (AHC) in this study included 25 pediatric individuals with a mean age of 27.64±11.96 months. Total genomic DNA was extracted from stool specimens were subjected to PCR detection of human adenovirus virus. SNP IFNG rs1861494 polymorphism was detected using the ARMS-PCR technique.

Results: The percentage of males to female ratio in this study was 54:46. The positive PCR result of HADV7 was 28% (14 of 50 cases) while the positive result of Cryptosporidium was 6% (6 of 75 cases). Genotyping studies showed the GG genotype increased at rate OR=2.67 as compared to AG and AA genotypes for the SNP IFNG rs1861494. The SNP IFNG rs1861494 results in gastroenteritis were highly correlated with HADV7 and Cryptosporidium (respectively: r = 0.968, P = 0.007 as well as r = 0.984, P = 0.008).

Conclusions: Adenovirus diarrhea in Iraqi pediatric patients is associated with SNP rs1861494 polymorphism and could possibly be used as a biomarker in assessing the susceptibility of Iraqi children to the virus.

Keywords: Cryptosporidium; HADV-7; IFNG rs1861494 polymorphism; gastroenteritis; paediatric patients.

INTRODUCTION

Human adenoviruses are recognized as a major cause of acute gastroenteritis. The genus Mast adenovirus contains more than 100 human adenoviral genotypes (HAdV), which are further classified into seven species (HAdV-A to HAdV-G) (1). Of these, HAdV F serotypes 40 and 41 are known as enteric adenoviruses because they are the most common cause of HAdV-associated gastroenteritis in young children, accounting for up to 20% of cases of diarrhea (2). Sporadic cases of diarrhea have also been linked to other serotypes in the HAdV-A, HAdV-B, HAdV-C, HAdV-D, and HAdV-G species. However, earlier studies were unable to draw a definitive conclusion regarding their role in causing diarrhea due to a lack of recruitment of healthy controls (3).

Cryptosporidiosis is typically a self-limiting infection in immune-competent hosts with a growing body of research indicating that human cryptosporidiosis may have distant effects, including a potential link to cancer. Typical cryptosporidiosis presents as an acute gastroenteritis and its severity may be influenced both by host as well as pathogen factors. Large-scale Cryptosporidium infection outbreaks, however, have been documented frequently in developed nations following public water supply contamination (4). Cryptosporidium parvum, are rare intestinal parasites spread through fecal–oral and/or zoonotic routes and known to primarily affect children under five, especially those who reside in areas with inadequate water supplies, poor sanitation, and poor hygiene practices (5). Cryptosporidium hominis and Cryptosporidium parvum infect mammals, including humans (4, 5). Despite extensive documentation of waterborne transmission, the precise route of infection and its natural reservoir remain unknown (4). The Interferon gamma (IFNG) a cytokine produced by natural killer cells and Th1 lymphocytes is considered an important modulator in the intestinal inflammation association with Crohn’s disease and ulcerative colitis (6). A SNP within the IFNG 3rd intron (rs1861494 T/C), has been residing in linkage disequilibrium in correlation with severe refractory ulcerative colitis development (7). Studies showed an increased amount of IFN-γ in response to prior exposure to the specific antigen of human

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cryptosporidial infections, where IFN-γ-dependent responses are important for both immune responses (innate and protective; 7, 8). It has been proposed that the exposure of Cryptosporidium-infected cells to an exogenous IFN-γ (a TNF-α expression activator) could inhibit Cryptosporidium growth (9). Moreover, CD8+ T-cells also play a respective role in response to gp15- specific antigen in cryptosporidial infection, where CD8+ T-cells probably also increase the IFN-γ production as stimulated by the cryptosporidium-infected cells (8). Moreover, antigen- sensitized CD8+ T cells may act through cytolysis to reduce the parasite load by potential lysing the infected intestinal cells (10). This study, the first of its kind, aimed to determine the co-occurrence of Cryptosporidium and human adenovirus infections in a cohort of pediatric gastroenteritis patients from Iraq. Additionally, it investigated the relationship between the IFNG SNP rs1861494 polymorphism and gastroenteritis caused by these organisms.

MATERIALS AND METHODS

Sample collection

This prospective case-control study enrolled 100 children, that 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 as 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.

Stool samples were collected in sterile screw capped bottles from each participant and processed for detection of adenovirus HADV-7 and the intestinal parasite Cryptosporidium. Detection of Cryptosporidium was done by microscopic examination of stool specimens as well as by acid-fast staining technique. The presence of adenovirus HADV 7 in stool sample was done by extracting the total DNA in 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′-CACAAAAAGCCTGCTATCAA-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′-AAATAGTGAGGAAGAACTGCA-3′; Allele G specific reverse 5′-AAATAGTGAGGAAGAACGCA-3′; Common forward 5′-CTTCTGCTGCTGAGTGG3′. 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 of the bands of size 272bp (G allele) and 163 bp (A allele).

