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RESEARCH ARTICLE

Detection Of Norovirus And Adenovirus Type 40/41 In Diarrhea Among Children Aged 1-60 Months, In Al-Najaf Governorate, Iraq

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Abstract

Noroviruses and Adenoviruses type 40/41 are from the most important etiologic factors in acute gastroenteritis of children. The aim of this study was to detect of norovirus and adenovirus type 40/41 among children with diarrhoea admitted to Al-Najaf governorate, in AL- Zahra teaching hospital for maternity and children, during the period from January, 2013 to December, 2014 including 314 stool samples from children with diarrheal, their age range (from 1 to 60 months). Stool samples were tested for norovirus and adenovirus antigens using immunochromatographic assay, which showed prevalence of 12% and 9% for norovirus and adenovirus, respectively. According to age groups , the results showed that the majority of infections with norovirus and adenovirus were recorded among children age (13-24) months. The positive cases were highly among infants fed with artificial dry milk (31 cases) compared with infants fed with mother's milk (14 cases). Regarding the clinical features of children with viral diarrheal infections the results showed that all of the cases of both viruses complained of watery or mucosal consistence stool (100%) followed by abdominal pain (94.5%) and (82.1%), vomiting (81%) and (78.5%) , and fever (78.3%) and (71.4%) for norovirus and adenovirus positive children respectively. The present study also revealed that a higher incidence of norovirus was detected from January - March 2014, when it was found in 81% (30/37) of the positive cases , whereas the incidence of adenovirus was higher from January - April 2014, during which it was found in 89.2% (25/28) of the positive cases.

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INTRODUCTION

Diarrheal diseases still has been a public health problem that particularly affect in infants and young children throughout the world and it is the largest killer cause after pneumonia in children, mainly in developing countries. In each year the diarrheal disease lead to 0.76 million death case which constitute 11% from all death cases in the world (WHO, 2013; CDC , 2013). There are many viruses that causing diarrhea, some of the most common include rotavirus, norovirus, astroviruses and adenovirus. Among the different enteric diarrheal viruses, norovirus is considered a major cause of severe gastroenteritis in infants and young children worldwide and it is recognized as the important cause of foodborne outbreaks (Nair *et al.* , 2010).

Norovirus is represented as the second very important causative agent of childhood diarrhea after rotavirus but the importance of norovirus is estimated to increase in the future, as a result of the application of routine rotavirus vaccination (Koo *et al.* , 2013). No specific antiviral drugs or vaccines are available until now for treatment

or prevention of norovirus infection, Although increasing the considerable in morbidity and mortality related with "norovirus gastroenteritis" (El-Kamary *et al.* , 2010; Vinje, 2010).

Adenovirus types 40 and 41, has been found to be associated with acute diarrheal diseases and is responsible for 3.2 to 12.5% cases of globally in infantile and childhood and the detection ratio is higher in developing countries. Enteric viruses including norovirus and types 40 and 41 adenoviruses specially infect the enterocytes of the small intestine and there is mainly affect in children less than two years of age, leading to persistent watery diarrhea that if left untreated, can cause severe dehydration (Nguyen *et al.* , 2007 ; Liu *et al.* , 2012).

Materials and methods :-

Three hundred fourteen stool samples suffering from children with diarrhoea attending to AL- Zahra teaching hospital for maternity and children in Al-Najaf governorate over period between January, 2013 to December, 2014 , each patient were collected stool amount from "2 gm to 20 gm" in disposable containers. In stool samples norovirus detection was done by direct chromatography technique by using manual principle of Rapid-VIDITEST company as a rapid detection and detection of adenovirus type 40 and 41 was done by direct chromatography technique by using Coris BioConcept company .

Results:-

1-Detection of human Norovirus in stool by Immunochromatographic test (IC):

Thirty seven positive samples for norovirus were identified by this method according to the presence of red colored line in the test reaction zone (T1 and T2) and blue control reaction zone indicating to positive result figure 1-A . While it is absent according to the red color in the test reaction zone for other samples of two hundred and seventy seven but a blue colored line was visible in the control reaction zone indicating to control only or negative result figure 1-B.

