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Enteric Viruses Co-infection with Giardiasis among Diarrheal Children in Diyala Province - Iraq

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Mixed infections of enteric viruses and parasite can infected the human alimentary canal and quite complex physiological changes can result from such infection. To study the association between enteric viruses and giardiasis among diarrheal children as well as to clearly the relationship with *Giardia lamblia* genotypes in Diyala province, Iraq. Descriptive cross sectional study was conducted in Al-Batool Teaching Hospital for Maternity and Children in Baqubah city, during the period from June 2017 till November 2017. One hundred and sixty stool samples were collected from children with gastroenteritis using a clean and dry container. Samples, screened for rotavirus, adenovirus, astrovirus and norovirus using Cer Test Biotec, and *G. lamblia* by ELISA kits.The positive *G. lamblia* samples were further analyzed by nested polymerase chain reaction. Rotavirus was detected in 20%, adenovirus in 18.75% and norovirus in 13.75% while no positive result with astrovirus. *G. lamblia* were detected in 42 cases (26.25%), amplification triose phosphate isomerase (tpi) gene was successful in 28/42 (66.66%) samples, 8 (28.57%) contained genotype A and 20 (71.43%) samples contained genotype B. The majority of infected children were males less than five years old. Rotavirus infection rate amongst children in Diyala appears to be relatively higher than other enteric viruses. Co-infection with *G. lamblia* type B have important role.

Keywords: Gastroenteritis, enteric viruses, *Giardia lamblia,* Chromatographic immunoassay.

Gastroenteritis, which is also well-known as 'infectious diarrhea', is the inflammation that affect the stomach and small intestine of the digestive system[1]. Its signs and symptoms are a combination of diarrhea, vomiting, and abdominal pain[2]. Furthermore, fever, lack of energy, and dehydration could be occur in such cases[3]. By the end of 2015, there were about 1.3 million cases of gastroenteritis out of two billion ones who were reported to be dead[4]. Children and those in the developing counties are most commonly affected[5]. Risk of infection is higher in children due to their lack of immunity and relatively poor hygiene[2]. The disease is less common in adults, partly due to the development of immunity[6].

Many microorganisms can cause gastroenteritis, such as: bacteria, parasites, fungus and viruses. However, viral gastroenteritis is regarded the most common one[7]. Rotavirus, norovirus, adenovirus, and astrovirus are known to cause viral gastroenteritis[6]. Rotavirus is the most common cause of gastroenteritis in children[8], and produces similar rates in both the developed and developing world[9]. Many studies has concluded that viruses could be estimated as nearly 70% as the main reason behind the incidents of infectious diarrhea in the pediatric age of human beings[10].

Likewise, gastroenteritis may be caused by a number of protozoa; most commonly *G. lamblia*.

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However, *Entamoeba histolytica*, *Cryptosporidium spp*. and other species have been additionally referred to in such related studies[10]. *G. lamblia* is the most common pathogenic intestinal parasite of humans worldwide and is a frequent cause of endemic and epidemic diarrhea. *G. lamblia* is divided into eight genotypes (A-H) which infect a wide range of mammals and humans, but human infections are caused by genotypes A and B[11].

There is some evidence that clinical signs associated with assemblage A depend on the *tpi* gene target, Sahagun *et al*., demonstrated an association for *G. lamblia* assemblage A with gastroenteritis in children less than 5 years old[12]. Another study found the same correlation in children after assemblage typing analysis of the SSU-rRNA[13]. Whereas, others found a strong relationship with assemblage B[14],[15]. Furthermore, one study showed that assemblage B exhibited more extensive association with persistent symptoms, while assemblage A was found in connection with intermittent diarrhea[16]. Most of the studies used one or two marker, and it is noticeable that the *tpi* gene was more often found to be associated with symptomatic assemblage A patients than the *gdh* and ²-giardin[13].