RESULTS

The age of pediatric gastroenteritis patients (n=75) included in this study ranged between 3-120 months (Mean 30.64±9.31months), while that of apparently healthy pediatric controls was 10-120 months (mean 27.64±11.96 months). No significant statistical difference (p=0.74, P>0.05) for age was seen among gastroenteritis patients and the healthy control group included in this study. Similarly, gender-wise no significant difference was seen between male and female children included in this study.

Detection of adenovirus

The PCR amplification of a 380 bp fragment of the HADV7 gene was considered positive for the presence of adenovirus (Fig.1). Among the 50 samples from pediatric patients with gastroenteritis tested, 28% were found positive for the virus.

Fig. 1: PCR detection of HADV7 gene M: DNA ladder 100-1100 bp. Lanes 1-10: Show samples positive for the presence of HADV7 gene band (380bp).

The 50 children with gastroenteritis were broadly divided into three groups based on their age range as shown in Table 1. As seen, the prevalence of the virus was highest in the age group of 3-36 months (8%), followed by 37-72 months (4%) and 73-120 months (2%). Similarly, gender-wise no significant difference
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(p>0.05) was for adenovirus infection among male and female children.

Table 1. Age-wise association of adenovirus infection in pediatric patients with gastroenteritis

<table>
<thead>
<tr>
<th>Age</th>
<th>No. (%)</th>
<th>Positive</th>
<th>Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-36</td>
<td>37 (74%)</td>
<td>8 (16%)</td>
<td>29 (58%)</td>
<td>Anova test</td>
</tr>
<tr>
<td>37-72</td>
<td>8 (16%)</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
<td>P=0.00 Significant</td>
</tr>
<tr>
<td>73-120</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td>(P&lt;0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100%)</td>
<td>14 (28%)</td>
<td>36 (72%)</td>
<td></td>
</tr>
</tbody>
</table>

Detection of Cryptosporidium

Stool samples of pediatric patients with gastroenteritis were checked for the presence of Cryptosporidium. Results indicated that 8% (6 out of 75) of the pediatric patients had diarrhea due to Cryptosporidiosis infection.

Detection of IFNG rs1861494 SNPs by ARMS PCR

ARMS PCR for the IFNG SNP rs1861494 revealed the presence of three bands of sizes 163bp (G allele), 109bp (A allele) and 272 bp (wild type allele) (Fig.2).

The GG genotype frequency was found to be significantly higher (46.5%) in pediatric gastroenteritis patients positive for the adenovirus HADV7 gene in relation to healthy pediatric children (Table 2). The genotypes AA and AG were present in lower frequencies and comparable to the genotype frequencies observed in healthy controls (Table 2).

The Odds ratio (OR) was calculated to find the association between the IFNG rs1861494 genotypes and adenovirus infection. Comparison of the GG genotype against AA yielded an OR value of 2.679 (95% CI=1.23-1.45). Similarly, the OR calculated for the AA and AG genotypes were 0.545 (95% CI=0.368-0.817) and 0.266 (95% CI=0.423-0.487) respectively (Table 2).

Fig 2: SNPs IFNG rs1861494 gene polymorphism by PCR; Lane M represent DNA marker size (100bps)

Table 2: Genotype frequency and odds ratio of Interferon gamma IFNG SNP rs1861494 in pediatric gastroenteritis patients and healthy controls

<table>
<thead>
<tr>
<th>IFNG SNP rs1861494 genotypes</th>
<th>Study Groups</th>
<th>P value</th>
<th>Odds Ratio (OR)</th>
<th>95% C.I for OR Lower</th>
<th>95% C.I for OR Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastroenteritis and positive for HADV7 gene (n=28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gastroenteritis negative for HADV7 gene (n=22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Healthy controls (n=25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA n (%)</td>
<td>8 (28.5%)</td>
<td>8 (36%)</td>
<td>9 (36%)</td>
<td>0.02</td>
<td>0.545</td>
</tr>
<tr>
<td>GG n (%)</td>
<td>13 (46.5%)</td>
<td>9 (41%)</td>
<td>8 (32%)</td>
<td>0.03</td>
<td>2.679</td>
</tr>
<tr>
<td>AG n (%)</td>
<td>7 (25%)</td>
<td>5 (23%)</td>
<td>8 (32%)</td>
<td>0.02</td>
<td>0.266</td>
</tr>
</tbody>
</table>

Table 3: Association between the IFNG SNP rs1861494 to adenovirus and Cryptosporidium infections in context to age and gender

<table>
<thead>
<tr>
<th>Spearman's rho</th>
<th>Age group months</th>
<th>IFNG rs1861494</th>
<th>Cryptosporidium</th>
<th>Adenovirus HADV7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADV7 gene</td>
<td>R 0.855**</td>
<td>0.986**</td>
<td>0.947</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.007</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>IFNG rs1861494</td>
<td>R 0.788**</td>
<td>0.732</td>
<td>0.855**</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.009</td>
<td>0.06</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td></td>
<td>0.732</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06</td>
<td>0.947</td>
<td></td>
</tr>
</tbody>
</table>

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A strong positive relationship (with highly significant correlation) was found between presence of HADV7 gene and SNP IFNG rs1861494 in patients (r = 0.986, P = 0.007). Similarly, there was a high significant correlation between Cryptosporidium and SNP IFNG rs1861494 in gastroenteritis patients (r = 0.788, P = 0.009). Additionally, HADV7 and IFNG SNP rs1861494 were found to have a high significant positive correlation to the ages of patients with gastroenteritis (r=0.855, P=0.001) and (r=0.788, P=0.009), respectively. However, non-significant relationship was found between Cryptosporidium and HADV7 gene (r=0.947, P=0.07) as well as Cryptosporidium and IFNG rs1861494 in patients with gastroenteritis (r=0.732, P= 0.06). A non-significant (r=0.663, P=0.08) association was observed for gender and Cryptosporidium infection (Table 3).