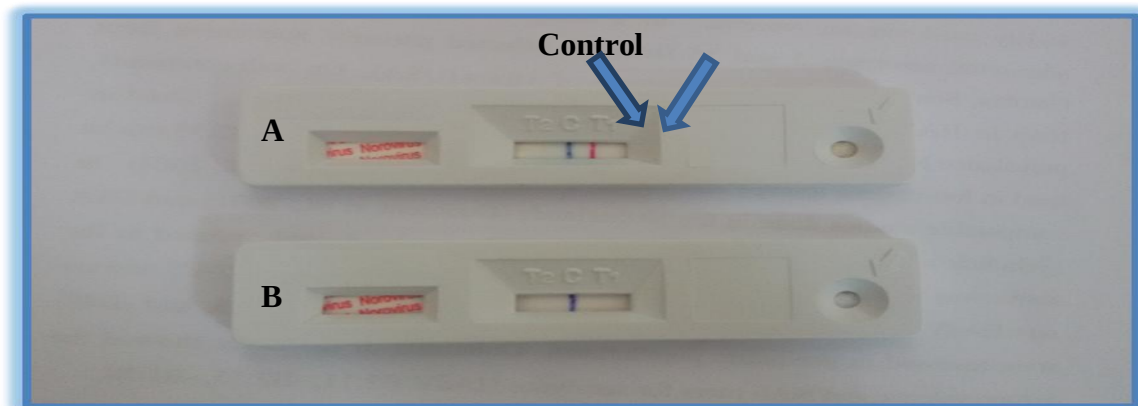
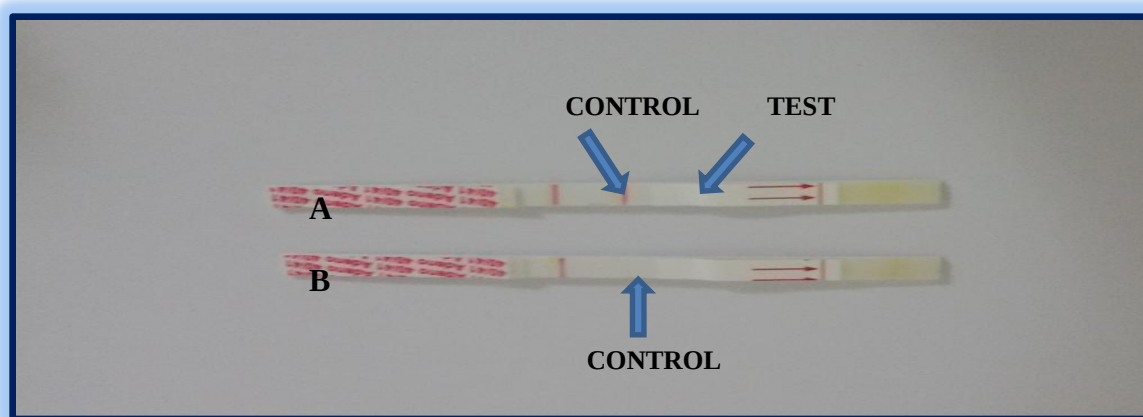


Figure (1): Norovirus rapid test. A-The red and blue colored indicate positive sample. B- The blue colored only indicate negative sample.

2- Detection of human adenovirus in stool by Immunochromatographic test(IC):

Of a total three hundred fourteen stool samples collected, only 28 cases were positive adenovirus as detected by rapid test ,Figure 2.



Figure(2): Adenovirus rapid test. A-The two red colored indicate positive sample. B- The only one red colored indicate negative sample.

