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RAPID IDENTIFICATION OF ROTAVIRUS, ADENOVIRUS AND NOROVIRUS USING IMMUNOCHROMATOGRAPHY TEST AMONG INFANTILE DIARRHEA, IRAQ

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ABSTRACT
One hundred three diarrheal stool samples were collected from infants with age (<1-12 months) admitted to Babylon Hospital for Maternity and Children, Iraq. All samples were subjected to rapid Immunochromatography test (ICGT). The results of rapid identification revealed that 50.5% (52/103), 29.1% (30/103) and 20.4% (21/103) were positive for Rotavirus, Norovirus and Adenovirus respectively. The commonlyeffected age group was (1- 4 months) which compile 51.5% and most of the infantile viral diarrhea were highly among rural infants, (two folds) than those in urban infants. Regarding the clinical features of infants with viral diarrheal infections the results display that (84.5%) of infants had watery or mucosal consistencestool with duration of diarrhea for more than 7 days. In addition (85.4%), (78.6%),(74.8%) and (68%) of infants had weakness, Fever > 38°C, Abdominal pain and vomiting respectively.As a conclusion, this study revealed that major viral enteropathogens causing infantile diarrhea in Hilla city were Rotavirus followed by Norovirus and Adenovirus. It was also evident that most patients were those who had mixed feeding methods using untreated water and lived in poor environmental conditions.The study highlights the need for continuous monitoring of viral enteropathogens for successful treatment and control of infantile diarrhea, and also for the development of public health strategy for residents in rural community.

KEYWORDS: Infantile diarrhea, Rotavirus, Norovirus, Adenovirus, Rural, Urban.

INTRODUCTION

Diarrhea remains the second leading cause of deatharound the world for children under 5 years of age. Diarrhea can be caused by a variety of microbes, or parasites, but most often it is caused by viruses. Approximately 10% of all cases of childhood diarrhea are caused by a rotavirus; Rotavirus frequently produces more severe diarrhea, than other microbes. It is known that among children with severe diarrhea, rotavirus is found in almost 50% of cases [1]. Rotavirus is the main cause of diarrhea-related illness and death in children worldwide. It is associated with more than 2-3 million hospitalizations and up to 600 000 deaths in children aged <5 years; the vast majority occurring in developing countries [2]. The clinical spectrum of rotavirus disease varies from asymptomatic infection to acute, severe, dehydrating diarrhea with vomiting that can be fatal [3, 4]. Initially, rotavirus replication was thought to be limited to the gastrointestinal tract in patients with gastroenteritis. This idea has prevailed despite repeated associations of rotavirus infection with systemic symptoms and non-gastro enteric clinical diseases, including respiratory illness and neurological syndromes. Rotavirus is transmitted by close contact, especially through the fecal-oral route, and possibly through the respiratory route [5]. Rotavirus is an important pathogen in day care-acquired illnesses. The virus can remain infectious on inanimate surfaces, such as toys, for several days, and up to four hours on human hands. Rotavirus infection is the foremost cause of severe gastroenteritis of young children worldwide[6]. As a rule, rotavirus infection is accompanied by fever, vomiting, and diarrhea followed by dehydration (loss of body water) and loss of electrolytes (minerals). Dehydration and loss of electrolytes is the cause of children's sickness when they develop diarrhea; therefore, management and prevention of dehydration is an important strategy in the treatment of diarrhea[7]. Viruses are one of the most important causes of gastrointestinal problems all over the world and infect especially children under 5 years of age in the third world and even developed countries [8]. Next to Rotavirus, one of the microorganisms causing acute diarrhea especially in infants and children is Adenovirus. Although Adenovirus is less prevalent than Rotavirus in causing acute diarrhea, its complications is very dangerous in children under 5 years of age [9]. Adenoviruses are second only to rotavirus as the most important causative agents of acute infantile gastroenteritis[10]. Adenovirus infection in children is associated with a variety of clinical manifestations and disease syndromes with different degrees of severity. Most of these infections can't be diagnosed based upon clinical criteria alone.Adenovirus is an important and common agent in causing gastroenteritis in children less than 6 years of age and is more dangerous than Rotavirus. Meanwhile there is no need to use antibiotics for treating these children and we should recognize the agent to cure the patients with conservative actions [9]. Noroviruswhich are non enveloped, positive-stranded RNA viruses with a polyadenylated genome of ≈6.4–8.4 kb, cause illness in animals and humans including gastroenteritis in human [11, 12].

