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Investigation of Norovirus among diarrheal children in Al- Najaf Provence

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Abstract

One hundred diarrheal stool samples were collected from infants with age (1-18 months) admitted to Al-ZahraaHospital for Maternity and Children& Al- Hakeem hospital in AL- Najaf governorate , Iraq , during the period extended from December 2014 to March 2015. All samples were subjected to rapid test specific of Norovirus . The results of rapid identification revealed that 29% (29/100) were positive for Norovirus ,eleven samples (37.9%) of 29 stool samples was detected RT.PCR technique. Most patients were those who had mixed feeding methods using untreated water and lived in poor environmental conditions.

Keywords: Norovirus; Gastroenteritis; Icosahedral

Introduction :

Human Noroviruses are nonenveloped ,icosahedral symmetry are small (26 to 35 nm in diameter), single-stranded positive-sense RNA genome. They are classified as a new genus in the Caliciviridae family, which also includes the genera Sapovirus, Lagovirus, and Vesivirus. Norovirus is the one of etiological agent of acute gastroenteritis and is often responsible for outbreaks in a wide spectrum of community and healthcare settings ,cause diarrhea and vomiting of abrupt onset and short duration ,diarrhea may occur without vomiting and vomiting without diarrhea but recognition of its importance as a pathogen has been limited because of the lack of available, sensitive, and routine diagnostic methods **[1]** .It is estimated that 1-2% of episodes of gastroenteritis in children are due to Norovirus ,there is a peak incidence in the winter months and these viruses contribute significantly as a cause of diarrhea in children in developing countries, children under the age of six months may be at an increased risk of prolonged viral shedding (greater than two weeks), even after the resolution of symptoms **[2]**. The great diversity of Norovirus strains and the lack of complete cross-protection, as well as the lack of long-term immunity, repeated infections can occur throughout life. Norovirus genome easily undergoes mutation that causes antigenic shift and recombination, which, in turn, result in the evolution of new strains that are able to infect susceptible hosts **[3]** .

People get a Norovirus infection directly from an ill individual who did not wash his hands adequately, or indirectly from food or water contaminated by the stool or vomit from an infected person, or from airborne particles produced by those vomiting. outbreaks occur most commonly in semi-closed communities such as schools, hospitals and military settings**[4],[5]** . Nosocomial norovirus outbreaks are one causes of hospital ward closures **[6]**.The aim of the current study : determine the incidence of Norovirus in children under two years of age in Najaf Governorate by detection of Norovirus in patients with diarrhea by rapid test and real-time PCR techniques, respectively.

METHODS

Patients and Samples

One hundred diarrheal stool samples were collected from infants with age (1-18 months) admitted to Al-ZahraaHospital for Maternity and Children& Al- Hakeem hospital in AL- Najaf governorate , Iraq , during the period extended from December 2013 to April 2014 Iraq. All samples

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(patients and 28 apparently healthy children) were collected and subjected to rapid test specific to Norovirus

1- Rapid test

The positive samples for rapid test were diluted with phosphate buffer saline or normal saline and stored at -20˚C in deep freeze.

2- Viral RNA extraction in stool samples

Each stool sample was added into 9 ml phosphate buffered saline (PBS) and mixed for 3 min. The samples were centrifuged at 3,000 rpm for 30 min at 4°C to remove debris, and then the clarified supernatant was collected . According to the manufacturer's instructions, viral RNA was extracted from 140 µl of the samples by using QIAamp microspin columns (viral RNA mini kit: QIAGEN) and stored at -20°C until analysis.

3- PCR

To detect noroviruses in stool, use sensitive reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays **[7].**

Results :

Samples examination

All stool samples that collected in this study examined for detection of Norovirus by rapid test,out of 29/100(29%) were positive , and then examined by RT-PCR technique , total numbers of positive samples were 11 (37.9%) for Norovirus (all patients children aged under two year ranged between 1- 18 months) as in table 1 . The results of rapid test for Norovirus were observed in (figure 4-2)

- Identification of Norovirus

1 – Rapid test :

Twenty nine positive samples for Norovirus were identified by this method according to the presence of two colored line in the test reaction zone on device indicating to positive result(figure 1A) . While a one colored line was visible in the test reaction zone for other samples and control indicating negative result (figure 1B).

Figure (1) Rapid test reaction for Norovirus (A) positive Norovirus (B) negative Norovirus

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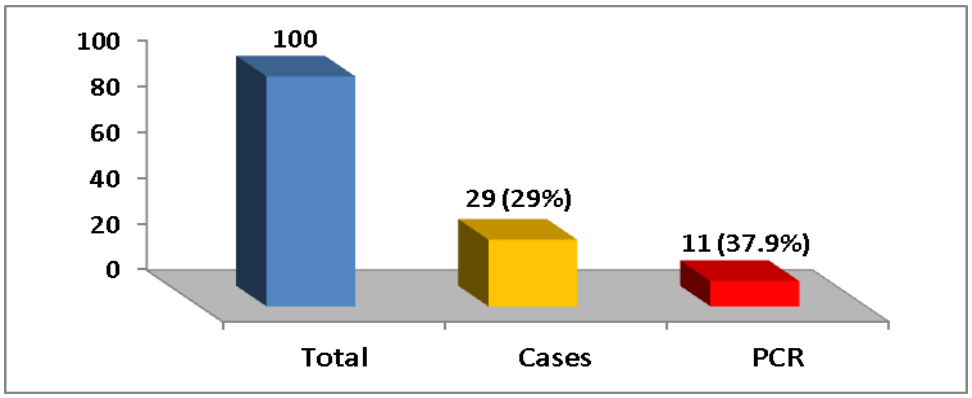

Figure (2): Using sensitive PCR diagnostics, Norovirus was detected in stools of infected individuals .

