

Molecular and Immunological Study of Rotaviral Infection among Children in Anbar Governorate

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Abstract

Background: Rotavirus infection is considered one of the most common causes of acute gastroenteritis in children less than 5 years and may be responsible for approximately 5% of all child deaths yearly. It is very contagious virus that causes diarrhoea in most babies and young children, vomiting and fever are also common. It is transmitted by fecal-oral contact and contaminated surfaces and hands also directly from person to person also. **Aim of the Study:** The current study aimed to study the role of rotaviral infection among all the clinically diagnosed children with watery diarrhoea, fever and vomiting detect by RT-PCR technique as a cut off molecular diagnosis tool in detection, evaluation the role of some immunological parameters in serum of rotaviral infected children act as mediators in immune response like the level of Interleukin-6 and Interferon gamma by ELISA technique. **Patients and Methods:** A total of (75) stool samples obtained from children <5 years with acute gastroenteritis were randomly collected from Maternity and Children at al-Anbar governorate from October 2019 January 2020. Rotavirus and Adenovirus was detected by rapid test for stool samples and Rotavirus, Norovirus and Astrovirus detect by real-time polymerase chain reaction. 5 ml of blood sample were also obtained to investigate the level of Interleukin-6 and Interferon gamma by ELISA technique. **Result:** Out of 75 enrolled in this study, rapid test rotavirus positive Ag was found in 75 (100%) and 28(37.3%) was adenovirus positive Ag (as mixed infection). RT-PCR were used for detection of viral genome from the total rotavirus result was 72(96.0%) as positive, 46(61.3%), Norovirus 29(38.7%) as positive result and Astrovirus 0.0(0.0%) as negative result. From 73 cases have positive rotavirus results, Interferon gamma recorded 31(43.1%) as positive results and 19(40.4%) as negative while IL-6 recorded 21(29.2%) as positive results and 51(70.8%) as negative result.

Key words: Rotavirus, acute gastroenteritis, IL-6, diarrhea

Introduction

Acute diarrhoea defined as three or more loose of liquid or watery stools or at least one bloody loose stool within 24 h ⁽¹⁾ It is one of the most common diseases in both developed and non-developed countries in infants and young children ⁽²⁾ The incidence of diarrhoea in children under 5 years of age in African, Latin American and Asian countries is estimated at over 1 billion, with approximately 3.3 million deaths per year ⁽³⁾. Rotavirus, a wheel-like virus with 11 double stranded (ds) segments

of RNA non-enveloped linear is responsible for acute gastroenteritis characterized by the sudden onset of watery diarrhoea, fever and vomiting. Seven classes of rotavirus (A to G) are classified according to VP6 antigenicity and RNA sequences, Groups A, B and C are known to infect populations of humans and animals ^(4,5,6). Rotavirus A is considered one of the most important causes of serious acute gastroenteritis in both developed and developing countries ⁽⁷⁾ One out of 67 children under the age of 5 in advanced countries are hospitalized for rotavirus-related disease at least once ⁽⁸⁾ Gastroenteritis of rotavirus usually occurs in children 6 to 24 months of age ^(9,10,11) Under conditions of low temperature, low pH, dry environment and ultraviolet light exposure, the

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virus can survive, making it easier to live for several days. It can also spread through the respiratory tract. (12,13) Rotavirus is transmitted by airborne droplets or through the fecal-oral route, and repeated exposure to viruses such as day care centers increases the risk of infection(14). In 2009, the World Health Organization (WHO) approved the international use of rotavirus vaccines in countries 'national immunization programs to help minimize the high disease burden associated with rotavirus diarrhoea(15) Two oral live attenuated rotavirus vaccines are officially approved for widespread use: Rotarix ® a monovalent rotavirus vaccine and RotaTeq™ a pentavalent rotavirus vaccine (16,17) It has been shown that both vaccines give homotypical and heterotypical safety. The use of these vaccines in Europe, Latin America and Africa has shown a marked decline in child morbidity and mortality due to diarrhoea associated with rotavirus (18). By the end of 2014, more than 70 countries had introduced rotavirus vaccine into their children's routine immunization programs. There are actually no antivirals effective for managing rotavirus disease. To prevent or correct dehydration, oral or parenteral fluids and electrolytes are given in. The current pillar of acute rotavirus gastroenteritis treatment is oral rehydration and early introduction of feedings. Several countries that have routinely implemented rotavirus infant vaccination documented a tremendous impact on severe diarrhoea and rotavirus disease requiring hospitalization(19).