Human Norovirus (HuCV) has been recognized as the most important cause of nonbacterial, acute gastroenteritis in humans of all age groups. Additionally, it is responsible for at least 50% of all gastroenteritis outbreaks globally; however, the incidence of this agent is rarely laboratory-registered in developing countries [13]. Gómez-Santiago *et al.* (2012)[14] indicating the frequency of HuCV related with acute diarrheal disease in children up to 5 years of age throughout Mexico. Interestingly, the age group most affected with HuCV infection corresponded to the group most commonly affected by rotavirus, with a median age of 12 months [15].

Moreover, it seems that infection with Rotavirus, Adenovirus and Nor virus is an important hygienic issue in our country. This study aimed to Identifying the accurate rate of prevalence and incidence of these viruses is of particular importance, as it will help us with our future programming for the correct control and choosing specific treatment methods for these viruses.

MATERIALS AND METHODS

Patients and Samples

One hundred three diarrheal stool samples were collected from infants with age (<1-12 months) admitted to Babylon Hospital for Maternity and Children, Iraq. All samples were collected during a period from October 2012 to May 2013 and subjected to rapid Immunochromatography test (ICGT).

Patients Data

The required data were recorded for each patient who includes: age, gender, geographical region, feeding methods and clinical features.

Rapid Immunochromatography Test (ICGT)

The cassettes for rapid detection test were used for Adenovirus, Rotavirus and Nor virus (Biofocus / Korea). The positive results indicated as two red lines for control and test. The stool specimens were tested, as soon as possible aftercollection, with Immunochromatographictest for Adenovirus, Rotavirus and Norovirus separately. This is a ready-to-use testbased on the use of homogenous membrane system withcolloidal gold particles. The fecal sample was diluted inthe dilution buffer supplied with the test. A nitrocellulosemembrane is sensitized with antibodies directed againstAdenovirus, Rotavirus and Norovirus(test lines). The test was carriedout as described by Weitzel et al. (2007) [16].

RESULTS

Concerning distribution of the Adenovirus, Rotavirus and Norovirus among infants with diarrhea, the results revealed that 50.5% (52/103), 29.1% (30/103) and 20.4% (21/103) were positive for Rotavirus, Norovirus and Adenovirus respectively as shown in figure (1). About the distribution of these viruses among age groups, the results show that the most effected age group was (1- 4 months) which compile 51.5% followed by (less than 1 month group) which consist 34% as mentioned in table (1).

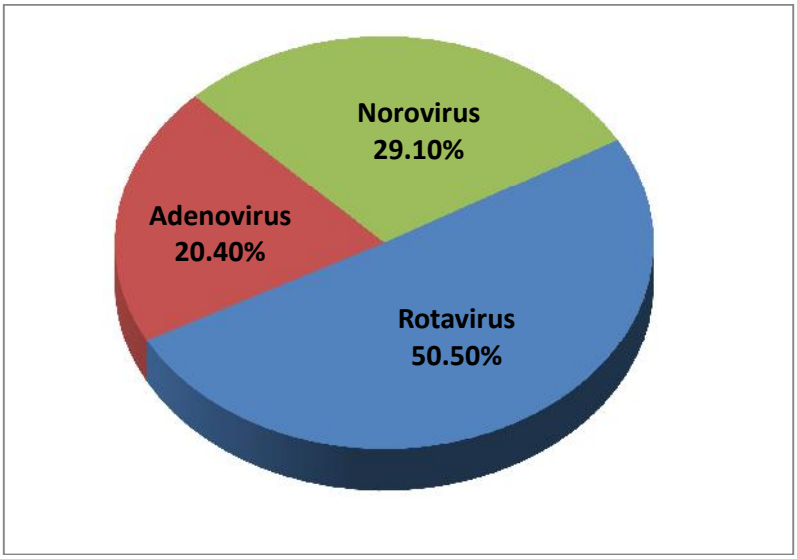

FIGURE 1: Frequency of Rotaviruses, Adenoviruses and Norovirus among children with diarrhea using Immunochromatography test (ICGT)