Table 1 ; distribution of patents according age

Age(months)	1-5	6-10	10-15	16-20	Total
Patients	**25**	**25**	**25**	**25**	**100**
Cases	**7**	**9**	**7**	**6**	**29**
PCR	**2**	**4**	**3**	**2**	**11**
Control	**7**	**7**	**7**	**7**	**28**

2- Molecular detection of Norovirus :

The Molecular detection of Norovirus resulted by RT-PCR technique . the results in figure (3) indicated to amplification plot of RT- PCR showing the positive samples of Norovirus.

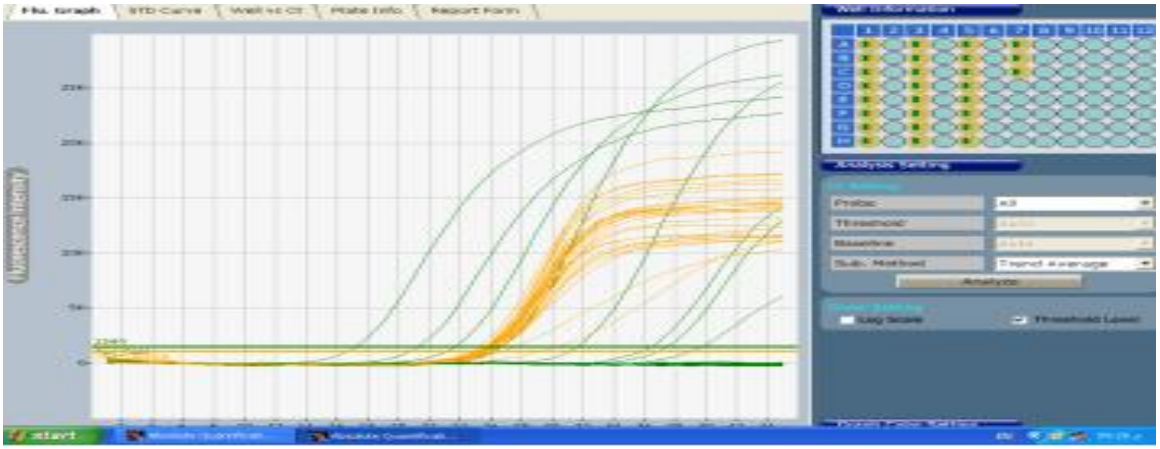

Figure (3) Amplification plot of Real-time PCR amplification of commercial standards and specimens ,internal control positive (orange) for the quantitative detection of Norovirus.

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Discussion

-Detection of Norovirus in stool samples:

According to the results were observed increase in the number of Norovirus infections in children aged (< 18 months) .This increase was explained as resulting from using solid food beside or instead of breast feeding, therefore exclusively depending on breast feeding belonged to the passive immunity that infants receiving from his mother during this period, gave him protecting against the common infections disease like the viruses infections, and decreases of the possible transmission from contaminated food **[8]**.

Comparing the results of the present study with the results of the prevalence of Norovirus in the world,it is likely that the observed increase in relative frequency of Norovirus gastroenteritis cases may be due to implementing a rotavirus vaccination **[9]**, or the results of several studies which depending on molecular methods to detection enteric viruses confirmed that Norovirus accounted for approximately 12% (range 4.4–30.7%) of severe gastroenteritis cases among children under five years of age therefore, many reports that show the importance of Norovirus as a cause of outbreaks, community acquired, sporadic cases of Norovirus gastroenteritis **[10],[11]**.

Detection of Norovirus in children by rapid test resulted in only twenty nine positive samples 29% of Norovirus from examined samples(all patient children aged under 18 months). These results were confirmed also by Real-time PCR and comparable with many studies from different countries of the world . A study in Iraq**[12]** mentioned that percentage of infection with Norovirus was 29.1% (30/103), while in Brazil it was 9.5% **[13]**. The results of the present study is also contrasted with other studies in Nigeria **[14]** which mentioned that the highest percentage of Norovirus infection recorded was 60/161 (37.3%). Poland **[15]** reached to (14.5%), while in Iran **[16]** mentioned that relative frequency of Norovirus in children suffering from gastroenteritis was 6.3% .. In other hand decrease the infection in children over one year age with Norovirus indicated that immunity from the several infections was acquired in the first year of their life **[17]**,on molecular methods were which applied for detection of enteric viruses confirmed that Norovirus accounted by RT-PCR in 43% (16/37) stool samples in Turkey**[18]**.

Conclusions

Norovirus is one of most viral gastroenteritis in developing countries and cause acute diarrhea occurring annually in children aged less than five years throughout the world .

Recommendations

-Performing further investigations for prevalence of Norovirus and Astrovirus in other Governorates of Iraq .

-Further research is needed to understand the correlation between prolonged shedding of norovirus and the risk of infection to susceptible patients .

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