ORIGINAL RESEARCH PAPER VOLUME-6 | ISSUE-8 | AUGUST - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

Comparison of Latex Agglutination with Enzyme Immunoassay for Detection of Rotavirus in Children with Acute Diarrhea in Diyala Province, Iraq

Biology

Ansam Dawood Salman Department of Biology, College of science, Diyala University, Diyala, Iraq.

Abdul-Razak SH. Hasan Department of Microbiology, College of Medicine, Diyala University, Diyala, Iraq

ABSTRACT

Rotavirus (RV) is the most important etiological agent of diarrhea in children under 5 years old, mainly in developing countries. The laboratory diagnosis is usually based on detection of viral antigen using enzyme immunoassay or direct latex agglutination techniques. **Objectives**: To compare the sensitivity and specificity of enzyme immunoassay versus direct agglutination test for the detection of rotavirus in stool of children less than 5 years of age with acute diarrhea in Diyala province. **Patients and methods**: The present study was conducted during the period from 1/August/2012 to 30/November/2013. 120 patients with acute diarrhea were included. The patients attended Al-Batool Maternity and children Teaching Hospital and other Primary Health Care Centers in Baquba, the center of Diyala province. Seventy (58.3%) patients were males and 50 (41.7%) were females. Detection of RV in stool specimens using direct agglutination test (Diaspot-USA) was performed as soon as possible after collection. The enzyme linked immunosorbant assay (ELISA) was carried out using the commercially available kit (DRG-Germany). Data were statistically analyzed and P value < 0.05 was considered significant. **Results**: The results showed that the detection rate of RV in stool specimens by DAT and ELISA were 70% and 93.3% respectively. The sensitivity, specificity, positive predictive value and negative predictive values of DAT was 75.56%, 66.67%, 97.22%, and 15.39% respectively, and for ELISA was 91.3%, 66.67%, 97.67%, and 33.33% respectively. Spearman's correlation analyses revealed that the age group 10-15 months was significantly affected (P= 0.046). **Conclusion**: Rotavirus antigen detection by EIA is a rapid, sensitive, and specific method, and could be used in large scale application for screening stool samples of patients with acute diarrhea.

KEYWORDS:

Rotavirus, Acute gastroenteritis, Diyala province

Introduction

Rotavirus is the leading cause of severe diarrhea worldwide among children aged <5 years. It account for5% of all deaths in children younger than 5 years in developing countries [1,2]. Based on data from the global surveillance network of 2009, which found that a median of 36% of enrolled and tested children aged < 5 years hospitalized with diarrhea (range: 25%-47% among thesix WHO regions) tested positive for rotavirus. Therefore, the WHO recommended inclusion of rotavirus vaccination in all national immunization programs [3].The RV belongs to the Reoviridae family; the viral genome consists of 11 segments of double-stranded RNA (ds-RNA), which encodes six structural proteins, and six non-structural proteins [4,5]. RVs are transmitted mainly by feco-oral spread, with an incubation period of 18-36 hours [6]. RV induce diarrhea is thought to be caused by a combination of factors beside the effect of the RV nonstructural protein 4, which is thought to be a viral enterotoxin [7.[The latex agglutination technique was affirmed to be a good, simple, and rapid tool for the detection of RV in stool specimens [8,9]. Previous studies using latex agglutination tests for detection of RV in stool specimens yielded variable detection rates ranged from 10%-60%[10,11]. On the other hand, enzyme linked immunosorbant assays are the most preferably used due to their high sensitivity and specificity for detection of RV in different pathological specimens [10,12,13]. In countries of the eastern Mediterranean region, studies among patients with acute diarrhea, RV was detected in 40% of inpatients and 23% of outpatients by 3 years of age [14]. In a population-based study on infants and young children with acute diarrhea in Saudi Arabia, American Journal of Infectious Diseases and Microbiology 71 RV was detected in 95% of stool specimens [15]. In Jordan, the RV was detected in 39.8% of stool specimen of hospitalized children below 5 years of age, and the most affected age group was less than 2 years [16]. In Iraq, it has been found that nearly two third of healthy population in Diyala province are liable for infection by RV as they lack anti-RV IgG antibodies [17]. Besides that, RV was found as a common cause of acute diarrhea particularly among children below 5 years of age [11]. In a recent molecular study using multiplex RT-PCR and sequence analyses of group A rotavirus detected from fecal specimens of Iraqi children with acute gastroenteritis, found that the G2 was the most prevalent genotype, followed by G1 genotype [18].