The data of this study were in accordance with those gathered by Elhag *et al.* (2013)[17] who found that Of the 99 cases of viral diarrhea, 83 (83.8%) were due to rotaviruses while 16 (16.2%) attributed to adenovirus. A recent review of 27 prospective studies from 20 countries published from 1990 to 2000 estimated the incidence of diarrhea as 3.8 episodes per child per year for children <11 months of age[16]. Kosek *et al.* (2003)[18] have recently made further update by reviewing 60 studies of diarrhea morbidity and mortality published from 1990 to 2000. They conclude that diarrhea accounts for 21%of all deaths at fewer than five years of age and causes 2.5 million deaths per year, although diarrhea morbidity remains relatively unchanged.

TABLE 1: Age and Gender distribution with viral diarrheal infections isolates from infants

Age group (Months)	No. of Patients						Total	%
	Rotavirus		Norovirus		Adenovirus			
	Male	Female	Male	Female	Male	Female		
< 1	12	11	5	-	2	5	35	34.0
1-4	8	10	12	12	3	8	53	51.5
5-8	2	6	-	-	1	-	9	8.7
9-12	-	3	-	1	1	1	6	5.8
%	21.4	29.1	16.5	12.6	6.8	13.6	100%	

According to the geographical distribution of the infant with diarrhea the results display that most cases of the viral diarrhea caused by Adenovirus, Rotavirus and Norovirus were highly among rural infants, two fold than those in urban infants as shown in table (2).

TABLE 2: Geographical distribution of the infant with diarrhea

Type of Virus	Residential distribution of infection		Total
	Urban infant no.	Rural infant no.	
Rotavirus	16	36	52
Norovirus	18	12	30
Adenovirus	3	18	21
Total	37	66	103

Regarding the relationship between viral (Adenovirus, Rotavirus and Norovirus) diarrheal infections and feeding method, the results revealed that the viral diarrheal infections among mixed feeding infants were three time (76.7%) lager than those of breast feeding (23.3%) as depicted in table (3).

TABLE 3: Feeding distribution with viral diarrheal infections isolates from infants

Type of Virus	Feeding distribution of infection		Total
	Breast feeding no.	Mixed feeding no.	
Rotavirus	10	42	52
Norovirus	11	19	30
Adenovirus	3	18	21
Total	24	79	103

On the subject of clinical features of infants with viral diarrheal infections, figure (2) and table (4) present ate that (84.5%) of infants had watery or mucosal consistence stool with duration of diarrhea for more than 7 days. In addition (85.4%), (78.6%), (74.8%) and (68%) of infants had weakness, Fever > 38°C, Abdominal pain and vomiting respectively.