3- Incidence Of Norovirus And Adenovirus:

During the study period 314 patients of the samples from children under five years of age with gastroenteritis symptoms, were screened based on Immunochromatographic test, among them 37 children (12%) were positive for norovirus, 28 children (9%) were positive for adenovirus while 249(79%) patients gave negative result for both norovirus and adenovirus as shown in figure 3.

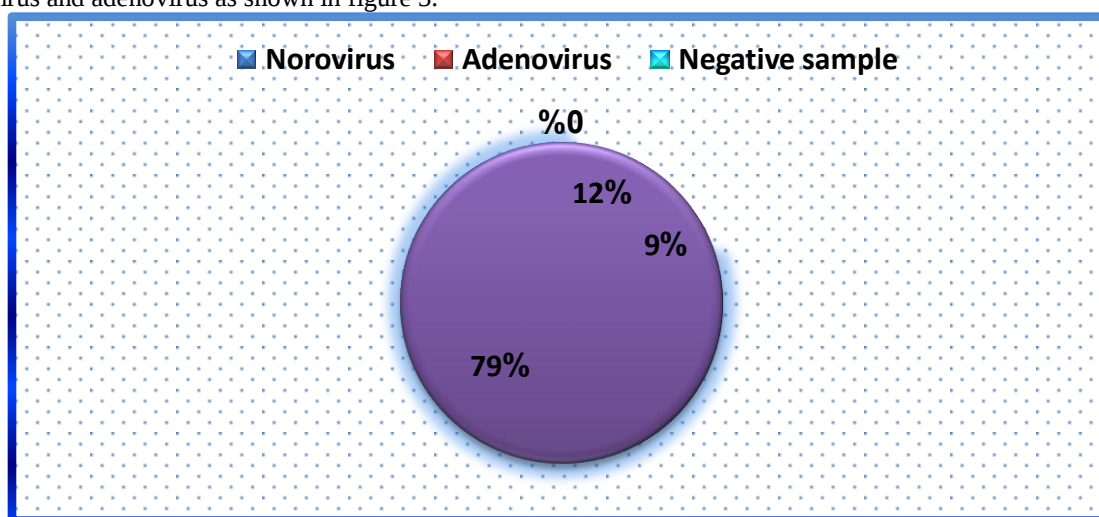


Figure (3): Incidence of norovirus and adenoviruses among diarrheic children .

4- Distribution Of Norovirus And Adenovirus Infections According To Age Groups:

The distribution of these viruses according to age groups , the total percentage results show that the majority of infections with norovirus and adenovirus was recorded among 13-24 months age which consist 24.6% followed by 7-12 months age which consist 23% ,while the minority of infection was recorded among 49-60 months age which consist 7.6%. As well as, the highest incidence of infection with norovirus and adenovirus was observed among male groups 19 and 18 patients respectively and less than among female groups 15 and 13 patient respectively as mentioned in table 1.

Table(1): Age and Gender distribution with positive cases of norovirus and adenovirus detection from children with diarrheal infections.

Age group (Months)	No. of Patients.						Total (%)
	Norovirus			Adenovirus			
	Male	Female	No.(%)	Male	Female	No.(%)	
1-6	1	4	5(13.5)	1	2	3(10.7)	8(12.3)
7-12	5	2	7(18.9)	5	3	8(28.6)	15(23)
13-24	4	6	10(27)	4	2	6(21.5)	16(24.6)
25-36	3	2	5(13.5)	2	3	5(17.8)	10(15.3)
37-48	3	3	6(16.3)	2	3	5(17.8)	11(16.9)
49-60	2	2	4(10.8)	1	0	1(3.6)	5(7.6)
Total	18	19	37(100)	15	13	28(100)	65(100)

5-Correlation Of Norovirus And Adenovirus Infections With Feeding Method:

Regarding the relationship between norovirus and adenovirus diarrheal infections and feeding method, an analysis type of milk results revealed that the most cases belonged to infants fed with dried milk (31 cases) and (14 cases) belonged to infants fed with mother's milk as depicted in table 2.