Method and Methods

A total of (75) stool samples obtained from children <5 years with acute gastroenteritis were randomly collected from Maternity and Children at al-Anbar governorate from October 2019 January 2020.

Rotavirus and Adenovirus were detected by rapid

test for stool samples and Rotavirus, Norovirus and Astrovirus were detected by real-time polymerase chain reaction . 5 ml of blood sample were obtained to investigated about level of Interleukin-6 and Interferon gamma by ELISA technique.

Detection of Rota Virus by Rapid Chromatographic Immunoassay, the chromatographic immunoassay performed to the first method, the SD Rota/Adeno Rapid test is a one-step lateral flow ICA that simultaneously detects group A rotavirus and adenovirus in stool samples. It uses colloidal gold-labeled monoclonal antibodies against the capsid protein of gene 6 (VP6) of rotaviruses and the hexon surface antigens of adenoviruses. Fresh stool sample was added to a tube containing 1 mL of diluents, and mixed well. Then, 4-5 drops (approximately 100-125 µL) of this mixed suspension were added to the sample well of the test device, and results were read after 15 min. All procedures were performed according to the manufacturer's instructions.

We were extracted RNA of 75 samples according to the Magnesia®Viral Nucleic Acid Extraction kit that is based on the principle of magnetic extraction method using the Fe₂O₃ magnetic beads coated with cellulose.

Samples were stored in clean Eppendorf tub at deep freeze; kit of extraction was stored at room temperature RT (15-30°C) until the day of the experiment, any lyophilized or dissolved substance must be stored at -20°C (like carrier RNA, Proteinase K) and wash buffer at RT.

Real-time PCR according multiplex RT-PCR for detection and identification of viruses causes acute intestinal infections in children Rotavirus/Norovirus/ Astrovirus (Sacace Company).

Results

Table 1: Detection of Rotavirus and Adenovirus Ag by rapid test

Total No. of Patients	Viral infection		
	Rotavirus	Adenovirus	Mixed infection
	No. (%)	No. (%)	No. (%)
75	75 (100%)	28 (37.3%)	28 (37.3%)

As shown in (Table 1), seventy five (75) caeses enrolled in this study , rotavirus positive Ag was found in 75 (100%) and 28(37.3%) was adenovirus positive Ag (as mixed infection) detected by rapid test.

Table 2: Level Of Interferon gamma in patient with Rotavirus infection

Total No. of Patients	Interferon gamma level	
	Positive	Negative
	No. (%)	No. (%)
75	32(42.67%)	43 (57.33%)

The distribution of positive and negative result of Interferon gamma with respect to rotavirus Ag positive results is represented in Table 2. The table shows that 32(42.67%) of all positive rotavirus cases recorded positive interferon gamma result and 43(57.33%) recorded negative interferon gamma result.

Table 3: Level of Interleukin-6 in patient with Rotavirus infection

Total No. of Patients	IL-6 level	
	Positive	Negative
	No. (%)	No. (%)
75	24 (32%)	51 (68%)

The distribution of positive and negative result of Interleukin-6 with respect to rotavirus Ag positive results is represented in Table 3. The table shows that 24(32%) of all positive rotavirus cases recorded positive IL-6 result and 51(68%) recorded negative IL-6 result.

in the presented study, the highest rate of infection 72(96.0%) was recorded with Rotavirus alone as show in (Figure 1). This percentage is substantially larger than any other percentages of infection. The next percentages were for Norovirus it was 29(38.7%) (Figure 2). and Astrovirus infection recorded (0.0%) (Figure 3).