Patients and methods:

The present study was conducted during the period from 1/August/2012 to 30/November/2013. One hundred twenty patients with acute diarrhea were included. The patients attended Al-Batool Maternity and children Teaching Hospital and other Primary Health Care Centers in Baquba, the center of Diyala province. Seventy (58.3%) of patients were males and 50 (41.7%) were females. The age range was 2 months to 5 years. The mean age of patients was 11.01 ± 9.71 months. Information regarding age, sex, residence, type of feeding and source of drinking water were collected. Human privacy was respected by taken children parent's consensus. Detection of RV in stool specimens using direct agglutination test (Diaspot-USA) was performed as soon as possible after collection. The enzyme linked immunosorbant assay (ELISA) was carried out using the commercially available kit (DRG-Germany). These techniques were performed according to the manufacturer's instructions. Positive or negative cases were determined by comparing the absorbance value of each sample in this study with that of the cut-off control value, samples with an absorbance value less than the cutoff value were considered as negative; samples with a value above the cut-off value were considered positive.The statistical package SPSS version 10.0 was used for statistical analyses. Data were statistically analyzed and P value < 0.05 was considered significant

Results

The results of the present study, generally showed that the detection rate by ELISA technique is highest than that of DAT technique (93.3% versus 70%), Table 1.

Table 1. Detection rate of RV by DAT versus ELISA techniques of 120 fecal samples

Techniques	No. positive (%)	No. negative (%)
DAT	84 (70)	36 (30)
ELISA	112 (93.33)	8 (6.67)

The sensitivity, specificity, positive predictive and negative predictive values of DAT was 75.56%, 66.67%, 97.22% and 15.39% respectively, while for ELISA was 91.3%, 66.67%, 97.67% and 33.33% respectively. Table 2, showed that the highest detection rate of RV in both ELISA and DAT was among children below 2 years of age. However, in both techniques there was no age group significantly affected. Regarding the sex distribution, the results revealed that in both DAT and ELISA techniques the detection rate of RV was highest among femalescompared to males. Nevertheless these differences failed to reach the levels of significance, Table 3

Table 2. Distribution of ELISA and DAT results according to age

Age (months)	Total	Result of DAT			Result of ELISA		
		Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
5≤	26	20(76.92)	6(23.07)	0.059 [NS]	25(96.15)	1(3.84	0.259 [NS]
>5 - ≤10	57	42(73.68)	15(26.31)		53(92.98)	4(7.01)	
>10 - ≤15	18	14(77.77)	4(22.22)		18(100.00)	0(0.00)	
>15 - ≤ 20	10	5(50)	5(50)		9(90.00)	1(10.00)	
>20	9	3 (33.33)	6 (66.66)		7(77.77)	2(22.22)	
Total	120	84	36		112	8	

NS: Insignificant

Table 3. Distribution of DAT and ELISA results according to sex

Sex	Total	Result of DAT			Result of ELISA		
		Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
Male	70	48(68.57)	22(31.42)	0.686 [NS]	64(91.42)	6(8.57)	0.323 [NS]
female	50	36(72)	14(28)		48(96.00)	2(4.00)	
Total	120	84	36		112	8	

NS: insignificant

Table 4. Distribution of DAT and ELISA results according to residence

Residence	Total	Result of DAT			Result of ELISA		
		Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
Urban	64	45(70.31)	19(29.68)	0.936 [NS]	61(95.31)	3(4.68)	0.353 [NS]
Rural	56	39(69.64)	17(30.35)		51(91.07)	5(8.92)	
Total	120	84	36		112	8	

NS: insignificant

Table 4, showed that the detection rate of RV was highest among children residing in urban areas compared to those in rural areas in both techniques. However, in either technique, these differences were insignificant. The results also showed that the highest detection rate of RV was among those children on mixed feeding (breast plus bottle feeding) in both techniques. However, in either case, the differences were statistically insignificant, Table 5.