Table(2): Distribution of norovirus and adenovirus according to feeding.

Type of Virus.	Type of Feeding No.			Total
	Mother's milk	Dried milk	Mothers & Dried milk	
Norovirus	9	18	10	37
Adenovirus	5	13	10	28
Total	14	31	20	65

6- Clinical Features Of Norovirus And Adenovirus Infections:

The clinical features of infants with norovirus and adenovirus infections in the current study are shown in figure 4, including duration of diarrhea for more than seven days in addition to watery or mucosal stool consistence, fever, abdominal pain and vomiting were the commonest symptoms seen in norovirus and adenovirus positive children. Duration of diarrhea for more than seven days which are observed in (83.7%) of cases are positive for norovirus and (57.1%) of cases are positive for adenovirus. All of the cases of both viruses complained of watery or mucosal consistence stool (100%) followed by abdominal pain (94.5%) and (82.1%), vomiting (81%) and (78.5%) and fever (78.3%) and (71.4%) for norovirus and adenovirus positive children respectively.

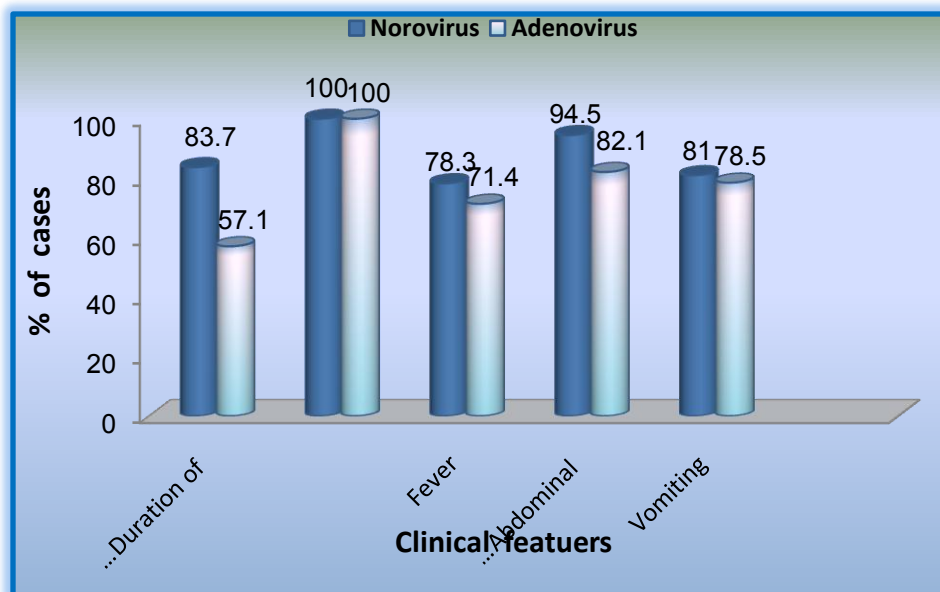


Figure (4): Clinical features of norovirus and adenovirus infections.

7- Seasonality Of Norovirus And Adenovirus Infections:

The data analyzed on a monthly basis for one year to limit whether seasonal changes play a role in the incidence of norovirus and adenovirus. Higher incidences of norovirus 81% (30/37) of samples were detected from January - March 2014, while it was detected at lower rates in the subsequent months. The incidence of adenovirus was higher from January - April 2014, during which it was found in 89.2% (25/28) of samples; however, the number of cases positive for adenovirus decreased in the subsequent months as shown in figure 5 .