A single and combined viral infections was recorded

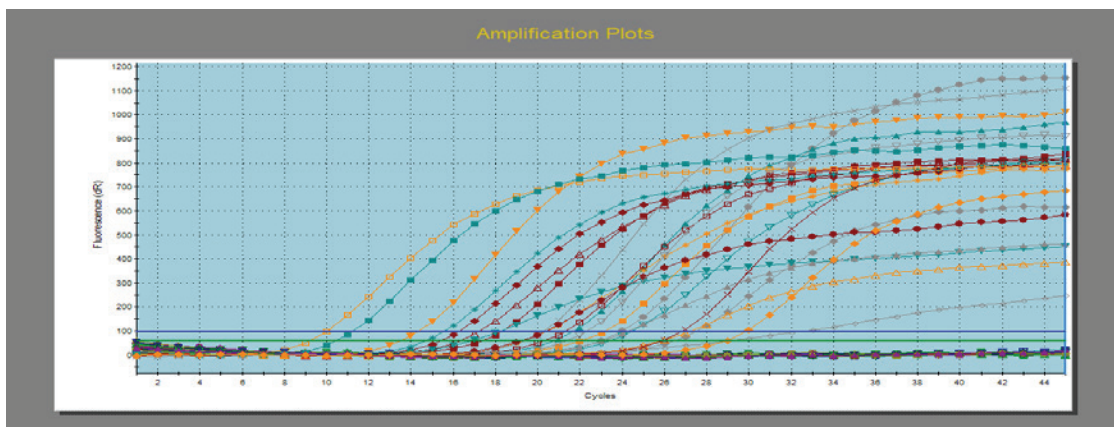


Fig. 1 : Optics Graph of RT-PCR – Human Rotavirus Results Using HEX and FAM Fluorescent Stain.
According to primary curve = 20.94 and threshold = 75.839.

FAM Positive Control threshold =9.877, ct= 44.5

FAM Negative Control threshold =9.877, ct= no ct.

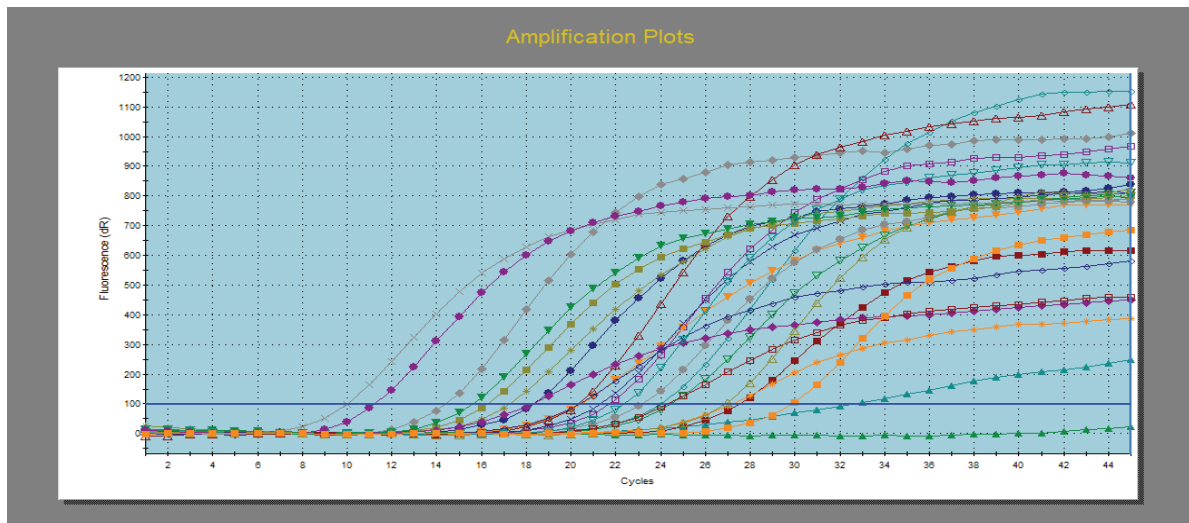


Fig 2: Optics Graph of RT-PCR – Human Norovirus Results Using HEX and FAM Fluorescent Stain.

