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Original Research Article

A study of rotavirus infection in acute diarrhoea in children less than 5 years of age

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ABSTRACT

Background: Diarrhoeal diseases account for an estimated 1.5 million deaths globally every year making it the second leading cause of childhood mortality. In India 1 out of every 250 children die of rotavirus diarrhoea each year.

Aim: To find out the incidence of rotavirus infection in acute diarrhoeal cases in children under 5 years of age.

Materials and Methods: A prospective study was conducted on 100 non repetitive stool samples of Children under 5 years of age, presenting with acute diarrhoea and hospitalized in the pediatric ward, during December 2015 to November 2016. Stool samples were processed according to premier rotacloone enzyme immunoassay protocol for the detection of rotavirus antigen, adhering to standard laboratory precautions.

Results: The incidence of acute diarrhoeal diseases was 5.86% in our setting. *Rotavirus* was detected in 29% cases by ELISA method.

Conclusion: The *rotavirus* antigen detection by EIA is a reliable test, as it is quantitative and also has high sensitivity and specificity. Hence, can be routinely employed to prevent major morbidity and mortality among children, especially less than 5 years of age.

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1. Introduction

Diarrheal diseases account for majority of deaths globally making it the second leading cause of death in childhood.¹ In India diarrhoea accounts to 14% deaths in children less than 5 years of age.²

Diarrhoea could be due to various causes like viruses, bacteria, protozoa and helminths. In developing countries 50-60% cases are bacterial in origin were *Escherichia coli* amounts to 25%, *Campylobacter jejuni* 10-15%, *Salmonella* species 5%, *Shigella* species 5%, followed by 35% viral etiology, where rotavirus accounts for 15-25% and in many the cause is unidentified or mixed.³

In India, acute diarrheal disease accounts to 8% deaths in children under 5 years age. During 2013, about 10.7

million cases with 1535 deaths have been reported in India.⁴ Although acute diarrheal disease is usually self limiting, infectious source needs proper work up and treatment.

Rotavirus infection is most common amongst the viral etiology leading to 20-30% hospitalized cases in India under 5 years age and estimated to cause approximately 5,27,000 deaths each year.⁵

Hence the present study was taken up for rapid diagnosis of rotaviral infections, to prevent the irrational use of antibiotic therapy and prolonged hospitalization.

2. Materials and Methods

The present study was a cross sectional, prospective study conducted at the department of Microbiology, from December 2015 to November 2016.

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2.1. Source of data

Stool samples from patients admitted in the paediatric ward of were received at Department of Microbiology.

2.2. Sample size

Total of 100 cases were included in the study.

2.3. Inclusion criteria

1. All children below 5 years of age presenting with acute diarrhoea and hospitalized for same, were included in the study.
2. All children enrolled were not vaccinated for rotavirus.

2.4. Exclusion criteria

1. Diarrhoea for more than 14 days / persistent diarrhoea.
2. Children with HIV positive status.
3. Children with dysentery.
4. Outdoor patient
5. Patient not giving consent

2.5. Methodology

A detailed clinical history was taken, thorough clinical examination was done and duly recorded in the case record forms. An evaluation of degree of hydration was done as per WHO criteria.

Subsequently stool sample was sent to department of microbiology for ELISA for Rotavirus detection.

1. KIT used: Premiere Rotaclone, Meridian Biosciences, Inc
2. LOT number: 6042.074
CINCINNATI, USA
3. Expiry date: 2017-02-04

2.6. Principle

Solid phase sandwich type EIA. Plastic microtitre wells are coated with a monoclonal antibody directed against the product of the sixth viral gene (VP6), which is the group specific antigen for all known human rotaviruses.

2.6.1. Procedure

1. Specimen preparation- Add 1 ml of sample diluent to a marked tube, using a transfer pipette.
2. Snap off sufficient number of wells for samples and the controls. Insert into the microtitre well holder.
3. Add 2 drops(100 μ l) each of diluted fecal sample, positive and negative control to the bottom of separate wells.
4. Add 2 drops(100 μ l) of enzyme conjugate to each well. Mix by gently swirling on the table top.
5. Incubate at room temperature for 60 minutes.

6. Pour the liquid out of the wells into a discard jar. Tap the microtitre plate holder upside down vigorously against the absorbent paper to ensure complete removal of liquid from the wells.
7. Fill all the wells to overflow with deionised water and pour the liquid out as in step 5
8. Repeat the washing procedure four times (a total of 5 washes)
9. Add 2 drops (100 μ l) of substrate solution to each well
10. Add 2 drops(100 μ l) of chromogen solution to each well
11. Incubate 10 minutes at room temperature

Spectrophotometric procedure :

This can be determined by adding 2 drops of stop solution (sulphuric acid) to each well.