Table 6, shows that in both techniques, the detection rate of RV was highest among those children drinking municipal water compared to those consume bottled water. However, none of these differences reach the levels of statistical significance. Table 7, revealed the Spearman's correlation between DAT and ELISA results and different demographic factors. The age of patients was significantly correlated with DAT results (p= 0.046) with a correlation coefficient (0.183). Of note, the effect of age was statistically insignificant in paired comparison as previously shown in Table 2. However, the ELISA results showed no correlation with any of these demographic factors.

Table 5. Distribution of DAT and ELISA results according to type of feeding (n=110)

Type of feeding	Total	Result of DAT			Result of ELISA		
		Positive (%)	Negative (%)	P value	Positive (%)	Negative (%)	P value
Breast	15	11(73.33)	4(26.66)	0.494 [NS]	13(86.66)	2(13.33)	0.314 [NS]
Bottled	59	41(69.49)	18(30.50)		56(94.91)	3(5.08)	
Mixed	36	29(80.55)	7(19.44)		35(97.22)	1(2.77)	
Total	110	81	29		104	6	

NS: Insignifica

Table 6. Distribution of DAT and ELISA results according to source of drinking water

Source of drinking water	Total	Result of DAT			Total	Result of ELISA		
		Positive (%)	Negative (%)	P value		Positive (%)	Negative (%)	P value
Municipal	184	68(80.95)	16(19.04)	0.288 [NS]	94	89(94.68)	5(5.31)	0.261 [NS]
Bottled	36	26(72.22)	10(27.77)		26	23(88.46)	3(11.53)	
Total	120	94	26		120	112	8	

NS: Insignificant

Table 7. Spearman correlation between DAT and ELISA results and different parameters in 120 children

Parameters test	DAT		ELISA test	
	r	p	r	p
Age	0.183	0.046	0.091	0.325
Sex	- 0.037	0.689	- 0.09	0.326
Residence	0.007	0.937	0.085	0.357
Type of feeding	- 0.087	0.368	- 0.126	0.191
Source of drinking water	0.097	0.291	0.103	0.264

r= correlation coefficient, p =probability (level of significance)

Discussion

Rotavirus is the most common cause of childhood gastroenteritis particularly in developing countries. In Diyala, as an Iraqi province, about 60 kilometers NorthEast Baghdad, the capital, previous studies had documented high prevalence of RV infection among general population [17]. Furthermore, RV was found as the major cause of acute diarrhea among children below 5 years old [11]. It is worth to mention that RV vaccine is not included in the expanded program of vaccination in Iraq yet. Therefore, searching for rapid and accurate diagnosis of RV infection is essential for early management and for prevention of severe complications of gastroenteritis [10]. In the present study, the detection rate of RV in stool specimens of patients with acute diarrhea was highest using ELISA versus DAT techniques. Additionally, the results revealed that ELISA technique is more sensitive and specific, and it is more suitable for large-scale screening of patients for detection of RV infection. These results are consistent with most previous studies worldwide, affirming that, although DAT are able to quickly give results, but it has lower sensitivity versus ELISA which appeared to be an optimal method for detection of RV infection [9,10,13]. On the other hand, it has been stated that DAT could be used as an alternative method for the rapid screening of RV in fecal specimens, especially during acute gastroenteritis outbreak season [15,19]. The high sensitivity and specificity of ELISA techniques are arise through the fact that these diagnostic kits incorporate highly specific anti-rotavirus monoclonal antibodies which have high affinity for capturing RV antigens in clinical specimens [10,12,20]. Other advantages of the enzyme immunoassays include their stability, simplicity and cost effectiveness, making these assays widely used for detection of RV antigen and antibodies and for epidemiological studies of rotavirus [21]. The results of both DAT and ELISA found that the age ≤ 2 years was significantly correlated with RV infection. Again these results are supporting our previous findings

[11], and also in agreement with other workers [2,14,22,23]. Additionally, both techniques consistently found that females were more affected than males. However, different studies had obtained different results in this regard [2,15,24]. Furthermore, the results of DAT and ELISA were concordant in that RV infection was higher among urban versus rural areas. Of note, similar results were obtained by other studies [14]. It seems that the high population density and better income in urban areas increase hospitalization for RV infection. Another finding that was consistently yielding by DAT as well as ELISA results, that RV infection was less frequent in breast-fed than among bottle-fed babies. Undoubtedly, this is related to the protective ability of maternal immunoglobulins transferred through the breast milk. These results were in agreement with most previous studies [21,23,25,26]. Moreover, the common result was that unsafe municipal water was associated with higher RV infection rate. This finding seems logical, as unsafe drinking water is an important source of infection by RV especially in developing countries [14,23,27]. It can be concluded that ELISA technique is the optimal method for detection of RV in stool specimens. However, DAT can be used as an alternative method for screening RV infection especially during outbreak season.

