

DETECTION OF ROTAVIRUS, NOROVIRUS AND ASTROVIRUS AMONG CHILDREN WITH ACUTE GASTROENTERITIS IN BABYLON GOVERNORATE, IRAQ

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ABSTRACT : Stool samples used in this study were collected from 130 randomly selected children with acute gastroenteritis of age ranging from 6 months to 5 years for both sexes were admitted to Babylon maternity and children's Hospital and some of primary health centers during the interval extended from June to November 2019, in Babylon Governorate, Iraq.

All these 130 samples were exposed to two diagnostic methods, including the rapid Chromatographic Immunoassay test and ELISA technique for identification of Rotavirus, Norovirus and Astrovirus antigens. The results of the rapid Chromatographic Immunoassay test revealed that Rotavirus isolates were observed in that 16 (12.3%) out of 130 samples, Norovirus isolates were 1 (0.8%) and Astrovirus isolates were 0 (0%) out of 130 samples. ELISA technique was utilized as a confirmation test and the results demonstrated that the identifying Rotavirus isolates were 18 (13.8%) out of 130 samples, followed by Norovirus isolates 4 (3.1%) and none of the 130 stool samples examined were positive for Astrovirus.

On the other hand, the percentage of viral gastroenteritis infection caused by Rotavirus and Norovirus was more in male (72.7%) than in female (27.3%). Likewise, the results indicated that the most viral infections (Rotavirus and Norovirus) were caused in the age between 6–24 months at percent (77.3%), while the peak incidence of the viral infection in children (45.5%) was between 6–12 months of age.

Furthermore, the results showed that the ratio of acute gastroenteritis infection caused by Rotavirus and Norovirus was higher in the rural area (63.6%) than in the urban area (36.4%).

Key words : Gastroenteritis, rotavirus, norovirus, astrovirus, chromatographic immunoassay, ELISA.

INTRODUCTION

Viral gastroenteritis is one of the most common infectious diseases throughout the world, causing significant illness and death in children under five years old (Colomba *et al*, 2007). Among enteric viruses, four categories of viruses are related to viral gastroenteritis, three of them are belong to RNA viruses, including group A Rotavirus, Norovirus and Astrovirus whereas the other belong to one DNA virus including Adenovirus 40/41 (subgenus F) (Levidiotou *et al*, 2009).

Rotavirus is the most common viral cause of acute gastroenteritis in infants and young children throughout the world and a leading cause the high rates of morbidity and mortality. Approximately 702,000 children die per year by Rotavirus infection (Parashar *et al*, 2009). Also, it has been caused gastroenteritis in both older children and adults, but in less commonly. The peak incidence of

infection has been appeared to happen in the winter season of the year (Hamkar *et al*, 2010). Rotaviruses are non-enveloped, icosahedral, double-stranded RNA viruses comprising a genus inside the family Reoviridae. Seven groups of Rotavirus (A–G) have been described, with group A rotaviruses being the leading cause of severe dehydrating gastroenteritis in children < 5 years of age worldwide (Nahar *et al*, 2013). It is transmitted by the fecal-oral route, person-to-person contact, the respiratory route, or contaminated environmental surfaces and fomites (Vanzyl *et al*, 2006). Norovirus gastroenteritis was accounted to be the second after Rotavirus gastroenteritis as the cause of children's hospital admissions worldwide, causing diarrhea and vomiting. Diarrhea may occur without vomiting and vomiting without diarrhea (Zeng *et al*, 2011). Norovirus is a single-stranded RNA virus, which is organized as an individual of the Caliciviridae family.

The virus is transmitted by fecal-oral route in a low dose leading to vomiting and diarrhea, particularly in the winter season (Robilotti *et al*, 2015). Astroviruses are a significant reason of gastroenteritis infection occurs predominantly in young children in addition to the adults. Astroviruses are non-enveloped, icosahedral symmetry, single-stranded RNA viruses and individuals of the Astroviridae family (Mozhgani *et al*, 2011). The tissue culture method not be useful for isolating the most viruses that associated with gastroenteritis infection, therefore the direct detection of viruses in stool samples by using electron microscope is still the main diagnostic method in spite of it was limited to the reference laboratories. The more sensitive methods using to detect virus antigen in stool samples, such as immunoassay and molecular techniques have been utilized for the detection of newly recognized viruses such as Norovirus and Sapovirus (Meqdam and Thwiny, 2007; Mohamed *et al*, 2015).