According to primary curve = 38.56 and threshold = 60.231.

FAM Positive Control threshold =9.877, ct= 44.5

FAM Negative Control threshold =9.877, ct= no ct.

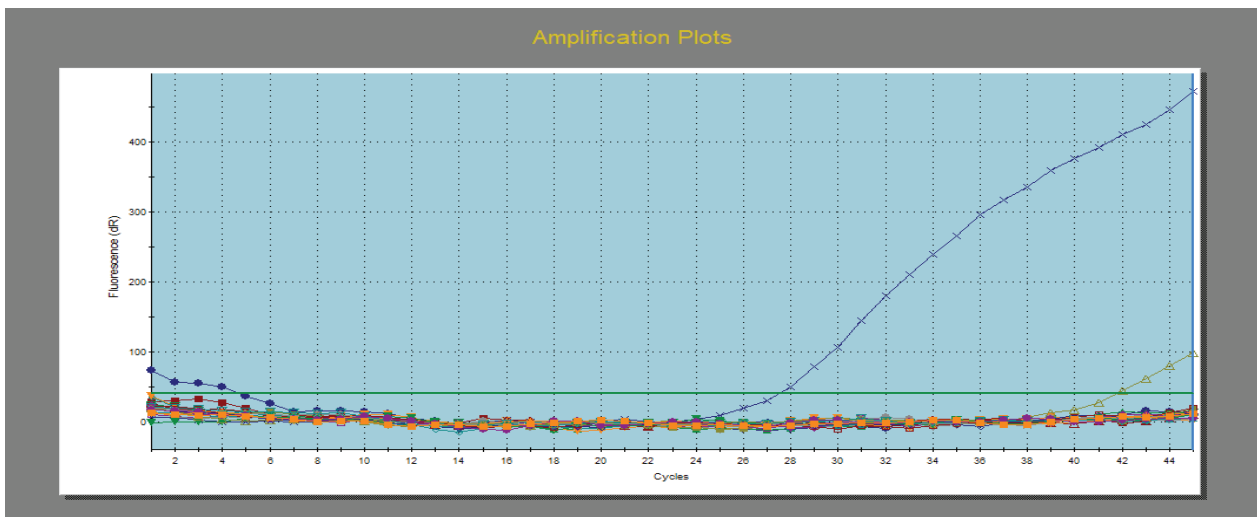


Fig 3 : Optics Graph of RT-PCR – Human Astrovirus Results Using HEX and FAM Fluorescent Stain.

According to primary curve = no c.t and threshold = 40.446.

FAM Positive Control threshold =40.446, ct= 27.59

FAM Negative Control threshold =40.446, ct= no ct.

Discussion

Rotavirus is still the main cause of diarrhea in children. The World Health Organization has indicated that more than half a million children under the age of five face death as a result of contracting rotavirus, and most of them are from poor countries ⁽²⁰⁾. These results are similar to many studies that show the same incidences that Rotavirus infection a main cause of

