Paediatrics

KEYWORDS: Rotavirus, Pediatric diarrhoea, clinical pattern, seasonal effect

CLINICAL PATTERN OF ROTAVIRUS INFECTION IN DIARRHEA CASES FROM NAVI MUMBAI: FOCUS ON SYMPTOMS NOT CONSIDERED FOR VESIKARI SCORING

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ABSTRACT:

Introduction:
Rotavirus diarrhea has been highest threat to neonates and toddlers all over the world. India contributes highest percentage with few other developing countries from Asia and South Africa. Hypermutability of the virus is due to reassortment of segmented genome which leads to generation of new recombinant genotypes. Continuous screening of pathology and clinical symptoms of rotavirus diarrhoea is inevitable to completely eradicate the disease.

Study design:
Proposed prospective observational study involved total 207 patients. Children less than 5 years showing symptoms of acute diarrhea were examined and standardized. Proforma was used to collect the demographic and clinical profile of the children. Severity of the illness was classified as per Vesikari scoring system and the dehydration was treated as per WHO protocol. Stool samples were collected and tested for rotavirus infection.

Results:
In the present studies, 207 cases of diarrhoea were screened for rotavirus infection of which 22 were found positive (10.62%) by technique of electropherotyping. Disease pattern comparison between rotavirus positive and negative groups showed some remarkable parameters associated with incidence rotavirus diarrhea such as, mean age, stool consistency, lethargy, vitamin deficiency and hospitalization. Seasonal variation in case of rotavirus infection remains unresolved.

Conclusions: Data from present study highlights current scenario of clinical pattern and disease pathology for rotavirus infection induced pediatric diarrhoea in Navi Mumbai and Raigad region.

INTRODUCTION:

Rotavirus is prime cause of Viral Gastroenteritis worldwide since more than a decade, followed by Norovirus, Astrovirus & then Adenovirus. Despite improving sanitation in many countries, certain viral infections have not been eradicated. Improving socio-economic conditions and collecting data or reports on viral infections in different countries are necessary for decision making health policies. National burden of rotavirus for india has been given by Jacob et al which postulates that 6,86,277 outpatient visits, 2,91,756 hospitalizations and 26,985 deaths can be prevalant in India.

Table : Frequency of rotavirus infection in various countries.

Sr. No	Country	HRV	Sr. No	Country	HRV
1.	USA	6.8%	2.	Uganda	9%
3.	UK	36%	4.	France	2.1%4
5.	South Africa	13%-55%	6.	Japan	58%
7.	Saudi Arabia	10%	8.	China	34.3%
9.	Western Arab	36.4%	10.	Malaysia	45.2%4
11.	Pakistan	13.7%	12.	Australia	74.54%
13.	Brazil	9.9%	14.	India	23.4%

HRV Human rotaviruses shows geographical variation in different countries all over the world.

Rotavirus infects the mature enterocytes in the mid and upper part of the villi of the small intestine, causing diarrhea. Studies of biopsies of the jejunal mucosa of infants infected with Rotavirus have revealed shortening and atrophy of villi, distended endoplasmic reticulum, mononuclear cell infiltration, mitochondrial swelling and denudation of microvilli.

MATERIALS AND METHODS:

Study design: Prospective observational study
Study period:
Sample size: 207 babies

Inclusion criteria:

1. All children having acute diarrhea as defined by WHO.
2. Age: under 5 years (U5C).
3. Parents willing to participate in the study after confirming their informed consent

Exclusion criteria:

1. Children having bacterial diarrhea after clinical and lab evaluation.
2. Small and frequent stools >15/day with or without tenesmus
3. Stool showing frank blood and mucous
4. Children going on Discharge against medical advice or subsequently not willing to participate in the study.

Methodology

U5C (Children under 5 years of age) presenting with diarrhea were studied attending paediatric OPD/IPD of MGM group of hospitals, Navi Mumbai. A fresh stool sample was obtained from the diarrhea patients and subjected to stool microscopy.

All presumed non-bacterial samples were transported within 2 hours to the testing laboratory (Central Research Lab, MGM Medical College, and Kamothe). They were processed by electropherotyping technique for detection of Rotavirus infection. All cases were clinically correlated for various symptoms.

RNA extraction using QIAamp Viral RNA mini kit:

PBS suspensions of faecal samples 10% were clarified by centrifugation at 10,000g for 10 minutes. Genomic RNA was extracted from 140µl of 10% stool suspensions using a spin column technique according to the manufacturer's instructions (QIAamp Viral RNA mini kit from QIAGEN Gmbh, Hilden, Germany).

RNA PAGE (Electropherotyping):

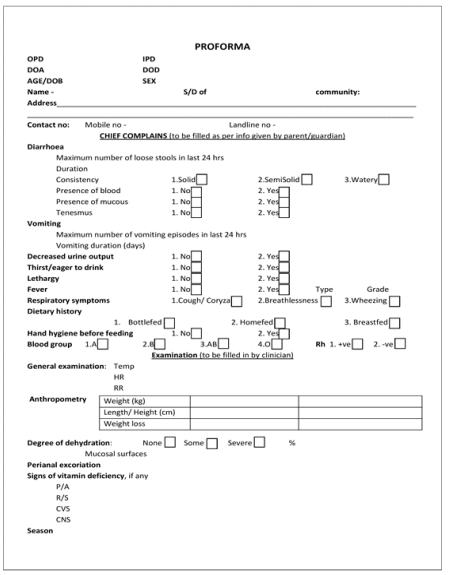
Rotavirus double-stranded RNA was extracted from stool of infected clinical samples by using Trizol-LS reagent (Life Technologies, Rockville, Md.). All faecal specimens were analyzed by polyacrylamide gel electrophoresis (PAGE) to identify the presence of rotavirus double-stranded RNA (dsRNA) to confirm the presence of rotaviruses (method explained in detail elsewhere).