2.6.2. Interpretation of result

1. Positive results by visual determination: Any sample with blue colour more than intense than that of negative control is considered positive.
2. Positive results by spectrophotometric determination: Specimens with absorbance unit A(450) greater than 0.150 are considered positive.

Statistical analysis of data was done using IBM SPSS statistical software version 22.

2.7. Ethical consideration

The ethical clearance of the study has been obtained from the institutional scientific committee.

3. Results

A total of 100 cases of acute diarrhoea in children less than 5 years, who were admitted in paediatric department, KIMS Hospital, Hubballi, were studied during the December 2015 to December 2016.

Table 1: Showing age and gender distribution of diarrhoeal cases and its comparison to rotavirus positive cases

Age in months	Male	Female	Rotavirus positive
Less than 6	06	04	02(6.89%)
6-12	24	24	16(55.17%)
1-2 years	17	09	09(31%)
>2-5years	07	09	02(6.89%)
Total	54	46	29

3.1. Observation

Maximum rotavirus positive cases were seen in the age group of 6-12 months (55.17%), followed by 1-2 years (31%) and least were seen among less than 6 months and 2-5 years. Also rotavirus positive cases in males and females

shows 62.06% and 37.93% respectively.

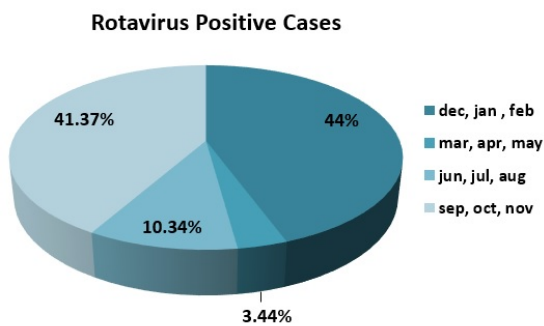
Table 2: Showing the urban - rural distribution of rotavirus cases

Place	No. of. cases of Rotavirus	Non Rotavirus
Urban	10(34.48%)	20
Rural	19 (65.5%)	51

The chi-square statistic is 0.3909. The p-value is .531852. This result is not significant at $p < 0.05$.

3.2. Observation

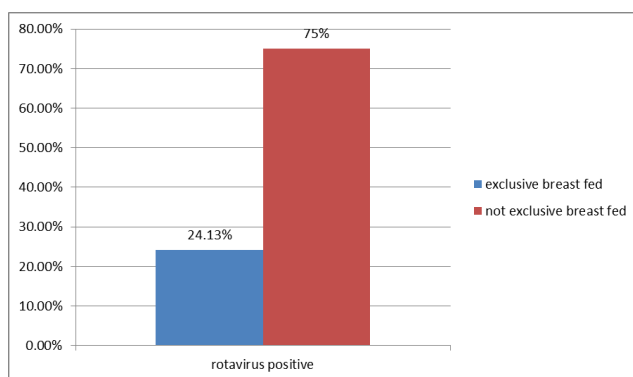
The Table 2 shows that rural rotavirus positive cases are 65.5% when compared to urban population which shows 34.48%.



Graph 1: Showing seasonal distribution of rotavirus infection

3.3. Observation

Above Table 2 that majority of the rotavirus positive cases were seen in the winter season which accounted to 85.37%, 10% in rainy season and 3.44% in summer season.



Graph 2: Diet and rotavirus correlation

3.4. Observation

Above Table 2 that maximum number of cases (75%) shows rotavirus positive when the children lack exclusive breast

feeding whereas 24.13% in children who were exclusively breast fed.

Table 3: Showing correlation of frequency of loose stools with rotavirus positive cases

No. of stools/day	Rotavirus positive	Rotavirus negative
≤ 5	05(17.24%)	21
$>5 < 10$	08(27.58%)	24
≥ 10	16(55.17%)	26

3.5. Observation

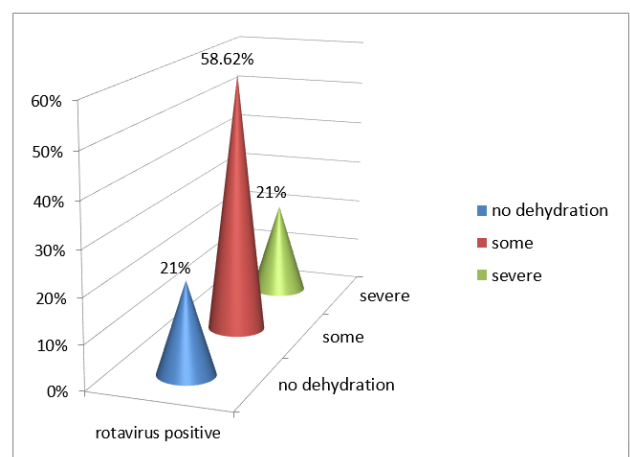
The Table 3 shows that samples having history of passing >10 stools per day had 55.17% of rotavirus positivity, followed by 27.58% when stools are <10 in number and 17.24% when it is less than 5 stools/day