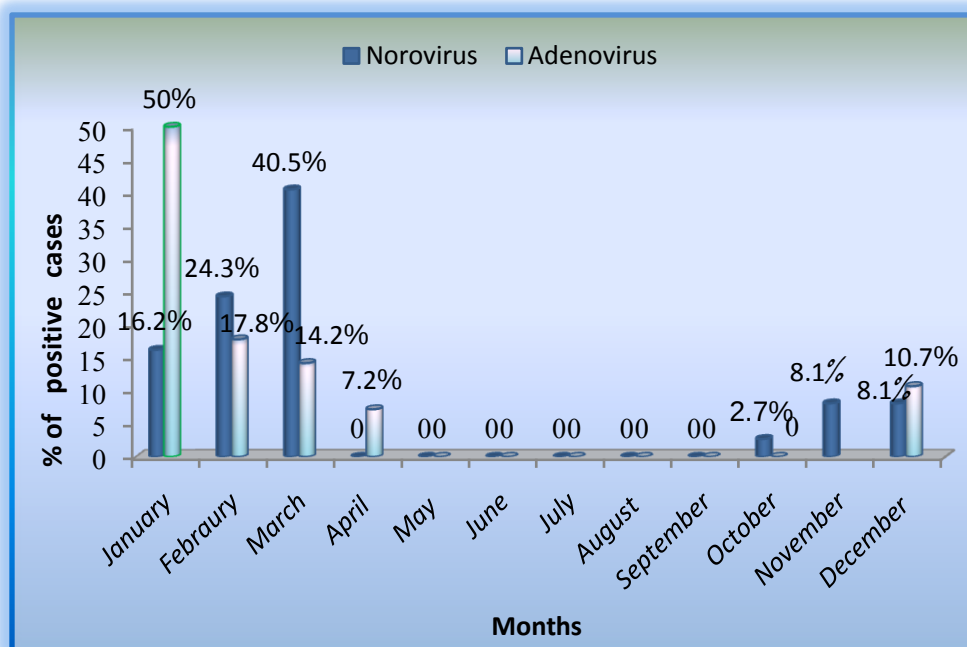


Figure (5): Seasonal distribution of norovirus and adenovirus from January to December 2014.

Discussion:-

In the present study, Norovirus was determined to be responsible for 12% (37/314) of the gastrointestinal infections in children. A previous study conducted in Europe also showed that norovirus is an important cause of acute

gastroenteritis (Levidiotou *et al.*, 2009; Tran *et al.*, 2010). In a study of diarrheic children in Nicaragua, Incidence rate of 24% (79/330) was found for norovirus infection, after the Rotavirus vaccine was implemented in Nicaragua, Norovirus became the main viral cause of acute gastroenteritis among children younger than five years of age (Bucardo *et al.*, 2014). Alike results reported in the America (Koo *et al.*, 2012; Payne *et al.*, 2013). Seeing that this is the first study conducted in Al-Najaf city to include norovirus as viral pathogens causing infantile diarrhea and that a positivity rate of 12% was observed for the entire study period. However, further studies of this virus are essential mainly after introduction of the Rotarix vaccine, which was correlated with an increase in the incidence of other viruses in many countries. Further studies are essential to investigate use of other methods for sensitive identifying these agents (Wikswa *et al.*, 2013; Rooney *et al.*, 2014). As well as in the present study, the percentage of positive samples for adenovirus type 40/41 (9%) , Adenovirus type 40/41 has been found to be associated with acute gastroenteritis and is responsible for 1 to 20% of the cases of diarrheal disease in children. Also adenovirus has detected in gastroenteritis studies in developing and developed countries with a incidence ranging from "2-35%" (Andreasi *et al.*, 2008; Verma *et al.*, 2009; Muller, 2010). In Japan the high rate 8% of adenovirus infection made adenovirus a second common agent of acute gastroenteritis (Shimizu *et al.*, 2007). One study done in the coastal belt during 2 month for 35 patients showed the analyzed stool samples were negative for Adenovirus infection and are contrary to the rates reported in other parts of our country. However this does not rule out complete absence of Adenoviral diarrhea in this region if not lower prevalence. A longer study period with greater sample size have given a better picture of the occurrence (Anup *et al.*, 2015). Norovirus and adenovirus considering highly contagious agents which distribute in hospitals ,schools, cruises and restaurants because transmission this agents by direct person to person contact , aerosolization and contaminated water or food .Also these viruses can be transmitted by droplet through vomitus infected person in rooms , as a results of survive fomites droplet on surfaces for long time these viruses have stable particles in the environment and extremely resistant to disinfections so this leads to outbreak infections especially in hospitals (Kirby *et al.*, 2011).