Very few studies have been performed in Diyala province to determine the enteric pathogens and co-infection. To the best of our knowledge, there is no study about relationship between enteric viruses and *G. lamblia* genotypes. Hence, the current study was designed to assess this points.

MATERIALS AND METHODS

Study design

This study was designed as descriptive cross sectional study and conducted in Al-Batool Teaching Hospital for Maternity and Children in Diyala province, Iraq during the period from July 2017 to November 2017.

Samples collection and processing

One hundred and sixty stool samples were collected from children with gastroenteritis using a clean and dry container. Age, gender and other demographic data were collected by direct interview with children's parents.

Chromatographic immunoassay

Cer Test Rota-Adeno-Astro-Noro one step combo card test is a colored chromatographic immunoassay (Cer Test Biotic S.L, Zaragoza-Spain)[17]. It was used for the simultaneous qualitative detection of rotavirus, adenovirus, astrovirus and norovirus. This test is highly sensitive and specific to make a presumptive diagnosis of these viruses.

Enzyme immunoassay for the qualitative assessment of *G. lamblia* in fecal samples was done according to the (RIDASCREEN® Giardia test).

Extraction of *G. lamblia* DNA from Stool

The DNA extraction was performed by using AccuPrep® Genomic DNA extraction Kit (Cat No. K-3032-Korea) for stool according to the manufactures' instructions. The extracted DNA was measured by Nano Drop 1000 spectrophotometer instrument and the purity was estimated with the OD260nm/OD280nm ratio, a ratio of 1.8 was generally accepted as pure for DNA. *G. lamblia* genotypes A identified by semi nested PCR through amplifying the *tpi* gene, for the first round of PCR, a PCR product of 605 bp was amplified by using primer set forward primer AL3543 and reverse primer AL3546 [18]. PCR mixture was prepared in 20 ¼l volume with 2 µl of extracted DNA in PCR master Mix (250 ¼M each of deoxynucleoside triphosphate (dNTP), {dATP, dTTP, dCTP, dGTP }, 1 U of Taq polymerase, 30 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl, tracking dye and stabilizer), 1 ¼l of each of forward and reverser primer, 16 ¼l distilled water. The PCR conditions were as follows: - denaturation step: -95°C for 5 minutes 35 cycles: -94°C for 45 second -50°C for 45 second -72°C for 60 second -Final extension step: -72°C for 10 minutes. The second round of PCR was prepared as separate reactions for A [19] and B [20] genotype. Genotypes specific primers presence of mixed infection was detected by visualizing the occurrence of bands in the agarose gel 1.5%, at 332 bp for assemblage A amplified using primer sets forward primer AssAF and reverse primer AssAR and at 400 bp for genotype B, PCR amplification mixture was prepared in 20 ¼l final volume with 10 ¼l of the first round of PCR as a template DNA in master Mix (250¼M each of deoxynucleoside triphosphate (dNTP), {dATP, dTTP, dCTP, dGTP }, 1 U of Taq polymerase, 30 mM KCl, 1.5mM MgCl2, 10 mM Tris-HCl, tracking dye and stabilizer), 1 ¼l of each primer, 16 ¼l distilled water. The PCR conditions were as follows: - denaturation step: -94°C for 10

minutes -35 cycles: -94°C for 45 second -64°C for 45 second -72°C for 60 second -Final extension step: -72°C for 10 minutes. The PCR products were reorganized by electrophoresis in 1.5% agarose gel stained with 0.5 mg/ml ethidium bromide.

Data analysis

The Chi-square-X^2 test and Fisher exact test were used to find out of different factors in study criteria; bellow or equal to (p d"0.05) was accepted as statistical significant difference.

RESULTS

The mean age of the 160 children infected with diarrhea was 4.53 year, range from (2 month- 15 year). The majority of diarrhea cases 108(67.50%) were observed in age group d"1-5 years than other groups; 52.50% were male and 47.50% were female, the result showed there was no gender significant preference to get the diarrhea as shown in (table 1).