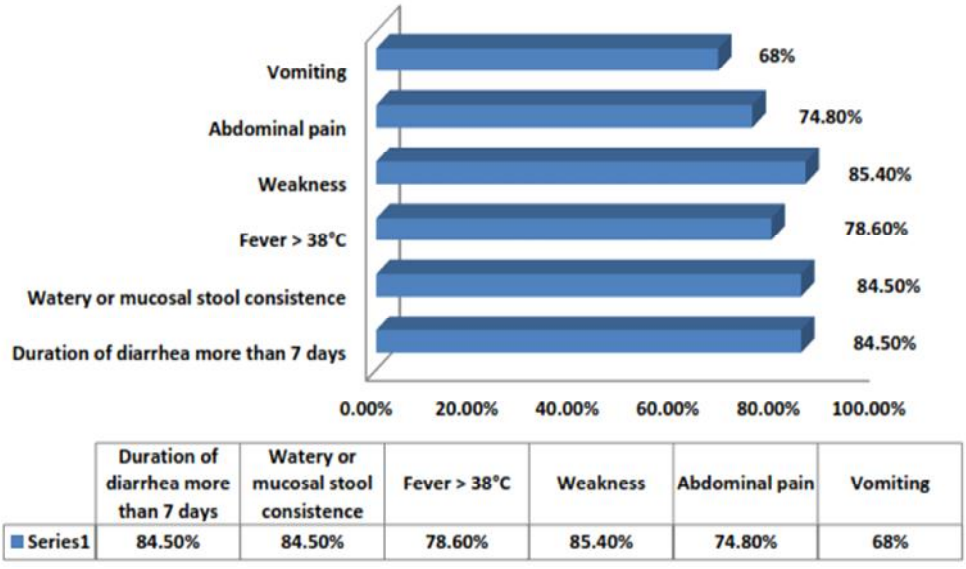

FIGURE 2: clinical features of viral diarrheal infections

TABLE 4: Clinical features of diarrheal infections

Clinical features	Type of Virus		
	Rotavirus	Norovirus	Adenovirus
Duration of diarrhea more than 7 days	50	22	15
Watery or mucosal stool consistence	42	24	21
Fever > 38°C	44	19	18
Weakness	49	21	18
Abdominal pain	40	26	11
Vomiting	40	16	14

The results of the current study were in agreement with those displayed by Ahmed *et al.* (2009)[19]who found that Vomiting was significantly higher in rotavirus diarrhea than non-Rota diarrhea.

DISCUSSION

Each year, rotavirus causes an estimated 111 million episodes of diarrhea requiring only home care, 25 million clinic visits, 2 million hospitalizations, and 352,000–592,000 deaths (median 440,000 deaths) in children <5 years of age. In other words, by 5 years of age, almost all children will have an episode of rotavirus gastroenteritis, 1 in 5 will require a clinic visit, 1 in 65 will require hospitalization, and approximately 1 in 293 will die [7]. The rapid Immunochromatography test (ICGT) is easy to perform at a low cost, may be an optimal method in place of latex test for the detection of Rotavirus, Adenovirus and Norovirus and ICGT showed a good agreement with EIA and RT-PCR [20].

The incidence of rotavirus disease is similar in children in both developed and developing nations. However, children in developing nations die more frequently, possibly because of several factors, including poorer access to hydration therapy and a greater prevalence of malnutrition. An estimated 1,205 children die from rotavirus disease each day, and 82% of these deaths occur in children in the poorest countries [20]. The proportion of diarrhea deaths attributable to rotavirus may have increased, given that this pathogen is often transmitted from person to person and is difficult to control through improvements in hygiene and sanitation. This hypothesis is supported by data from Mexico, demonstrating that whereas deaths from diarrhea declined substantially from 1989 to 1995, the decline was less evident for winter seasonal deaths in children <2 years of age whose illness met the epidemiologic features of rotavirus diarrhea. In addition, some recent studies of rotavirus based on hospital surveillance in developing countries have demonstrated detection rates in excess of 50%[21]. Dalgıç *et al.* (2010)[22] state that, the symptoms and signs commonly associated with rotavirus gastroenteritis including diarrhea, vomiting, fever, lethargy and dehydration were recorded, as well as decrease in oral intake. Rotavirus gastroenteritis may be associated with high fever and consequently, typical febrile seizures may occur. On the other hand, there are several reports of febrile seizures associated with rotavirus gastroenteritis [23].

The fever regarded as prominent clinical signs of Rotavirus diarrheal infections and this finding was in accordance with those exhibited by Ahmed *et al*. (2009)[19] who found that fever was present in 58.4% of infants with Rotavirus diarrheal infections. The grade of the fever was categorized as “mild” (37.7-38.5 °C), “moderate” (38.6-39.5 °C) and “severe” (>39.6 °C) according to the narration of the parents and temperature record of the OPD and hospitalized patients[19]. Regarding vomiting the previous study [19], state that (63.7%) of patients, there was history of vomiting. The vomiting was either preceded or followed by diarrhea. Unprovoked and spontaneous vomiting was significantly higher inRotavirus diarrheal infections. As a conclusion, this study revealed that most of the infants (≤12 months) were infected by viral enteropathogens and the major viral pathogens causing infantile diarrhea in Hilla city were Rotavirus followed by Norovirus and Adenovirus. Also this study regards the first for Norovirus documentation as viral pathogens causing infantile diarrhea in Hilla city. The study highlights the need for continuous monitoring of viral enteropathogens for successful treatment and control of infantile diarrhea, and also for the development of public health strategy for residents in rural community.

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