Acknowledgements

The authors would like to thank Al-Batool Maternity and children Teaching Hospital central health laboratory and primary health care centers in Diyala province for their support.

References

1. Agocs, M.M.; Serhan, F.; Yen, C.; Mwenda, J.M.; de Oliveira, L.H.; Teleb, N,; Wasle,y A.; Wijesinghe, P.R.; Fox, K.; Tate, J.E.; Gentsch, J.R.; Parashar, U.D. and Kang, G. WHO global rotavirus surveillance network: a strategic review of the first 5 years, 2008-2012. MMWR Morb. Mort. Wkly Rep. 2014; 63 (29): 634-7.
2. John, B.M.; Devgan, A. and Mitra, B. Prevalence of rotavirus infection in children below two years presenting with diarrhea. Med. J. Armed Forces India, 2014; 70 (2): 116-9.
3. Frieden, T.R.; Jaffe, H.W.; Stephens, J.W.; Thacker, S.B. and Zaza, S. Rotavirus surveillance --- worldwide, 2009. MMWR Morb.
4. Bishop, R. Discovery of rotavirus: Implications for child health. J.Gastroenterol. Hepatol. 2009; 24 (3): S81-5.
5. Pesavento, J.B.; Crawford, S.E.; Ester, M.K. and Prasad, B.V. Rotavirus proteins: Structural and assembly. Curr. Trop. Microbiol. Immunol. 2006; 309: 189-219.
6. Strauss, J.H. and Strauss, E.G. Viruses that contain doublestranded RNA: Family Reoviridae. In: Viruses and human disease. 2nd. Ed. 2008. Academic press. pp 193-210.
7. Borghan, M.A.; Mori, Y.; El-Mahmoudy, A.B.; Ito, N.; Sugiyama, M.; Takewaki, T. and Minamoto, N. Induction of nitric oxide synthase by rotavirus enterotoxinNSP4: Implication for rotavirus pathogenicity. J. Gen. Virol. 2007; 88 (7): 2064-72.
8. Dusetty, P.; Velazquez, F.R.; Gutierrez-Escolano, A.L. and Ludert, J.E. Evaluation of the second generation of the commercial latex agglutination test for the detection of rotavirus antigens in fecal samples. J. Clin. Virol. 2013; 57 (1): 88-90.
9. Chieochansin, T.; Vutithanachot, V.; Theambooniers, A. and Poovorawan, Y. Evaluation of rapid test for human rotavirus A in Thai children with acute gastroenteritis. Clin. Lab. 2014; 60 (3):511-4.
10. Lee, S.Y.; Hong, J.H.; Lee, S.W. and Lee, M. Comparison of latex agglutination, immunochromatography and enzyme immunoassay methods for the detection of rotavirus antigen. Korian J. Lab. Med. 2007; 27 (6): 437-41.
11. Hasan, A.SH.; Al-Duliami, A.A.; Mehdi, SH. and Al-Azawi, A.Y. The prevalence of rotavirus infection in Baquba-Diyala province. Iraqi J. Commun. Med. 2011b; 24 (3): 200-4.
12. Vainio, K.; Nordbo, S.A.; Njolstad, G.; Storvold, G.; Dollner, H.; Midgaard, C.; Bosse, F.J.; Rognlien, A.G.; Rojahn, A.; Wathne, K.O. and Flem, E. Detection and characterization of group A rotavirus in children hospitalized with acute gastroenteritis in Norway 2006-2008. J. Med. Virol. 2009; 81 (10):1839-44.
13. Forster, J.; Guarino, A.; Parez, N.; Moraga, F.; Roman, E.; Mory, O.; Tozzi, A.E.; de Aguileta, A.L.; wahn, U.; Graham, C.; Berner, R.; Ninan, T.; Barberousse, C.; Meyer, N. and Soriano-Gabarro, M. The rotavirus study group. Hospital-based surveillance to estimate the burden of rotavirus gastroenteritis among European children younger than 5 years of age. Pediatrics, 2009; 123 (3): 393-400.