Regarding enteric viruses infection, Cer Test one step detect 32 cases positive for rotavirus, 30 cases positive for adenovirus and 22 cases positive for norovirus while no positive cases with astrovirus as shown in table (2).

According to results of enzyme linked immunosorbant assay positive *G. lamblia* antigen was detected in 42 out of 160 cases (26.25%). Majority of infection among age group d"1-5 years also high percentage of infection was noticed in males 24/42 than females 18/42 as shown in table (3). And statistical analysis showed significant differences

The results of tpi gene amplification has shown that *G. lamblia* was successful among 28/42 (66.66%) samples. Eight (28.57%) of samples were having genotype A (figure 1) while 20 (71.43%) of samples were having genotype B (figure 2). The distribution of genotypes among giardiasis patients in Diyala province was highly significant at Pd"0.001 as shown in table (4).

Regarding co-infection between *G. lamblia* and enteric viruses, high number (14 cases) was recorded with rotavirus followed by 6 cases with norovirus and 4 with adenovirus and statistically significant as shown in table (5)

Table 1. Distribution of study group according to their age and gender

Criteria	No. (%)	Comparison of Significance P-value Sig.
Age (years)		< 0.00001 *
d"1-5 years	108(67.50%)	
6-10 years	38(23.75%)	
11-15 years	14(8.75%)	
Total	160(100%)	
Gender		0 .371**
Males	84(52.50%)	
Females	76(47.50%)	
	160(100%)	

X2: Chi square, P: Probability,*Significant,**Non- significant.

Table 2. The intestinal viruses identified from study group by Cer Test one step

Intestinal viruses	No. (%)	Comparison of Significance P-value Sig.
Rotavirus	32(20%)	< 0.00001 *
Adenovirus	30(18.75%)	
Norovirus	22(13.75%)	
Astrovirus	Zero	
Total	160	

* Significant

Table 3. *G. lamblia* infection and related with age and gender in diarrheal children

Criteria	No. (%)	Comparison of Significance P-value Sig.
Age (years)		0.094 *
d"1-5 years	34(80.95%)	
6-10 years	6(14.28%)	
11-15 years	2(4.77%)	
Total	42(100%)	
Gender		0.482 *
Males	24(57.15%)	
Females	18(42.82%)	
	42(100%)	

* Non-Significant at p < 0.05

DISCUSSION

In the present study, the infection rate of rotavirus was higher 20% than rate of other enteric viruses, this rate was comparable with result of study done by Mahmood *et al*[21] in Baghdad (21%), Jaff *et al*.,[22] in Sulaimani province with the (22%), and lower than that observed in Babylon (56%)[23] and Erbil city (37%)[24].

The percentages of adenovirus among diarrhea cases was18.75% and this percent was lower than Al-Khoweledy study in Al-Najaf province (23.33%), also this result agreement study of Mahmood *et al*[21] rotavirus infections were higher than adenovirus in infants and young children in Baghdad, this may be related with similarity in studied area as well as transmission may occur via consumption of contaminated water, contaminated found or when people share personal objects.

Enteric viruses analysis showed that lower percent(13.75%) with norovirus and this result was lower than study of Ahmed[26]. Norovirus is the source in about 18% of all cases. Children and those in the developing world are most commonly affected[27]. Especially those below age five[28]. Also in the developing world children less than two years of age frequently get six or more infections a year[29].

The present study also not recorded any positive astrovirus and this result disagreement with study done by Thewiny *et al*., in Basrah city, Who found prevalence of astrovirus was 2.6% among children under five years of age who were hospitalized with acute diarrhea in Basrah, Iraq. But astrovirus infection could be related to a mild diarrheal disease which would not require frequent hospitalization[31,32]

The present study showed that *G. lamblia* infection rate was (26.25%) this may be related with protozoan cysts are highly resistant to chlorine disinfection[33].

