Comparison of Diagnostic Modalities for Diagnosis of Rotavirus Infection Using Rapid Antigen Diagnostic Kit and ELISA with Epidemiological Prevalence in Children of Acute Diarrhea below 5 Years of Age

Dr Jugal Kishor Agarwal[1], Dr Deepak Gupta[2], Dr. Dipty Agrawal[3], Dr. Manoj Kumar[4], Mr.Matloob Ahmad[5].

1. Assistant Professor, Microbiology, Rama Medical College, Hapur, U.P
2. Professor, Microbiology, Rama Medical College, Hapur, U.P
3. Assistant Professor, Anasthesia, Rama Medical College, Hapur, U.P
4. Assistant Professor, Microbiology, Rama Medical College, Hapur, U.P
5. Assistant Professor, Microbiology, Rama Medical College, Hapur, U.P.
corresponding author- Dr. Manoj Kumar,
Assistant Professor, Microbiology, Rama Medical College, Hapur, U.P

ABSTRACT
Introduction- Rotavirus is one of the leading cause of Pediatric diarrhea. There is a need for data analysis on the prevalence of rotavirus diarrhea. This study was carried out to determine the prevalence of rotavirus infection in children up to the age group of 5 year who presented with acute diarrhea. The study also ascertains factors associated with rotavirus infection in them. **Material and Method**-In the present study Rotavirus antigen is detected by ELISA and with one step rapid antigen kit based on immuno-chromatography principle, consider ELISA as gold standard rapid card test was compared with it. Rapid diagnosis of Rotavirus associated diarrhea can prevent inappropriate administration of antibiotics and help in preventing the spread of multi-drug resistance. **Result-** Out of 240 children 150 were males and 90 were females with more representative age group were 6 months- 2 years of age. These suspected acute diarrhea children were analyzed for rotavirus in present study and of these 52 (21.66%) were found to be positive for rotavirus antigen by ELISA and 50(20.8%) were positive via rapid antigen card test. Sensitivity of Rapid card test was 96.2% while specificity of rapid test was found to be 98.93%. Positive predictive value (PPV) was 96.2% while Negative Predictive Value of Rapid Card test (NPV) was 98.93%. p value was <0.00001 which is statistically significant and in favour of ELISA. **Conclusion**-There is urgent need to do the investigation of rotavirus antigen detection in paediatric acute diarrhea cases, for preventing in the MDR cases.

I. INTRODUCTION:
Acute diarrheal disease is a major public health concern and is also a leading cause of infant morbidity and mortality in both developed and especially in developing countries like India. It is the second leading cause of death in children below 5 years of age with approximately 10% of death each year [1]. Amongst the etiological agents of diarrhea, viruses are contributing about 80% of cases. Among the viruses, rotavirus infection is third most common cause of severe diarrhea in young children worldwide [1]. Almost all the kids have had one episode of rotavirus infection by the time they are 5 years old.

Rotavirus is a genus of reoviridae family and is currently classified into seven sergroups (A-G) , of these seven groups, group A virus is the most important cause of severe acute diarrhea in infant and young children globally[2]. Rotavirus causes approximately 11.37 million episodes of acute gastroenteritis (AGE) in children <5 years annually in India requiring at least 3.27 million OPD and 8.7 lakh in patients admissions, resulting in total health expending costs of 10.37 billion per year Indian rupee (INR). According to 2011 rotavirus infection data analyses , it is estimated that there were 78000 deaths due to rotavirus associated AGE in India, with the majority (75.6%) in the first 2 year in life[2]. World health organization (WHO) estimated that diarrhea is responsible for about 18 % of death among children <5 years of age[3].

For prevention of rotavirus associated AGE, two orally administered rotavirus vaccines named Rotarix RV1, monovalent G1P; (Glaxo smith kline Biologicals, Belgium) and Rota Teq C RV5; pentavalent G1, G2, G3,G4,P(8); (Merk vaccines, NJ, USA), has been commercially available in India since 2006 . In 2015, the ROTAVAC vaccine (Bharat Biotech, India), containing Live 116 E rotavirus strain (G9P), was introduced as the first indigenously developed vaccine with lower cost [13]. In April 2016, vaccine of rotavirus was included into universal immunization programme (UIP) by Govt. of India in 4 states (Andra Pradesh, Haryana, Himachal Pradesh and Odisha) with the subsequent introduction into 5 additional states by September 2017 (Rajasthan, Madhya Pradesh, Assam, Tripura, Tamil Nadu).

In the view of high incidence of morbidity and mortality, there is a need of rapid diagnostic methods with high sensitivity as well as specificity, which can be used in routine diagnostic laboratory, performing antigen detection using either enzyme immunoassay (EIA), latex agglutination assay [7] and immunochromatography tests [8,9]. Evidence by direct virus detection using electron microscopy is not feasible by routine laboratories. Rotavirus can be isolated from stool sample by culture, but it is a tedious job and needs sophisticated laboratory with skilled personnel.

The recent advance in antigen detection technique is based on immunological principles using monoclonal antibodies, which has gained the attention of the researchers for identification of rotavirus at point of care. Therefore, direct detection of antigen in stool samples by rapid one step assay is easy, inexpensive, non-invasive procedure, no specialized instrument is required and has high sensitivity [10].
The present study aims to detect Rotavirus antigen using rapid diagnostic kits and ELISA at the point of care for earliest diagnosis of rotavirus diarrhea in children < 5 years of age.

II. RESULT AND DISCUSSION:

Out of 240 children 150 were males and 90 were females with more representative age group were 6 months- 2 years of age (as shown in table 1),which is statistically significant ($x^2= 7.82$, p value <0.05), it is in accordance with various recent and previous studies done in India that ranged from 20-75%. As study done by Saravanan P. etal [9] who reported 29.95% rotavirus positive children among 6-12 months of age groups. Another study done by Banerjee I.et al [10] and Jijoho Mischael M.A.et al[1] , reported that median age of

detection of rotavirus infection in children admitted in hospital was 10 months and 11 months respectively. Robin SM.et al [11] also reported that majority of rotavirus diarrhea cases occurred in children younger than 2 years, which is in concordance with the present study.

With regard to the dehydration states of the patients, more than half of patients were dehydrated (60%), 25% of childrens were moderately dehydrated, remaining 15% of the children were severely dehydrated, which in concordance with study done by Jijoho Mischal M.A. et al [1] (Fig 1). Majority of infected children in our study were between 6-24 month of age 42/240 (17.5%), which showed a significant difference between other age group of children and infection (P<.001) (Table 2), which is in concordance with the studies done by Saravanan P. etal[9] and Banergee I. et al[10]. It appeared that children between 6 months of age were initially protected by passively transferred maternal antibodies to rotavirus infection and by acquired immunity after 24 months of age 9. The study result is similar to other studies done in Eastern Nepal and other countries [14].

In present study majority of cases presented with watery diarrhea with dehydration of various degree due to elaboration of a potent enterotoxin, which causes profuse watery diarrhea, destroys the intestinal epithelial surface leading to blunted villi, extensive mucosal damage and shedding of massive quantities of virus in stools [15].

A total of 240 suspected acute diarrhea children were analyzed for rotavirus in present study and of these 52 (21.66%) were found to be positive for rotavirus antigen by ELISA and 50(20.8%) were positive via rapid antigen card test(Table 3). The present study showed lower positivity in comparison to study done by Saravanan P et al (29.95%)[9] and by Jijoho Mischael et al (39.2%)[1]. In our study. Sensitivity of Rapid card test was 96.2% while specificity of rapid test was found to be 98.93%. Positive predictive value (PPV) was 96.2% while Negative Predictive Value of Rapid Card test (NPV) was 98.93%. p value was <0.00001 which is statistically significant and in favour of ELISA (Table 4). Considered ELISA as gold standard screening test but it also requires sophisticated instruments alongwith trained staff, therefore rapid card testing can be taken as point of care diagnostic test, although it is inferior to ELISA in diagnostic accuracy for detection of rotavirus antigen.

III. LIMITATION:

Study of duration was of short duration (10 months), because of this we do not conclude exact prevalence of rotavirus in that localized area. Also there was no electrotyping done which is a potential tool for studying the molecular epidemiology of human rotavirus infection.

IV. CONCLUSION:

A Rotavirus associated acute diarrhea is more prevalent in children between 6 months to 24 month age group. It is more in male as compared to female. It is not routinely diagnosed in most of the hospital due to non availability of tests. Present study shows that rapid card tests are inferior as compared to ELISA but ELISA is not used routinely in India, only few hospitals uses ELISA for the diagnosis of Rotavirus infection. Mostly rapid card test use in routine investigation which is easy to handle and having low cost, and not required trained staff. By doing so, if we investigate that infection is due to rotavirus we restrict inappropriate misuse of antibiotics, so as to prevent multidrug resistance and with only symptomatic treatment we can treat acute diarrhea cases.

REFERENCES

[1]. Jijoho.M.M.A et al. Epidemiological survey of Rotaviruses responsible for Infantile diarrhea by Immunomolecular Technique in Cotonou (Benin, west Africa). International Journal of Microbilogy. Volume 2018, Article Id 3602967,7 pages.

[2]. Giri et al. BMC public Health (2019) 19:69. Rota virus gastroenteritis in Indian children <5 years hospitalized for diarrhea,2012 to 2016.

[3]. Kapikan AZ, Chamock, R.M. 1996. Rotavirus In: Fields BN Knipe DM, Howley PM et al. eds. Fields virology. 3rdedn. Philadelphia:Lippincott- Raven, PP 1657-1708.

[4]. Parashar, U.D., Hummel mane, G. 2003. Breese JS, Miller MA, Glass RI. Global illness and death. Caused by Rotavirus disease in children. Emerg. Infect. Dis., 9: 565-572.

[5]. Bryce, J., Boschi – Pinto, C., Shibuya, K., Black, R.E. 2005. WHO estimate so the causes of death in children Lancet, 365(9465): 1147-52.

[6]. Dennehy, P.H. ,Hartin M., Nelson, S.M. and Rebing S.F. 1999. Evaluation of immune card STAT Rota virus assay for detection of group- A Rota virus in fecal specimen. J. Clin. Microbiol, 37(6);1977-1979.

[7]. Dewar J., De Beer M., Elliott E. ,Monaisa P., Semenya D., Steele A. 005. Rapid detection of Rota viruses- Are labotatories underestimating infection in infants. South African Med. J. 95(7);494-5.

[8]. Cukor. G. 1984. Detection of Rotavirus in human stools by using monoclonal antibody. J. Clin. Microbial., 19: 888-892.

[9]. Saravanan, P., Ananthan, S., Ananthasubramanian, M. 20004. Rotavirus Infection among infants and young childrenin Chennai, South India. Indian J. Med. Microbiol., 22(4): 212–21.

[10]. BanerjeeI,Ramani S, Primerose B, Mosis P, Iturriza GM, Gray J et al. Comparative study of epidemiology of Rotavirus in children from Community based cohort and a hospital in South India. J Clin Micro 2006 July; 44(7):2468-74

[11]. Robin SM, Nogueira MB, Hakim VM,Torrecilha VTG, Lerner H and Tsuchiya LRV.Comparison of latex Agglutination with Enzyme Immuno assay for detection of Rotavirus in fecal specimens. Am J ClinPathol 200;117:392-394

[12]. Broor S., Ghosh D., Mathur P., 003. Molecular epidemiology of Rota virus in India. Indian J. Res., 118;59-67.

[13]. BenerjeeI, Ramani S, Primerose B, Mosis P, Iturriza GM, Gray J et al. modification of rotavirus multiplex RT-PCR for detection of G12 strain based on characterization of emerging G12 rotavirus strains from South India. J Med Virol. 2007;79:1413-21.

[14]. Shariff, M., Deb, M., Singh, R. 2003. A study of diarrhea among children in eastern Nepal with reference to rotavirus. Indian J. Med. Microbiol., 21(2): 87–90.

[15]. Glass, R. I., Parashar, U.D., Bresee, J.S., Tureios, R., Fischer, T.K., Widdowsen, M.A., et al. 2006.Rotavirus Vaccines: Current Prospects and Future Challenges. Lancet, 368: 323-32.