The current study aimed to determine the frequency of Rotavirus, Norovirus and Astrovirus among children under five years of age, suffering from acute gastroenteritis in Babylon Governorate, Iraq.

MATERIALS AND METHODS

Patients and samples collection

One hundred and thirty stool samples were obtained from infants and young children of both genders of age groups ranging from 6 months to 5 years suffering from acute diarrhea and were admitted to Babylon maternity and children's Hospital and some of primary health centers during the interval from June to November 2019, in Babylon Governorate, Iraq. Clinical features of viral gastroenteritis cases and some other data assent such as age, sex and residence were acquired from either the mother or the father of the child and afterward records. Also, all patients must don't get any type of antibiotics to prevent the negative false results. We collected 5-8 gm of stool in a clean, sterile plastic container with tightly fixed caps, transferred to the laboratory within two hours on ice in sealed bags and stored in the freezer at -20°C until further tested for the viral antigens.

Laboratory diagnosis

Chromatographic immunoassay test

All 130 stool samples were examined for the presence of Rotavirus and Astrovirus antigen by using commercially available kit COMBO CARD TEST supplied by CerTestBiotec, Spain and examined the presence of Norovirus antigen by using commercially kit supplied by Biofocus Company, South Korea. The procedures were done according to the manufacturer's instructions.

Enzyme-Linked Immunosorbant Assay (ELISA) technique

The all stool samples which tested for Rotavirus, Norovirus and Astrovirus antigen by rapid Chromatographic Immunoassay were retested also by ELISA technique for Rotavirus and Norovirus antigen using commercially available kits such as (DRG-Germany) and RIDASCREEN Norovirus (R-Bio-pharm AG, Darmstadt, Germany), respectively. Also, stool samples examined for detection of Astrovirus antigen by using commercially kit supplied from ABO company, Switzerland. The procedures were done as described in the manufacturer's instructions.

RESULTS

One hundred and thirty stool samples were involved in this study and processed for identification of Rotavirus, Norovirus and Astrovirus antigens by using two methods, including the rapid Chromatographic Immunoassay test and ELISA. The results showed that the prevalence of identifying Rotavirus isolates by the rapid test were 16 (12.3%) out of 130 samples, Norovirus isolates were 1 (0.8%) and Astrovirus isolates were 0 (0%) out of 130 samples. Whereas the results showed that the prevalence of identifying Rotavirus isolates by ELISA technique were 18 (13.8%) out of 130 samples, Norovirus isolates were 4 (3.1%) and Astrovirus isolates were (0) out of 130 samples as shown in Table 1.

Table 2 reveals that Rotavirus and Norovirus infections were more in male 13 (72.2%) and 3 (75%) respectively, than in female 5 (27.8%) and 1 (25%), respectively.

Table 1 : Profile percentage of Rotavirus, Norovirus and Astrovirus in stool samples using chromatographic immunoassay test and ELISA technique.

Viruses	Chromatographic Immunoassay test		ELISA technique	
	No.	%	No.	%
Rotavirus	16	12.3	18	13.8
Norovirus	1	0.8	4	3.1
Astrovirus	0	0	0	0
Negative	113	86.9	108	83.1
Total	130	100	130	100

Table 2 : Gender distribution of patients with Rotavirus and Norovirus detected by ELISA technique.

Gender	Viral Pathogens		
	Rotavirus(%)	Norovirus(%)	Total viruses(%)
Male	13(72.2%)	3(75%)	16(72.7%)
Female	5(27.8%)	1(25%)	6(27.3%)
Total	18(100%)	4(100%)	22(100%)

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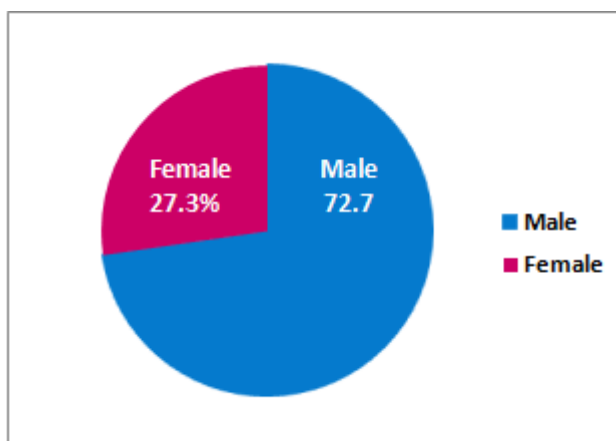


Fig. 1 : Distribution of patients with viral gastroenteritis according to the gender.

