Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 289-294

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 289-294**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.507.030

A Comparative Analysis of Enzyme-Linked Immunosorbent Assay and Rapid Card Test for Diagnosis of Rotavirus Antigen in Acute Diarrhea Below Five Years Children

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Keywords

ELISA, Group A Rotavirus, Immuno-chromatography, Watery diarrhea.

Article Info

Accepted:
12 June 2016
Available Online:
10 July 2016

A B S T R A C T

Group-A Rotavirus are responsible for severe watery diarrhea in young children. Rapid diagnosis of Rotavirus associated diarrhea can prevent inappropriate administration of antibiotics and help in preventing the spread of multi- drug resistance. Rotavirus antigen is detected by ELISA and with one step rapid antigen test showed an incidence of 22% using ELISA and 21.33% using Rapid test respectively. Rapid test showed a sensitivity of 96.97% and specificity of 100% in comparison to ELISA. The Rotavirus infection was highest in age group of 6 months to 24 months (62.77%) and in male (63.64%). The infection was maximum during winter and presented with triad of diarrhea, vomiting and fever. Majority of cases had watery diarrhea with severe dehydration.

Introduction

Acute diarrheal disease is a major public health problem leading to high morbidity and significant mortality in both developed and developing countries like India. Rota virus infection is the third most common cause of severe diarrhea in young children Worldwide (Kapikian *et al*., 1996) Almost all kids have had a Rota virus infection by the time they are 5 years old. It is estimated that Rota Virus infection annually causes 111 million episodes of gastroenteritis requiring home care, 5 million clinic visits, million hospitalization and approximately 600,000 deaths in children less than 5 years of age Worldwide (Parashar *et al*., 2003). There are seven Rota virus groups known to infect the humans, among them the most dominant is group A (Parashar *et al*., 2001). WHO estimated that diarrhea is responsible for 18% deaths among children younger than 5 years of age (Bryce *et al*., 2005).

In view of high incidence of morbidity and mortality in developing countries like India

there is a need for rapid and sensitive detection method in routine diagnostic laboratory, which perform antigen detection using enzyme immunoassay (EIA), latex agglutination assay (Bryce *et al*., 2005) or immuno chromatography. Evidence by direct virus detection using electron microscopy is not practical by routine laboratories. Although Rota virus can be isolated from stool sample by culture, but it is a cumbersome process and needs equipped laboratory require skilled personnel. The recent advance in antigen detection based on immunological techniques using monoclonal antibodies has gained the attention of researchers. Therefore the direct detection of antigen in stool sample by rapid one step assay is inexpensive, easy to handle, non invasive procedure, no specialized instrument is required and have high sensitivity (Cukor *et al*., 1984).

Materials and Methods

A prospective study was undertaken between January 2014 to October 2014 in the Microbiology and Pediatrics Department, SIMS, Hapur after obtaining a written & Informed consent by parents of children below five years with acute diarrhea. Clearance was taken from the ethical committee of the institute.

150 Freshly passed stool, samples were collected in wide mouth sterilized container from hospitalized children and OPD patients of SIMS, Hapur of acute diarrhea by the help of their parents or caretaker and transported to the Microbiology department as soon as possible. Samples were kept at 4°C and tested within 24 hours of collection. Rotavirus antigen is detected by immunochromatographic test (SD Bioline test kit) and ELISA kit (premier rotaclones) according to manufacturer's instruction (One step rotavirus antigen test Korea, 2011; Rotavirus antigen ELISA, 2012).

Results and Discussion

Rotavirus was found to be a common cause of diarrhea in children less than 5 years of age with an incidence of 22% using ELISA and 21.33% using Rapid test.ICG was compared to standard ELISA showed a sensitivity of 96.97% and specificity of 100%. The infection was maximum during the winter months. Male children (62.7%) were affected more than female (37.3%). There was no statistically significant difference in the frequency of rotavirus infection among patients from urban and rural area. A high number of (72.6%) of rotavirus positive cases by ELISA presented with a triad of diarrhea, vomiting and fever followed by vomiting preceding diarrhea (21%) and only diarrhea (6.4%). Dehydration was significant in children 72% showed severe dehydration.

In present study, out of 150 patients there were 32 rotavirus positive cases, with Rapid test. When compared to standard test - ELISA, Rapid test showed a sensitivity of 96.97% and specificity of 100%. ELISA is clearly the most sensitive method for detection of rotaviruses and is ideal for screening of large number of fecal specimens in a single sitting.

Now Rapid tests for rotavirus detection are also available such as ICG. This test facilitates qualitative information of rotavirus infection based on the presence of a rotavirus specific band. In addition, the Rapid test (ICG) requires less handling of the sample and quick as the results are available in short time (Momenzadeh *et al*., 2008).

The present study is matched with observations made by other workers. Momenzadeh *et al*., compared Rapid test with ELISA for detection of rotavirus. They

considered ELISA as standard test and found the sensitivity and specificity of ICG to be 87.7%, 98.6%, respectively, which is comparable to present study.

Another study done by Dennehy P H reported the sensitivity, specificity of ICG as 94% and 100%, respectively and that of ELISA, 95.5% and 100% respectively. ICG test was reported as a sensitive, specific and relatively simple test (Dennehy *et al*., 1999).

Dewar J *et al*., found out sensitivity of 88% (66/75) and a specificity of 100% of Rapid test when compared with the ELISA. Wilhelmi I *et al*., compared Rapid test method, with enzyme immunoassay. The statistical values of the enzyme immunoassay, and Rapid test method were respectively 96% and 99% for sensitivity; 99% and 96% for specificity (Wilhelmi *et al*., 2001).