**DISCUSSION**

In the past few years, infantile diarrhea has been identified as a serious public health concern worldwide, accounting for the third highest death toll among both developed and developing nations (11). Viral agents are reported to be the most common cause of pediatric diarrheal conditions (12). Enteric adenovirus is thought to be the most significant virus among these, ranking second only to rotavirus in terms of its association with pediatric diarrhea-related deaths (2). Human adenoviruses, more especially types 40 and 41, are the most common cause of acute gastroenteritis in children under two years old (13). In the current study, we found that 28% of the pediatric gastroenteritis patients to be infected by adenovirus. Similar investigations in Australia, Brazil, Indonesia, Saudi Arabia, Korea, Iran, UK, Turkey, Hungary and Sweden, reported enteric adenovirus detection rates among diarrheal patients to range from 1% to 96.3% of (14). Zaghloul et al., (15), examined 638 stool diarrheal samples of Tunisian children, and reported that rotaviruses, astroviruses, and adenoviruses types 40/41 to be present in 30% of diarrheal specimens and the frequency of adenovirus strains to be 6%. 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). In the study done by Zaghloul et al., (15) found the percentage of HADV in stool samples was 28.33%. Moyo et al., (16) reported higher rates of adenovirus in those under one year of infants. However, the adenoviral frequency was not related to the clinical state of presence of diarrhea in the examined children (17). The reported HADV prevalence in recent studies (during 2017 and 2019) in acute diarrheal patients in China ranged from 3.1%-4.44% (18). In pediatric diarrheal patients in African countries such as Nigeria and Gabon (19.6%), HADV infections were reported to be (19.3%) and (19.6%), respectively (19, 20). However, it reportedly had a higher stool detection rate of HADV-B or HADV-C than that of HADV-F in pediatric patients with diarrhea but still un-explained whether directly caused by either HADV-B or HADV-C (12). The reported differences in these frequencies in different studies may be attributed to the difference in the diagnostic techniques used for their detections, different economic levels, age differences in the studied children, and the differences related to the geographical regions of the studied areas. The limited number of previously reporting studies obstacles the comparison to the previous reports in children.

Worldwide, cryptosporidiosis is underdiagnosed, since probably did not have a proper consultation or laboratory testing for Cryptosporidium spp (14). Worldwide, the epidemiologically evidenced characteristics of previous cases showed transmission of this waterborne parasite during rainy seasons (21). In the study by Chalmers et al., (22) C. hominis was detected in 64% of patients, suggesting direct human contacts or water-borne epidemic transmission of these agents. The positive result for the current study of Cryptosporidium was 6%. This assumes significance as infections by Cryptosporidium spp. represent an increased risk in children to contact other severe infections. The mucosal expression of IFN-γ and other pro-inflammatory cytokines found to be critical both to the development and maintenance of inflammation, and to modulate cryptosporidiosis disease severity (23). A study by Peresi et al., (24) on IBD found that IFNG rs1861494 T allele carriage in patients with IBD enhanced the secretion of IFN-γ and was associated with severe disease progression and complications (25). Genotyping studies for IFNG SNP rs1861494 in this study showed the prevalence of GG genotype to be higher compared to AG and AA genotypes. Further, the GG genotypes were found associated with adenovirus infection in pediatric children with diarrhea as compared to the high frequency of AA genotypes prevalent in normal healthy children of the same age. Previous studies on tuberculosis revealed that rs1861494 T allele carriage is associated with susceptibility and a more severe form of the disease (26). Likewise, in hepatic schistosomiasis, T allele carriage has poor control of disease and severe hepatic fibrosis (25). Also in chronic myeloid leukemia, T allele carriage was linked to poor response to monoclonal antibody therapy. Hence, the identified enteric co-infection of adenovirus and Cryptosporidium, as well as IFNG rs1861494 polymorphism among Iraqi pediatric patients may throw light for possible pathogenic roles in the gastroenteritis which requires further studies.

**CONCLUSION**

<table>
<thead>
<tr>
<th>Gender</th>
<th>R</th>
<th>-0.149</th>
<th>0.663</th>
<th>0.145</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.166</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.249</td>
<td>0.477</td>
<td>0.08</td>
<td>0.034</td>
</tr>
</tbody>
</table>

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Our study indicates a link to exist between the SNP rs1861494 polymorphism and adenovirus-induced diarrhea in pediatric children from Iraq. This polymorphism observed could probably be used as a biomarker for assessing the susceptibility of Iraqi children to be infected by this virus.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES