

ORIGINAL RESEARCH ARTICLE

PREVELANCE OF ROTA VIRUS DIARRHOEA IN CHILDREN AND COMPARATIVE EVALUATION OF DIAGNOSTIC MODALITIES- A STUDY DONE AT TERTIARY LEVEL HOSPITAL OF WESTERN RAJASTHAN

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ABSTRACT:

Background: Rota virus is most common cause of severe diarrhoea in infants and young children worldwide. In India it is estimated that 20-30% of hospitalised diarrhoea[3] cases are caused by Rotavirus, a pathogen that is not affected by improvement in clean water and hygiene.

Results: The present study is a prospective study of total 447 patients whose stool samples were submitted for examination from May 2016 to April 2017. Out of them 126 (28.19%) samples were positive by Rotavirus LCT. Prevalence of Rotavirus diarrhoea in children less than 3 years was 32.83% (110/335) and in children more than 3 year were 14.28% (16/112). This difference was statically significant (P value 0.0013). The total agreement of LCT in comparison to ELISA was 85.32%. The Cohen's Kappa coefficient for this comparison was 65.21%.

Conclusions: Our finding suggest that chances of Rotavirus infection has not related to living standard and sex distribution and in resource poor setting where ELISA is not performed LCT can be an alternative to ELISA for Rotavirus diagnosis.

Key Words: LCT, ELISA ,EM,PAGE,RT -PCR,

Introduction: The World Health Organization estimatd 1.5 bilion episode of diarrhoea occures in children <5 year old annually resulting in 3 million deaths¹. Rota virus is most common cause of severe diarrhoea in infants and young children worldwide. Nearly 25-35% of childhood hospitalisation for severe diarrhoea result from infection with rotavirus, a pathogen that is not affected by improvement in clean water and hygiene². In India it is estimated that 20-30% of hospitalised diarrhoea[3] cases are caused by Rotavirus and India spend approximately 2-3.4 billion (US\$ 41-72 million) annually for treatment of Rota virus diarrhoea in children⁴.

Rota virus is member of family Reoviridae. The Rotavirus genome contains double stranded RNA (dsRNA) genome with 11 segments. Mature Rota virus particle are 75 nm in size with icosahedral symmetry. The capsid made up of three layer – the outer layer, intermediate layer and inner core layer. The route of transmission is feco-oral route with symptoms develop after 1-2 days. Majority of children infected with rotavirus with in the first three year of life, with peak incidence of Rotavirus diarrhoea between 6 to 24 months[5]. The presenting sympyoms are vomitting and diarrhoea with little or no fever. The diarrhea was generally considered to be malabsorptive, secondary to enterocyte destruction. There is no blood and mucus in stool. Oral rehydration therapy for patient are most likely to fail because of vomitting, which is also a common presenting symptom[2]. Government of India included the Rotavirus vaccine in universal immunization programme in Rajasthan in April 2017 in fourth phase (Rotavac- an indigenously developed vaccine). Rotavirus vaccine reduces the Rotavirus associated death and decreases number of severe cases and hospitalization.

Laboratory procedures for diagnosis of rotavirus include electron microscopy (EM), passive latex agglutination assays (LA), electro phenotyping using polyacrylamide gel electrophoresis (PAGE), enzyme linked immunosorbent assays (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR)[6]. Enzyme linked Immuno Sorbent Assay (ELISA) is method of choice for routine screening⁶. It is rapid (4 hours), easy to perform, sensitive and specific. Documentation of Rota virus disease in rural and semi urban area is a challenge so Lateral Chromatographic Test (LCT) is economical alternative to ELISA. This is a bed side test which is performed without need for special equipment.

This study aims to determine the prevalence of rotavirus diarrhoea and various influencing factors. This study also try to determine correlation between LCT and ELISA at tertiary level hospital.

Material and Method: This is a hospital based prospective study carried out in Umaid Hospital Microbiology Lab, Department of Microbiology Dr. S.N. Medical College Jodhpur for a period of 12 month from May 2016 to April 2017. Samples were taken from patients having complaints of ‘Diarrhoea’ attending OPD/Hospitalised for same in Dept. of Paediatrics, Umaid hospital and Mathura Das Mathur Hospital. Informed and written consent was taken from patients and their attendants. The study protocol was approved by the Ethical committee of Dr. S. N. Medical College Jodhpur, Rajasthan.

Laboratory procedures: All patients who gave consent were instructed to collect freshly passed stool sample without contamination of urine. After proper labeling sample was

transported to microbiology laboratory and processed immediately for Macroscopic, Microscopic examination and Culture. All Macroscopic and Microscopic examination was done on same stool sample. No preservative were added during sample collection and examination.

Rotavirus antigen detection was done by LCT (SD Bio line) and ELISA (Rotavirus ELISA – Premier Rotaclone) according to manufacturer’s instructions. Samples were preserved at -20⁰C for ELISA.

RESULTS:

The present study is a prospective study of 447 patients whose stool samples were submitted for examination from May 2016 to April 2017. Out of 447 diarrhoea patients 259(57.94%) patients were male and 188(42.06%) patients were females. Maximum number of patients were enrolled in age group 6-24 month were 261(58.39%) followed by age group 2-5 year were 107(23.94%). In this study 233(52.13%) patients from rural background and 214(47.87%) from urban background.

In this study 447 stool sample were tested for Rotavirus antigen detection. Out of them 126(28.19%) samples were positive for Rotavirus by LCT. Most Rotavirus positive patient was from age group 6-24 month 94(74.60%) followed by age group 2-5 year 25(19.84%), age group >5 year 4(3.17%) and in age group 0-6 month 3(2.38%).

Prevalence of Rotavirus were maximum in 6-24 month (36.02%) followed by age group 2-5 year (23.36%), 0-6 month (9.68%) and > 5 year (7.69%). Prevalence of rotavirus diarrhoea were significantly high in age group 6-24 month with P value (0.0006). Rotavirus diarrhoea was more prevalent in rural patients (28.76%) then urban patients (27.57%), but this difference was statically not significant (P value 0.80).

Out of total 447 stool sample tested for Rotavirus most positive samples were received during month of November-2016 54(42.86%) followed by in month December-2016 32(25.40%) and January-2017 14(11.11%). The prevalence of Rotavirus was also highest during month of November-2016 (52.94%) followed by December-2016 (38.55%) and January-2017 (26.42%). Rotavirus diarrhoea was more prevalent during winter months of a year.

Due to financial reason Rotavirus ELISA were done for all LCT positive samples and randomly selected LCT negative samples. Out of 184 samples Rotavirus ELISA were positive for 131(71.20%) and negative for 53(28.80%) samples.

Comparative results of LCT and ELISA shown in table no. 13. The total agreement of LCT in comparison to ELISA was 85.32%. The Cohen's Kappa coefficient for this comparison was 65.21%.

(There isn't clear-cut agreement on what constitutes good or poor levels of agreement based on Cohen's kappa, although a common set of criteria is: less than 0% no agreement, 0-20% poor, 20-40% fair, 40-60% moderate, 60-80% good, 80% or higher very good.)

DISCUSSION:

This is a hospital based prospective study carried out in Umaid Hospital Microbiology Lab, Dr. S.N. Medical College Jodhpur. This study was done from May 2016 to April 2017 for a period of 16 month. During the study period a total of 447 stool samples were examined for Rotavirus antigen.

In present study Rotavirus associated with 28.19% (126/447) of diarrhoea cases as a sole agent (94.44%) or associated with another parasitic pathogen (5.56%). Rotavirus mostly presented with acute watery diarrhoea in age group 6-24 month of age as compared to other age group (P value 0.0006). Prevalence of Rotavirus in present study coincides with different studies done across the world.

According to study done by Nair et. al. (2010)[6] in Kolkata Rotavirus was detected in 19.6% of diarrhoea patient and as the sole pathogen in 42.2% cases and mixed pathogen seen in 57.8% (285/493) of cases. Mixed infection of Rotavirus with parasite was seen in 23.7%. Prevalence of rotavirus in this study is lower than present study but mixed infection is much higher than the present study this may be due to difference in age structure of patient population, geographical and environmental condition. In this study Rotavirus was associated with patients in <5 years age group (48.1%) than in the higher age group (9.7%) and this association was statistically significant.

According to study done by Moyo et. Al.[7] in 2011, they found enteric viruses in 32.2%. In children aged 7-12 months diarrhoea was more often due to viruses, mainly Rotavirus and Norovirus. Prevalence in this study coincides with present study and study from Jordan[12] also have similar result. Another study from Saudi Arabia⁹⁰ have lower prevalence (22%) and study From Utter Pradesh India⁹⁸ showed higher prevalence (41.2%) of diarrhoea in hospitalized patient.

Prevalence of Rotavirus associated diarrhoea in present study in male and female are 30.5% and 25% respectively, but the difference is statically not significant. A study by Teotia et. al.[8] in U.P. India have similar findings as Rotavirus positivity rate did not differ significantly by sex (34.7% among boys vs. 24.3% among girls).

Rotavirus diarrhoea has seasonal variability with peak seen during the month of November to January with prevalence ranging from 26.42% to 52.94%. Similar result has showed by Nair et. al.[6] in Kolkata according to this study Rotavirus mediated diarrhoea had distinct seasonality with peaks during December-February (winter).

In present study the total agreement rate between LCT and ELISA is 85.32% and Cohen's Kappa coefficient is 65.16%. Which show a good agreement between these two tests. Study done by Kim et. al.[9] in 2014 shows total agreement between LCT and ELISA 95.8% and Kappa coefficient 0.857, which show excellent agreement between these two tests.

CONCLUSION:

In this study most of cases were detected during winter months and didn't find the significant difference of prevalence according to sex and demography. This finding shows that chances of Rotavirus infection has not related to living standard and sex distribution. So only mode of prevention is vaccination. Rotavirus vaccine reduces the Rotavirus associated death and decreases number of severe cases and hospitalization. There is good agreement ($K=65.21\%$) between Rotavirus LCT and ELISA, so in resource poor setting where ELISA is not performed LCT can be an alternative to ELISA for Rotavirus diagnosis.

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Table No. 1

Distribution of Rotavirus according to Age group

Age group	Total	Positive	
		N	Percentage
0_1	138	45	35.71%
1_3	197	65	51.59%
3_5	64	12	9.52%
5_10	32	2	1.59%
11 & more	16	2	1.59%
	447	126	100%
SEX			
Male	259	79	62.70%
Female	188	47	37.30%
Demography			
Rural	233	67	53.17%
Urban	155	59	46.83%

Fig.1. Distribution of Rotavirus according to Sex

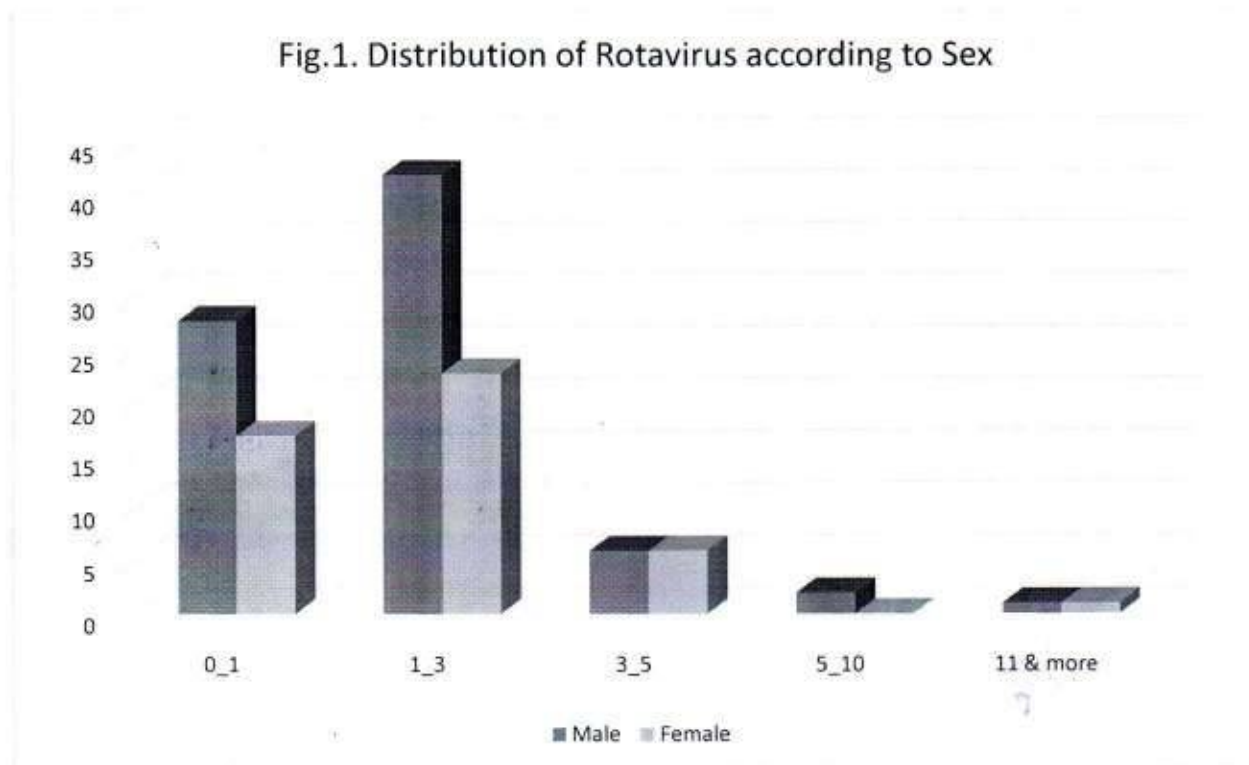


Fig.2. Distribution of Rotavirus according to Demography

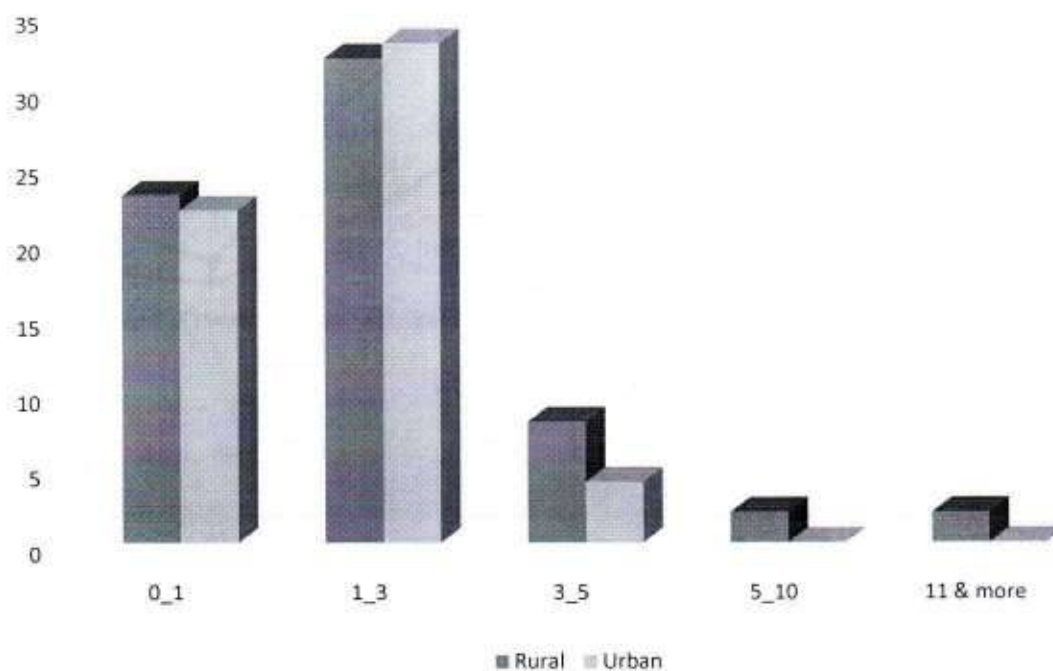


Table No. 2

Seasonal variation in Rotavirus

Month	Total	Positive	Percentage
May-16	10	0	0.00%
Jun-16	6	0	0.00%
Jul-16	11	0	0.00%
Aug-16	66	5	3.97%
Sep-16	29	6	4.76%
Oct-16	44	6	4.76%
Nov-16	102	54	42.86%
Dec-16	83	32	25.40%
Jan-17	53	14	11.11%
Feb-17	7	0	0.00%
Mar-17	19	3	2.38%
Apr-17	17	6	4.76%

Fig.3. Seasonal variation of Rotavirus

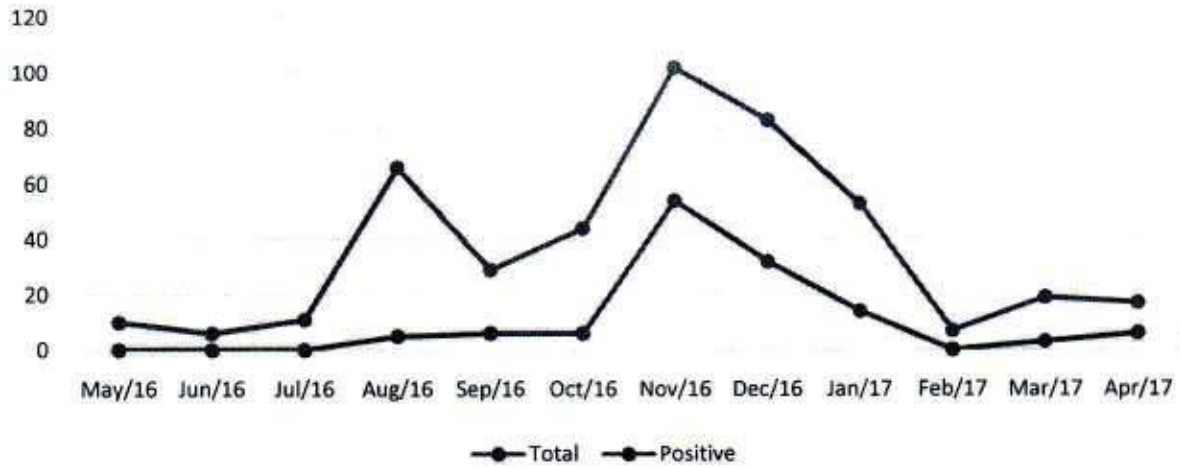


Table No. 3

Result of Lateral chromatography in comparison to ELISA

		ELISA		
		Positive	Negative	Total
LCT	Positive	115	11	126
	Negative	16	42	58
Total		131	53	184