In the above study Rapid test technique showed high sensitivity and specificity and was rapid and easy to perform in the routine clinical laboratory. ELISA is the standard test for detection of rotaviruses but because of limited availability and rather high cost we compared ICG to ELISA. In our study sensitivity (96.97%) and specificity (100%) of ICG was comparable to ELISA and made the diagnosis simple, rapid, cost-effective and convenient. A study conducted by Momenzadeh, *et al*., and Kim J *et al*., also showed similar results (Kim *et al*., 2014). Rotavirus accounting 20-50% of hospitalization for Diarrhea in children worldwide and is the most important cause of death of children (14). The present study showed an incidence of 22% of rotavirus diarrhea by ELISA in children less than five years of age. The result of this study is supported by other studies from Chandigarh (16-19%), Kolkata (5-22%) and Chennai (20.8%) (Broor *et al*., 2003).

Majority of infected children in present study were between 6 to 24 months of age (62.77%). It appeared that children below 6 months of age were initially protected by maternal antibodies to rotavirus infection and by acquired active immunity after 24 months of age (Saravanan *et al*., 2004). This result is similar to other studies done in Eastern Nepal and other countries (Shariff *et al*., 2003).

In present study 90.47% cases had watery diarrhea and majority (76.19%) are presented with severe dehydration due to elaboration of a potent enterotoxin which causes profuse watery diarrhea, destroys the intestinal epithelial surface leading to blunted villi, extensive damage, and shedding of massive quantities of virus in stools (Glass *et al*., 2006).

Table.1 Age & Sex Distribution of Diarrhea Cases

Age	Male	%	Female	%	Total	%
6 - 12 months	25	16.67%	20	13.33%	45	30.00%
1 - 2 years	32	21.33%	15	10.00%	47	31.33%
2 - 3 years	17	11.33%	13	8.67%	30	20.00%
3 - 4 years	9	6.00%	6	4.00%	15	10.00%
4- 5 years	7	4.67%	6	4.00%	13	8.67%
Total	**90**	**60.00%**	**60**	**40.00%**	**150**	**100.00%**

Table.2 Monthly Distribution of Rotavirus Positive Cases

Month	Number of Cases	Number of Rotavirus Positive Cases by ELISA	% Rotavirus Positive Cases
Jan	20	4	12.12%
Feb	13	4	12.12%
Mar	20	6	18.18%
April	30	8	24.24%
May	14	3	9.09%
June	11	0	0.00%
July	11	2	6.06%
Aug	10	1	3.03%
Sep	11	2	6.06%
Oct	10	3	9.09%
Total	**150**	**33**	**100%**

Table.3 Age & Sex Distribution of Rotavirus Positive Cases by ELISA

Age	Male	%	Female	%	Total no. of cases	Total %
6 - 12 months	8	24.24%	3	9.09%	11	33.33%
1 - 2 years	8	24.24%	5	15.15%	13	39.39%
2 - 3 years	3	9.09%	3	9.09%	6	18.18%
3 - 4 years	2	6.06%	1	3.03%	3	9.09%
4- 5 years	0	0.00%	0	0.00%	0	0.00%
Total	**21**	**63.64%**	**12**	**36.36%**	**33**	**100.00%**

Table.4 Diarrhea in various Age Groups

Age	Total No. of Cases	ELISA No. (+/-)	Rapid Test No. (+/-)
6 - 12 months	45	11/34	10/35
1 - 2 years	47	13/34	13/34
2 - 3 years	30	6/24	6/24
3 - 4 years	15	3/12	3/12
4- 5 years	13	0/13	0/13
Total	**150**	**33/117 22.00%**	**32/118 21.33%**

Table.5 Comparison of Rapid Test with ELISA

Sensitivity	96.97%
Specificity	100.00%
Positive Predictive Value	100.00%
Negative Predictive Value	99.15%
Accuracy	99.33%

Table.6 Statistical Significance of Rotavirus with respect to months

Observed Value		**Cooler Months (Oct - Feb)**	**Hotter Months (Mar-Sep)**	**Total**
Rotavirus Cases	**Positive**	11	22	33
	Negative	32	85	117
Total		**43**	**107**	**150**

Table.7 Statistical Significance of Rotavirus with respect to Age Group

Observed Value		**6 months to 2 years**	**More than 2 years**	**Total**
Rotavirus Cases	**Positive**	24	9	**33**
	Negative	68	49	**117**
Total		**92**	**58**	**150**

In conclusion, rotavirus was significantly associated with diarrhea in children of 6-24 months of age, more in male children, associated with severe dehydration, vomiting, and fever. It is not routinely diagnosed in most of the hospitals due to non availability of tests and its clinical spectrum of signs and symptoms which are mimics to other types of diarrhea. In India few hospitals use ELISA for the diagnosis of Rota Virus infection because of limited availability and cost. On the other hand Rapid test showed a sensitivity of 96.97% and specificity of 100% in comparison to ELISA, that is good agreement with ELISA. It has the advantage of being a quicker, cost-effective, and useful for testing even a single specimen, convenient, not requiring additional equipments, readily available, simple to perform and easy-to-read results.

Acknowledgement

JKA and DC collected the data, SPG guided the study, and DA critically reviews the article.

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How to cite this article:

Jugal Kishor Agarwal, S.P. Garg, Dayachand and Dipty Agrawal. 2016. A Comparative Analysis of Enzyme-Linked Immunosorbent Assay and Rapid Card Test for Diagnosis of Rotavirus Antigen in Acute Diarrhea Below Five Years Children. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 289-294. doi: http://dx.doi.org/10.20546/ijcmas.2016.507.030