14. Malek, M.A.; Teleb, N.; Abu-Elyazeed,R.; Riddle, M.S.; Sherif, M.E.; Steele, A.D.; Glass, R.I. and Bresee, J.S. The epidemiology of rotavirus diarrhea in countries in the Eastern Mediterranean Region. J. infect. Dis. 2010; 202 Suppl: S12-22.
15. Ghazi, H.O.; Khan, M.A.; Telmesani, A.M.; Idress, B. and Mohamed M.F. Rotavirus infection in infants and young children in Mekkah, Saudi Arabia. J. Pak. Med. Assoc. 3005; 55 (6): 231-4.
16. Nafi, O. Rotavirus gastroenteritis among children aged under 5 years in Al-Karak, Jordan. East Mediterr. Health J. 2010; 16 (10): 1064-9.
17. Hasan, A.SH.; Al-Azzawi, D.S.; Al-Duliami, A.A. and Al-Azawi, A.Y. Antirotavirus IgG seropositivity among healthy population and patients with acute diarrhea in Baquba-Diyala province. Iraqi J. Commun. Med. 2011a; 24 (2): 100-4.
18. Ahmed, S.; Klena, J.; Albana, A.; Alhamandi, F.; Oskoff, J.; Soliman, M.; Heylen, E.; Teleb, N.; Husain, T. and Matthijnssens, J. Characterization of human rotaviruses circulating in Iraq in 2008: Atypical G8 and high prevalence of P [6] strains. Infect. Genet. Evol. 2013; 16:212-7.
19. Khamrin, P.; Tran, D.N.; Chan-it, W.; Thongprachum, A.; Okitsu, S.; Maneekarn, N. and Ushijima, H. Comparison of rapid methods for screening of group A rotavirus in stool samples. J. Trop. Pediatr. 2011; 57(5): 375-7.
20. Knisley, C.V.; Bednarz-Prashad, A.J. and Pickering, L.K. Detection of rotaviris in stool specimens with monoclonal and polyclonal antibody-based assay system. J. Clin. Microbiol. 1986; 23(5): 897-900.
21. Angel, J.; Franco, M.A. and Greenberg H.B. Rotavirus immune responses and correlates of protection. Curr. Opin. Virol. 2012; 2 (4): 419-25.
22. Kota, M.; Bino, S.; Delogu, R.; Simaku, A.; Neza, B.; Ruggeri, F.M. and Fiore, L. Epidemiology of rotavirus diarrhoea in Albania. Arch. Virol. 2014; 159 (9): 2491-5.
23. Panda, S.; Deb, A.K.; Chawla-Sarkar, M.; Ramamurthy, T.; Ganguly, S.; Pradhan, P.; Chakraborty, A.; Desai, S.; Gupte, M.D. and Dhere, R. Factors associated with diarrhoea in young children and incidence of symptomatic rotavirus infection in rural West Bengal, India. Epidemiol. Infect. 2014; 142 (9): 1848-58.
24. Salim, H.; Karyana, I.P.; Sanjaya-Putra, I.G.; Budiarsa, S. and Soenarto, Y. Risk factors of rotavirus diarrhea in hospitalized children in Sanglah Hospital, Denpasar: a prospective cohort study. BMC Gastroenterol. 2014 Mar 26; 14: 54. 25.
[25] Bonkoungou, I.J.; Sanou, I.; Bon, F.; Benon, B.; Coulibaly, S.O.; Haukka, K.; Traore, A.S. and Barro, N. Epidemiology of rotavirus infection among young children with acute diarrhoea in Burkina Faso. BMC Pediatr. 2010; 10: 94.
26. Plenge-Bonig A.; Soto-Ramirez, N.; Karmaus, W.; Petersen, G.; Davis, S. and Forster, J. Breastfeeding protects against acute gastroenteritis due to rotavirus in infants. Eur. J. Pediatr. 2010; 169 (12): 1471-6.
27. Naficy, A.B.; Abu-Elyazeed, R.; Holmes, J.L.; Rao, M.R.; Savarino, S.J.; Kim, Y.; Wierzba, T.F.; Peruski, L.; Lee, Y.J.; Gentsch, J.R.; Glass, R.I. and Clemens, J.D. Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. Am J. Epidemiol. 1999; 150 (7): 770-7