The highest prevalence with norovirus and adenovirus between 13-24 months (24.6%) followed by 7-12 months group (23%). The low ratio of norovirus and adenovirus disease in infants under 7 months of age has been attributed to a higher rate of breast feeding providing partial protection in this age group due to presence of maternal antibodies in the breast milk. In addition ,the results observed increase in the number of infections in children aged < one years because of decreased protection from maternal antibodies which starts to decline at this age as well as using solid food which may be contaminated from environment. The data of this study were in accordance with those gathered by Liu *et al.*, (2014) who found that the positive rate lower in infants under 6 months of age compared to the positive rates of the other age groups. These findings correlate with the observation done by Samarbaf-Zadeh *et al.*, (2010) who found that adenovirus gastroenteritis is very common in children with age 7-12 months , a prevalence of adenovirus infection in age group 7-12 was 33.3% that was higher than other age groups. Also other study found the incidence of adenovirus infection was greater among patients 12-24 months of age 75% than children of <30 months of age 25% (Hamide *et al.*, 2010). Furthermore, the data of this study were in accordance with those gathered by Oldak *et al.*, (2012) who found that majority of infections with norovirus was recorded in children up to two years of age and most commonly affected children between 12 and 23 months of age. A numerous studies have revealed that norovirus infection mostly occurs in children younger than 2 years old (Siqueira *et al.*, 2013). In other study done by Maria *et al.*, (2015) who found that the peak norovirus infection rates occurred in children with age " 0-18 "months . Norovirus and adenovirus infections among mother's milk-fed infants under one year are less than the other age groups. This confirms the highest number of viral infection found in infants being fed with dried milk alone or plus mother's milk. Moreover, the reason for prevalence infection among other age groups infants using dried milk or mixed feeding may be because of using untreated water for preparing milk ,which is one of important the methods for transmission of norovirus and adenovirus, as well as, the proteins in powdered or pasteurized milk can reduce the amount of antibody in breast milk absorption, these kinds of antibodies play a protective role against infectious agents. Furthermore the data of this study were in accordance with those gathered by Al-Dahmoshi *et al.*, (2013) who found that majority of infections with norovirus and adenovirus were recorded in children feeding on dried milk alone or plus mother's milk.

According to the results, diarrhea was the main clinical feature of viral gastroenteritis among norovirus and adenovirus positive patients in the present study. Also important relations between norovirus and adenovirus infection with three parameters of abdominal pain, vomiting and fever were detected in this study. Therefore the clinical features of these patients are similar to other studies conducted in different countries, including Iran Hamkar *et al* (2010), Brazil Maria *et al* (2015) and Iraq Al-Khafaji (2015). There are no significant differences in these symptoms among norovirus and adenovirus positive patients . Seasonality is an important feature of norovirus and adenovirus infection, most studies have demonstrated that norovirus and adenovirus infection peak during winter season. The present study, observed two peak waves of norovirus infections were observed during the cooler

months of the year, one is detected between January - March (16.2%, 24.3% and 40.5% respectively corresponding to 81% of the detected cases) and the second one from October – December (2.7%, 8.1% and 8.1% respectively corresponding to 19% of the detected cases). This is in agreement with studies conducted during cool months of the year (Sabrina, 2014; Maria *et al.*, 2015). The findings of adenovirus infection observed a high number of it occur during January, which is regarded as a month of long rains and beginning of the cool months of the year in our study setting. Therefore, these findings partly support winter month seasonality of adenovirus associated diarrhea as documented by several studies in the past (Rakhi *et al.*, 2011; Al-khafaji, 2015).

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