Table 4: Showing correlation of associated complaints with rotavirus positive cases:

Complaints	No. of cases with rotavirus	Non rotavirus cases
Only diarrhoea	05(17.24%)	19
Diarrhoea with fever	06(20.6%)	17
Diarrhoea with vomiting	03(10.34%)	18
Diarrhoea with vomiting and fever	15(51.72%)	17

3.6. Observation

Shows that almost 51.72% cases of rotavirus had complaints of diarrhoea, fever and vomiting, followed by 20.6% with diarrhoea and fever, 17% with only diarrhoea and 10.34% with diarrhoea and vomiting.



Graph 3: Showing assessment of dehydration with rotavirus positive cases

3.7. Observation

Maximum number of cases of rotavirus positive samples were showing some dehydration(58.62%), followed by equal distribution of cases in no dehydration and severe dehydration which had 17, 6 and 6 respectively. Among severe dehydration Rotavirus cases were 66.66%.

Table 5: Showing stool macroscopy in acute diarrhoea cases

Type	No. of Cases
Solid stools	08
Yellowish stool	70
Greenish stools	22

3.8. Observation

Above Table 5 that 70% cases were presenting with yellowish coloured stools, followed by 22% showing greenish stools and 8% with solid stools. Showing macroscopic picture of greenish frothy stools with mucous flakes of rotavirus positive specimen:

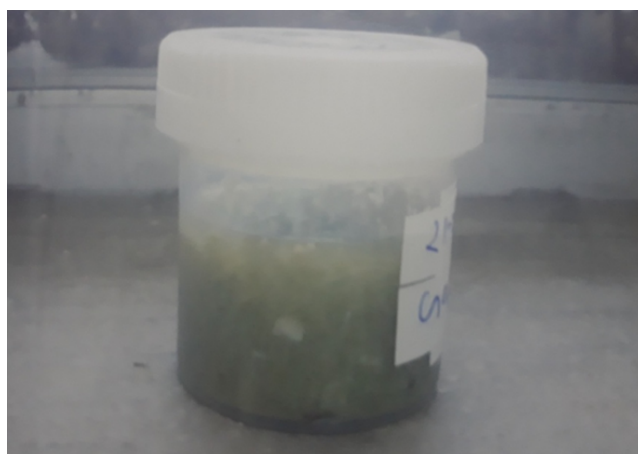


Fig. 1: Showing macroscopic picture of greenish frothy stools with mucous flakes of rotavirus positive specimen

4. Discussion

This study was conducted in the department of microbiology from December 2015- December 2016.

The total number of acute diarrhoeal cases admitted to paediatric ward during this period were 186 in accounting to prevalence rate of 5.86% in our set up.

Among them, 100 cases were included in the present study for acute diarrhoea which were within our inclusion criteria.

In the present study maximum rotavirus positive cases were seen in the age group of 6-12 months (55.17%), followed by 1-2 years(31%) and least were seen among less than 6 months and 2-5 years. Also rotavirus positive cases in

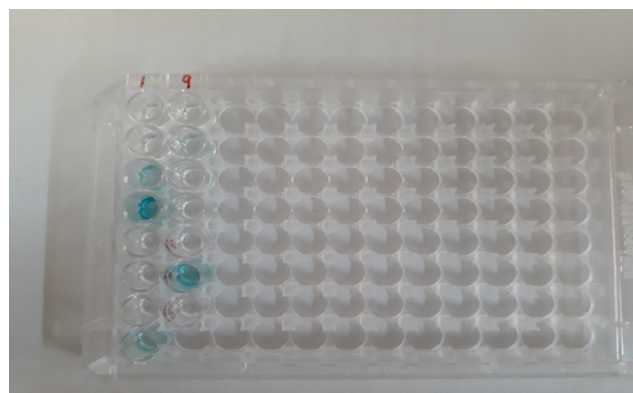


Fig. 2: Showing ELISA plate: visual determination of rotavirus antigen detection Blue colour well: positive, colourless well: negative