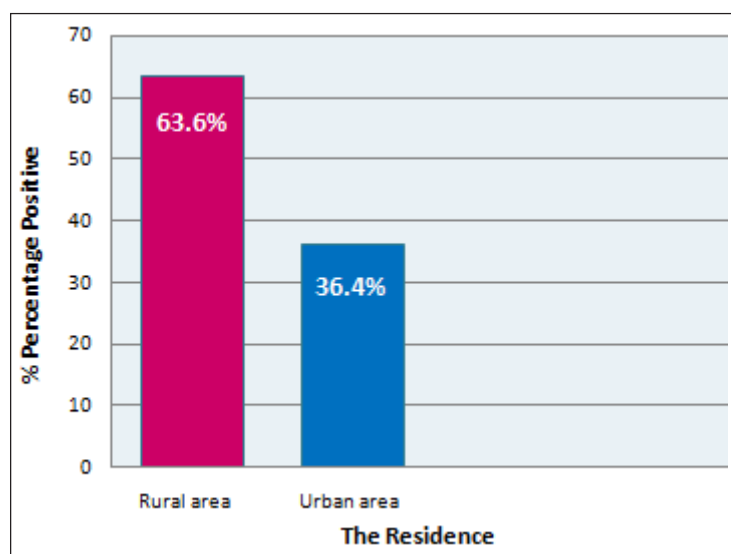


Fig. 2 : Incidences of viral gastroenteritis according to rural and urban area.

Table 3 : Age distribution of patients with Rotavirus and Norovirus detected by ELISA technique.

Age groups (months)	Viral pathogens		
	Rotavirus(%)	Norovirus(%)	Total viruses(%)
6–12	7(38.9 %)	3(75 %)	10(45.5 %)
13–24	6(33.3%)	1(25 %)	7(31.8 %)
25–36	2(11.1%)	0(0)	2(9.1 %)
37–48	2(11.1 %)	0(0)	2(9.1%)
49–60	1(5.6%)	0(0)	1(4.5 %)
Total	18(100%)	4(100 %)	22(100 %)

Thus, the total percentage of viral gastroenteritis infection caused by Rotavirus and Norovirus was more in male (72.7%) than in female (27.3%) as shown in Fig. 1.

Furthermore, the results showed that the most infections with Rotavirus and Norovirus were caused in the age between 6 – 24 months in percent (77.3%) and the greatest ratio in the age 6–12 months in percent (45.5%) as shown in Table 3.

Table 4 : Distribution of patients with Rotavirus and Norovirus according to rural and urban area.

Residency	Viral Pathogens		
	Rotavirus%	Norovirus%	Total viruses%
Rural	11(61.1%)	3(75 %)	14(63.6 %)
Urban	7(38.9 %)	1(25 %)	8(36.4 %)
Total	18(100%)	4(100 %)	22(100 %)

The result in Table 4 showed that the high prevalence of positive Rotavirus and Norovirus in rural area (61.1% and 75%), respectively than in urban area (38.9% and 25%), respectively.

Thus, the total percentage of viral gastroenteritis infection caused by Rotavirus and Norovirus was higher in the rural area (63.6%) than in urban area (36.4%).

DISCUSSION

A total of (130) stool samples, the findings demonstrated that the Rotavirus was the major cause of acute diarrhea in infants and young children in percent (12.3%) by Chromatographic Immunoassay test and in percent (13.8%) by the ELISA technique. It was observed that the Chromatographic Immunoassay test is a rapid, simple, useful diagnostic method and that will serve physicians for early diagnosis viral gastroenteritis (Rotavirus, Norovirus and Astrovirus), reducing unnecessary treatment, whereas the ELISA technique have been shown more specific diagnostic technique for the investigation of viral agents.

A negative result may occur in the Chromatographic Immunoassay test due to the level of antigen is lower than the detection limits of this test, but the ELISA is a sensitive, specific method for the determination of viral pathogen and large quantities of sample can be processed in a short time (Kelkar *et al*, 2004).