Table 1. **Age & Sex distribution of acute diarrhea cases:**

Age	Male	%	Female	%	Total	%
6-12 months	52	21.66	28	11.66	80	33.33
13-24months	48	20	32	13.33	80	33.33
25-36 months	28	11.66	16	6.66	44	18.34
37-48months	12	5	9	3.75	21	8.75
49-60 months	10	4.16	5	2.08	15	6.25
Total	150	62.48	90	37.52	240	100%

Table 2. **Age & sex distribution of rotavirus positive acute diarrhea cases**:

Age groups	Male	%	Female	%	Total	% Total
6-12 months	10	20.83	6	12.5	16	33.33
13-24 months	15	28.85	11	21.15	26	50
25-36 months	5	10	2	3	7	13.46
37-48 months	2	3	1	1	3	5.71
49-60 months	0	0	0	0	0	0
Total	32	62.68	20	37.32	52	100%

Table 3. **Rotavirus antigen detection via ELISA and Rapid test**:

Age groups	Total	ELISA +VE	ELISA –ve	Rapid test +VE	Rapid Test -ve
6-12 months	80	18	62	17	63
13-24 months	80	25	55	24	56
25-36 months	44	6	38	6	38
37-48 months	21	3	18	3	18
49-60 months	15	0	15	0	15
Total	240	52	188	50	190

Table 4. Total number of Positive and Negative cases observed with Rapid Card Test compared to ELISA

		ELISA		Total
		Positive	Negative	
Rapid Card Test	Positive	50	2	52
	Negative	2	186	188
Total		52	188	240

Table 5. Diagnostic efficacy of Rapid Card Test compared with ELISA

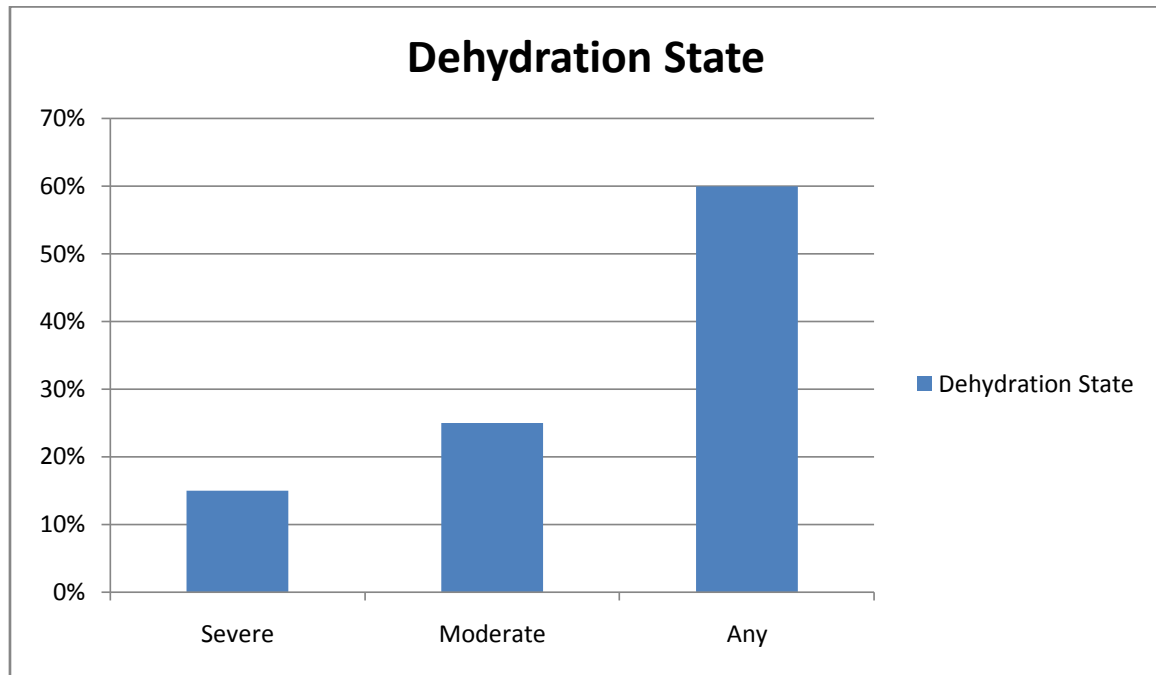|	
Sensitivity of Rapid card test	96.2%
Specificity of Rapid card test	98.93%
Positive predictive Value of Rapid card test	96.2%
Negative Predictive Value Of Rapid Card test	98.93%
Accuracy	98.36%
p Value	<0.00001

Figure1.   Distribution of study population according to dehydration state