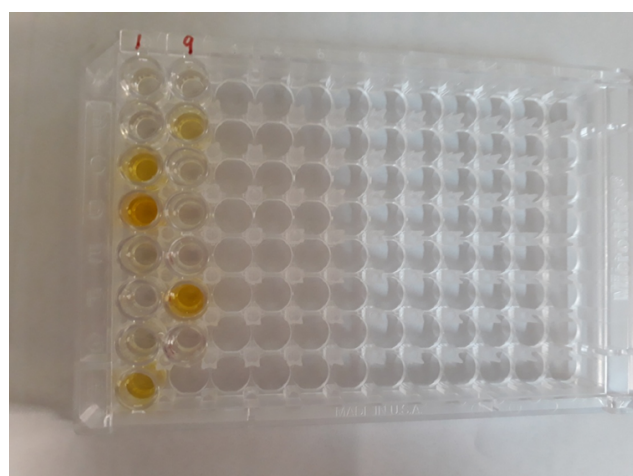


Fig. 3: ELISA plate showing rotavirus positive after adding STOP solution: spectrophotometric determination: yellow colour shows Positive for Rotavirus antigen

males and females shows 62.06% and 37.93% respectively. Also in a study conducted by Satarupa Mullick et al⁶ in 2014 most cases of ADD belonged to 6months to 2 years, followed by 0-6months of age. In another study done by Shaveta Dhiman et al⁷ in 2015 at Amritsar maximum cases of ADD (69%) belonged to 6 months - 2 years of age. These findings were similar to the present study. Predominant number of cases in the age group of 6-12 months may be due to the commencement of weaning period, which leads to exposure to the water and other contaminants and also during this period infant starts toddling and acquire the habit of mouthing.⁸

In the present study out of 100 children presenting with acute diarrhoea 54% were males and females accounted for 46%. In a study at Tumkur, Karnataka, from Nov 2007 –Aug 2009, the prevalence of diarrhea in children accounted for 10.12%. Out of 150 cases enrolled, maximum cases of diarrhea occurred in the first 24 months of life 107

Table 6: Showing commonly affected age group due to rotavirus infection

S.No	Author	Year	Place	Total no. of study cases	Commonly involved age group
1	Shaveta Dhiman et al ⁷	2015	Amritsar	100	6months-2 years
2	Anup kumar Shetty et al ⁹	2014	Karnataka	35	7months- 2 years
3	Ma Mathew et al ¹⁰	2013	Kerala	1827	6 months to 2 years
4	Muhammed Irfan Habib et al ¹¹	2007	Karachi	300	6months-2 years
5	Pierre Van Damme et al ¹²	2005	Europe	2846	6months-2 years
6	Present study	2016	Hubballi	100	6months-2 years

(71.3%), followed by 24–60 months (15.3%) and 6–12 years (13.3%).¹³ In a study conducted by Manohar Badur et al in 2015 in Tirupati, predominant number of males were affected which is around 57.2% and females were 42.8%.¹⁴ Thus the results are almost similar to the present study. The rotavirus cases in present study accounted for a total of 29%, with a predominant male pattern with 62.06% and females showing 37.93%, showing ratio of 1.5:1. In a study conducted at Lucknow during August 2010–July 2012, rotavirus was the most frequent cause of viral gastroenteritis in both hospitalized and non-hospitalized children accounting for 19.5% and 9.1% respectively,¹⁵ Rajiv Bahl et al¹⁶ in 2005 at New Delhi showed 26% positivity, Satarupa Mullick et al⁶ in 2014 at Kolkata showed 25.2%, Manohar Badur et al¹⁴ in 2015 at Tirupati showed 25.67%. In a study conducted by Shobha Ram et al (1990) at Chandigarh observed M:F of 3.3:1, for rotavirus infection.¹⁷ The present study correlates with study by P. Sarvanan et al (2004),¹⁸ Pune,¹⁹ Karachi²⁰ and Turkey²¹ which detected rotavirus antigen in equal frequency in both males and females.

Seasonwise distribution of Rotavirus diarrhoea:

In the present study 88% cases were detected in the winter, 3.44% in summer and 10% during rainy season.

In a study conducted in southern Orissa from Jan 2007–Dec 2010 among children less than 5 years of age; higher prevalence of diarrhoea was noticed in July to October that is during monsoon period.²²

M.R. Saha et al (1984) at Calcutta detected frequent rotavirus infection during cooler months of the year.²³ A study at Nepal by M. Shariff et al (2003) observed that rotavirus infections occurred throughout the year.²⁴

A study conducted at Vietnam reported that rotavirus infection occurred all round the year, with a peak in winter season.²⁵

A study demonstrated that the degree of sensitivity and specificity of LA test showed a roughly linear relationship with the degree of ELISA sensitivity and specificity, and the positive predictive value of the test was high.²⁶ According to Raj et al, the LA kit studied was a specific and rapid method. While it may be useful in certain situations, such as in outbreaks, its low sensitivity can make it unsuitable for use in routine clinical practice.²⁷

5. Conclusion

Incidence of rotavirus cases in and around Hubballi is 29% in children less than 5 years of age. The study shows that maximum rotavirus cases were seen in the age group of 6–24 months. As the samples collected were from unvaccinated children, it also relates the importance of vaccination which could prevent dehydration resulting from rotavirus infection. Also, a rapid diagnosis of these infections with EIA, may be valuable in the formulation of prognosis for children with acute diarrhoea and may prevent the irrational use of antibiotic therapy and prolonged hospitalization.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.


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