The results of this study corresponded to the results recorded in Baghdad city by AL-Shuwaikh (2016) indicated that the prevalence of Rotavirus and Astrovirus investigated by rapid chromatographic immunoassay were found to be 14.36% and 0 (0%) respectively, while by using ELISA technique, it was found to be the prevalence of identifying the Rotavirus was (18.62%), and there was no availability for Astroviruses, respectively. Also, the other local previous study in Babylon province carried out after the introduction of Rotavirus vaccine by Mutlak *et al* (2018) showed that the changing prevalence rate of Rotavirus (48%). In addition to that Zaman *et al* (2012) in Kirkuk city found out that the prevalence rate of Rotavirus was (33.3%) and in

Sulaimani city the prevalence rate of Rotavirus was recorded in percent (22%) by Jaff *et al* (2016), while, Alani *et al* (2012) in western Iraq, Anbar city mentioned that the prevalence rate of Rotavirus estimated as (39%). These findings showed that the differences in the results between multiple cities in Iraq probably due to geographical area effects, fresh or frozen stool samples, the timely collection of samples (seasons), duration of the study and using different kits for the same method and the personal hygiene.

On the other hand, the results revealed that Norovirus has been considered to be second to Rotavirus have been identified and none of the 130 stool samples examined were positive for Astrovirus.

A study carried out by Zaki and Abo Elkheir (2017) in Egypt demonstrated that the higher rates of Rotavirus, Norovirus and Astrovirus infections than those found in the current study (44%, 30% and 14%) respectively. Also, another study recorded in Porto Velho, state of Rondônia, Brazil, done by Amaral *et al* (2015) demonstrated higher Norovirus and Astrovirus infection rates (7.8% and 0.8%), respectively. About 32.27% of Norovirus have been detected in Baghdad with new genotypes incidence (Mohamed *et al*, 2015).

Additionally, the results revealed that viral gastroenteritis infection (Rotavirus and Norovirus) had been affected both sexes, but the frequency of infection in male (72.7%) higher than in female (27.3%). This result is in agreement with results study in Kirkuk city done by Zaman *et al* (2012), who have observed that Rotavirus infected the malein (63.3%) more than female (36.3%). Another study recorded in Basrah city by Hussein and Hassan (2000) indicated that the majority of Rotavirus infections was significantly occurred in males more than female. The explanation of these results probably due to the fact that the males are more in touch with the environment than female especially in agricultural communities and it may involve gender specific immunological differences. However, the result of the present study is in contrast with results recorded in North Iran by Ozdemir *et al* (2010), who found that no differences in viral infection rate among male and female.

Moreover, the results indicated that the age group between 6 – 24 months (≤ 2 years old) showed the highest prevalence of viral gastroenteritis infection with Rotavirus and Norovirus(77.3%) than other age groups and prevalence decrease in the percentage of viral infections with increasing age and the peak incidence of viral infection in children (45.5%) was between 6 – 12 months of age. This is probably due to that using solid food beside

of breast feeding that lead to increases of the possible virus transmission from contaminated food, the other reason is at this age the feeding will starting and the children become to put any things in their mouths, while in older children the percentages of viral infections were decline probably due to acquired protective immunity from previous exposures to the virus leading to give them more resistance for infection with the virus.

These results were agreed with the results of some local studies reported in Kirkuk and Basra province by Ali *et al* (2010), AL-Ameen *et al* (2008), respectively. Additionally, different other previous studies reported in Kirkuk city, Iraq and in Taiz, Yemen by Zaman *et al* (2012), AL-Badani *et al* (2014) found that the peak of Rotavirus infection in children, who aged ranging from 6 – 12 months.

Furthermore, the results showed that rural area has been associated with higher incidences of viral gastroenteritis infection caused by Rotavirus and Norovirus (63.6%) than in urban area (36.4%). The difference in distribution probably due to the low level of hygiene and many rural areas using river waters for drinking and swimming and therefore constitute as a source of infection with the virus. A similar result was recorded in Baquba Diyala Cities, Iraq by Abdul-Razak *et al* (2011) observed that fewer incidences of Rotavirus infection in urban area than rural area.

CONCLUSION

Rotavirus is one of the most important enteric viruses followed by Norovirus causing acute gastroenteritis occurring annually among children aged less than five years, particularly those ≤ 2 years old and viral pathogens should be investigated routinely in diarrhea stool samples, and required careful clinical interest.

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