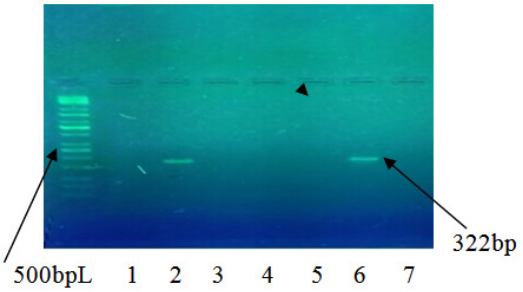

Fig. 1. *G. lamblia* genotyping A in agarose gel electrophoresis with an amplicon of 322bp (lanes 2,6). M: 100-10000 bp molecular marker. fragments were resolved on 1.5% agarose gel and visualized with red stain

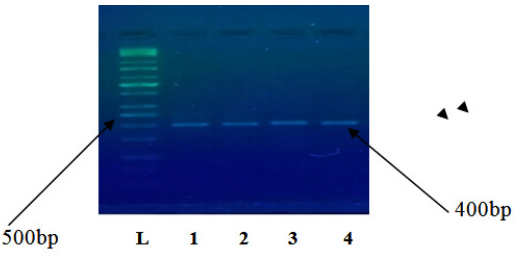

Fig. 2. *G. lamblia* genotyping B in agarose gel electrophoresis with an amplicon of 400bp (lanes 1-4). M: 100-10000 bp molecular marker. fragments were resolved on 1.5% agarose gel and visualized with red stain

Table 4. Identification of *G. lamblia* genotypes by PCR technique

Genotypes	No. of samples (%)
A	8 (28.57%)
B	20 (71.43%)
Total	28(100%)

Significant P= 0.001

Table 4. Co-infection between *G. lamblia* and enteric viruses

G. lamblia	Rotavirus	Adenovirus	Norovirus	p-value
G. lamblia - positive (42)	14	4	6	0.013*
G. lamblia - Negative (118)	18	26	16	0.185**
Total 160	32	30	22	0.297**

P: Probability,*Significant,**Non- significant

This result comparable with study of Hussein [34] who found 23.7% in Thi-Qar province and higher than study of Salman *et al*.[35] in Kirkuk province (9.35%). Recently, infection rate of *G. lamblia* was 9.5% in Duhok city and 5.7% in Erbil city [36], and 3.9% in Samarra[37].

The result of current study has shown that high frequency associated with *G. lamblia* assemblage B (71.43%). This finding in agreement with other studies done in different places and had similar result such as[38-43]. This may be related with fact all patients with assemblage B show a greater rate of elimination of cysts. Also genotype A is also identified to most often responsible for the zoonotic spread with a broad range of animals offered as reservoir hosts, although assemblage B probably spread from human to human, it has been testified in some animals and may also be an animal potential[44].

The differences in environmental and social condition might have contributed to the variations in the distribution of *G. lamblia* assemblages[45]. Therefore, the detection these factors may be lead to control on the parasitic infections.

According to co-infection with giardiasis, 14/42 cases had co-infection with rotavirus, 6/42 cases had co-infection with norovirus and 4/42 cases had co-infection with adenovirus and statistically significant. Co-infection can also increase treatment costs, probably as a result of clinical complications due to interactions among co-infecting pathogens.

Regarding age group most infection less than 5 years this causes could relate to the contaminated drinking water, and artificial milk in bottle, contaminated eating food or sucking pacifiers and personal hygiene measures, especially that age of creeping on ground as most parasites were belonging and they not realize the good sanitation in compare with other ages[46].

Concerning the gender, this study revealed a high number of males patients than females patients which seem to be similar with study done by Mahmood *et al* (2015) in Baghdad, but statistically non-significant and this may be related with male and female were exposed to chance of infection due to all of them were living under the same conditions and climates of disease[48].

In conclusion, infection rate of rotavirus amongst children in Diyala province appears to be relatively high than other enteric viruses. Co-infection with *G. lamblia* type B have important role in gastroenteritis. However, continued enteric virus surveillance and epidemiology amongst this group is required.

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