gastroenteritis⁽²¹⁾. The sample of real time data were 75 admitted cases. the highest rate of infection 72(96.0%) was recorded with Rotavirus alone as show in (Figure 1). This percentage is substantially larger than any other percentages of infection. The next percentages were for Norovirus it was 29(38.7%) (Figure 2). and Astrovirus infection recorded (0.0%)(Figure 3). Most of the admitted cases approximately 65(87%) \leq 15 months , whereas 8 (10%) cases age from 6 – 29 months were above 30 months 2 (3%) cases. , there was a many studies similar to this results⁽²²⁾. In this context, sensitivity of RT-PCR techniques is found to be equals to 0.947 and specificity equals to 0.935. It seems that this test is very accurate in detecting the positive and negative case. Co-infection were recorded in some patients and the highest rate of co-infection was recorded with rotavirus and Norovirus infection. Adults who had Rotavirus as children typically develop a lifelong immunity to it, but with Norovirus the patient may have repeated infection with the age because Norovirus have highly mutant. However, infection with Norovirus can combined with or without fever while, co-infection with Rotavirus and Norovirus had differences in the degree of fever and intensity of diarrhea and vomiting and frequency of symptoms⁽²³⁾. Our design study also provided a useful way to descriptive the T-cell subset secretion to Interferon gamma cytokines. Aging is associated with a decline immune function, IFN-gamma is important immune deviation related cytokines and according to our results age group \leq 15 months show high positivity in the level of IFN-gamma which decline with increasing of age. Human IFN-gamma inhibited adenovirus multiplication in vitro and prevent viral adsorption and penetration⁽²⁴⁾. This study examined the association between serum IL-6 and disease severity in children and involved in both Rotavirus and Norovirus. There were significant correlation between the severity of illness with rotaviral infection and IL-6 levels, it show a significant discriminating ability between rotavirus and Norovirus infection^(25,26). The table 2 shows that 24(32.%) was found with positive interleukin-6 , 51(68%) was found with patient with negative interleukin-6. these results are similar to many studies that show the role of hnterleukin-6 in patient with Rotavirus infection^(27,28). To study the variation and significant of serum interferon gamma level in children with Rotavirus enteritis, there was significant correlation between serum IFN-Y and gender. Serum IFN-Y associated with

the severity of Rotavirus enteritis and have protection effects against acute Rotavirus infection at the early stage^(29,30).

Conclusions

Children are more affected with Rotaviral infection than adults and high incidence of infection is found in winter and spring, In this study there is no significant correlation between Rotavirus Ag and IFN-Y, but there is a correlation between Rotavirus Ag and IL-6. Others viruses can cause acute gastroenteritis like Norovirus, Adenovirus and Astrovirus.

Conflict of Interest: None

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Ethical Clearance: Not required

References

1. Nguyen TV, Le Van P, Le Huy C, Weintraub A. Diarrhea caused by rotavirus in children less than 5 years of age in Hanoi, Vietnam. *J Clin Microbiol*. 2004;42:5745-50.
2. Mokomane M, Kasvosve I, Melo E, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. *Ther Adv Infect Dis*. 2018;5:29-43.
3. GBD Diarrhoeal Diseases Collaborators. Estimates of global, diarrhoeal diseases: a systematic analysis for the Global Burden regional, and national morbidity, mortality, and aetiologies of Disease Study 2015. *Lancet Infect Dis*. 2017;17:909-48.
4. Kapikan AZ, Hishino Y, Chanock RM. Rotaviruses. In: Knipe DM, Hudson MJ, editors. *Fields virology*. 4th ed;2001.p.1787-833.
5. Anderson EJ, Weber SG. Rotavirus infection in adults. *Lancet Infect Dis*. 2004;4(2):91-99.
6. Matthijnsens J, Otto PH, Ciarlet M, Desselberger U, van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol*. 2012;157(6):1177–1182.
7. Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, et al. Rotavirus infection. *Nat Rev Dis Primers*. 2017;3:17083.
8. Committee PRE (2006) The paediatric burden of rotavirus disease in Europe. *Epidemiol Infect*

134:908–916.

9. Chae JH, Kim MJ, Kim DH, Lee KY, Kang JH, Lee JS(2007) Epidemiologic study of rotaviral gastroenteritis in Daejeon, Korea, 2001–2005. *Korean J Pediatr Infect Dis*14:155–161.
10. Kim JS, Kang JO, Cho SC, Jang YT, Min SA, Park TH et al (2005) Epidemiologic profile of rotavirus infection in the Republic of Korea: results from prospective surveillance in the Jeongeub district, 1 July 2002 through 30 June 2004. *J Infect Dis* 192(Suppl 1): S49–S56.
11. Seo JK, Sim JG (2000) Overview of rotavirus infections in Korea. *Pediatr Int* 42:406–410.
12. Fischer TK, Breese JS, Glass RI (2004) Rotavirus vaccines and the prevention of hospital-acquired diarrhea in children. *Vaccine* 22 (Suppl 1):S49–S54.
13. Parez N (2008) Rotavirus gastroenteritis: why to back up to development of new vaccines? *Comp Immunol Microbiol Infect Dis* 31:253-269.
14. P. H. Dennehy. Transmission of rotavirus and other enteric pathogens in the home. *Pediatr. Infect. Dis. J.*,19 (10):S103{S105, 2000.
15. World Health Organization. Rotavirus vaccines: an update. *Wkly Epidemiol Rec.* 2009;84:533–40.
16. World Health Organization. Rotavirus vaccines WHO position paper-January 2013. *Wkly Epidemiol Rec.* 2013;88:49–64.
17. World Health Organization. Meeting of the strategic advisory Group of recommendations. *Wkly Epidemiol Rec.* 2009;84:517–32.
18. Hassine-Zaafrane M, et al. The molecular epidemiology of circulating rotaviruses: three-year surveillance in the region of Monastir, Tunisia. *BMC Infect Dis.* 2011;11(266).
19. PatelMM, Glass R, Desai R, Tate JE, Parashar UD. Fulfilling the promise of rotavirus Vaccines: how far have we come since licensure? *Lancet Infect Dis* 2012; 12:561–70.
20. Al-Shuwaikh AM, Ibrahim IA, Al-Shwaikh RM. Detection of E. coli and Rotavirus in Diarrhea among Children Under Five Years Old. *Iraqi J Biotechnol.* 2015;14(1):85–92.
21. John O, Sastry G, David A, et al. Applied regression analysis: a research Tool. *Applied Statistical Methods.* University of Florida. Springer-Verlag, New York. 2nd ed 2009.
22. Chen, Shan-Ming, et al. “Diagnostic performance of serum interleukin-6 and interleukin-10 levels and clinical predictors in children with rotavirus and norovirus gastroenteritis.” *Cytokine* 59.2 (2012): 299-304.
23. Peculiarities of Norovirus and Rotavirus infection in hospitalized young children Irena Narkeviciute etal. *J pediatr Gastroenterd Nutr.* 2008 .Mar; 46(3):289-292.
24. Yen C J 1, Lin S L and Huang K T .2000 Age-associated changes in interferon-gamma and interleukin-4 secretion by purified human CD4+ and CD8+ T cells *J Biomed Sci.* Jul-Aug 2000;7(4):317-21
25. Shan-Ming Chen 1: Min-Sho Ku: Ming-Yung Lee; et al. 2012. Diagnostic performance of serum interleukin-6 and interleukin-10 levels and clinical predictors in children with rotavirus and norovirus gastroenteritis. *PubMed.* 59(2):299-304
26. Diagnostic performance of serum interleukin-6 and interleukin-10 level and clinical predictors in children with Rotavirus and Norovirus gastroenteritis. Shan-Mingchenet al. *Cytokine.* 2012.Aug Pusmed. National Library of Medicine.
27. Jiang, B., et al. “Cytokines as mediators for or effectors against rotavirus disease in children.” *Clinical and diagnostic laboratory immunology* 10.6 (2003): 995-1001.
28. Chen, Shan-Ming, et al. “Diagnostic performance of serum interleukin-6 and interleukin-10 levels and clinical predictors in children with rotavirus and norovirus gastroenteritis.” *Cytokine* 59.2 (2012): 299-304.
29. Variation and significance of serum and stool IL-6 and IFN-γ level in children with Rotavirus enteritis. You-Geu Gaoetal. Aug 2006 ; 8(4): 304-6 Pusmed. National Library of Medicine.
30. Diagnostic performance of serum interleukin-6 and interleukin-10 level and clinical predictors in children with Rotavirus and Norovirus gastroenteritis. Shan-Mingchenet al. *Cytokine.* 2012.Aug Pusmed. National Library of Medicine.