STUDY PROFORMA:

In detail proforma (Figure 1) was filled in by counselling parent or guardian of the patient. It included demographic parameters such as age, sex, season, symptoms included in vesikari system as well as certain miscellaneous symptoms.

Filled in by clinician after counselling parent / guardian

Table 1 explains parameters included in vesikari scoring. In 1990, Ruuska and Vesikari described a numerical scale to assess severity of gastroenteritis on the basis of duration and frequency of diarrhea, vomiting, fever, dehydration, and type of treatment required. These have been used to grade the severity of rotaviral diarrhea in epidemiological studies in form of Vesikari score from 0-20 (table 1) and interpreted as mild, moderate and severe depending on the score.



PROFORMA

OPD IPD
DOA DOD
AGE/DOB SEX
Name - S/D of community:
Address

Contact no: Mobile no - Landline no -

CHIEF COMPLAINS (to be filled as per info given by parent/guardian)

Diarrhoea
Maximum number of loose stools in last 24 hrs
Duration
Consistency 1.Solid 2.SemiSolid 3.Watery
Presence of blood 1. No 2. Yes
Presence of mucous 1. No 2. Yes
Tenesmus 1. No 2. Yes

Vomiting
Maximum number of vomiting episodes in last 24 hrs
Vomiting duration (days)

Decreased urine output 1. No 2. Yes
Thirst/eager to drink 1. No 2. Yes
Lethargy 1. No 2. Yes
Fever 1. No 2. Yes Type Grade
Respiratory symptoms 1.Cough/ Coryza 2.Breathlessness 3.Wheezing

Dietary history
1. Bottlefed 2. Homefed 3. Breastfed
Hand hygiene before feeding 1. No 2. Yes
Blood group 1.A 2.B 3.AB 4.O Rh 1. +ve 2. -ve

Examination (to be filled in by clinician)

General examination: Temp
HR
RR

Anthropometry			
	Weight (kg)		
	Length/ Height (cm)		
	Weight loss		

Degree of dehydration: None Some Severe %
Mucosal surfaces
Perianal excoriation
Signs of vitamin deficiency, if any
P/A
R/S
CVS
CNS
Season

Figure 1: Proforma used in present study for recording history of diarrhoea patients.

Table 2: Vesikari scoring

Paramete r Sr. No.	Parameter	Score		
		1	2	3
	Diarrhoea			
P1	Maximum no stools/day	1-3	4-5	≥6
P2	Diarrhoea duration (Days)	1-4	5	≥6
	Vomiting			
P3	Maximum no vomiting/day	1	2-4	≥5
P4	Vomiting duration(Days)	1	2	≥3
P5	Temperature	37.1 – 38.4	38.5 – 38.9	≥39.0
P6	Dehydration	N/A	1-5 %	≥6%
P7	Treatment	Rehydration	Hospitalization	Hospitalization

7 various parameters were included for vesikari scoring as mentioned in the table from P1 to P7. Scores were given as 1, 2 or 3 depending on the frequency or grade of particular parameter. Table adapted from rotavirus clinical trial utilising Vesikari clinical severity scoring system [Ruuska and vesikari, 1990]

Mild	Moderate	Severe	Maximum score
<7	7-10	≥11	20

Scores of all the seven parameters were added and final value predicts severity of the disease. Less than 7 score shows mild disease, score between 7 to 10 shows moderate disease while vesikari scoring above 11 shows severe disease.

Statistical analysis:

The data was analysed with SPSS version 17 software. Independent sample t-test was used for comparison. Significance was taken at P value of <0.05.

Results:

207 patients were scrutinised in detail for clinical symptoms and related parameters, to obtain Clinical profiles of the Patients. Symptoms included in vesikari system were in particular recorded. In each of the cases, detail proforma (Annexure I) was filled in with information which was obtained by interviewing the parent / guardian of the respective patient. Also counselling of parent or guardian of the patient was also carried out, whereever necessary. The proforma was updated with parameters such as age, sex, season in which the case reported to the OPD. Following that a stool sample was collected in a sterile container (stored in deep freezer, if necessary) to screen the patient for rotavirus and norovirus infection.

The disease severity was assessed according to vesikari scoring. It was found that in the whole study group, 90/ 207 (43.5%) had moderately severe disease, 66 (31.9%) had mild and 51/207 (24.6%) had severe disease. (Figure 2)

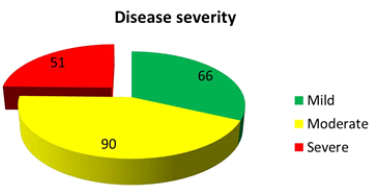


Figure 2: Disease severity according to Vesikari score

Screening of diarrhoea cases by electropherotyping

Figure 3: Electropherotyping of rotavirus isolated from clinical samplesElectropherotyping permits monitoring of Rotavirus infection (Figure 3). Rotavirus is a segmented virus having dsRNA double stranded Ribonucleic Acid. The genome extracted using Trisol shows 11 bands on gel for 11 genes of the virus. Bands with lowest base pairs runs faster being lighter in weight. Whilst longer genes are heavier hence run slower and remain near the wells. The gel shown in figure 3 shows four wells all showing RNA extracted from stool specimens positive for rotavirus. The gel is stained by silver nitrate.

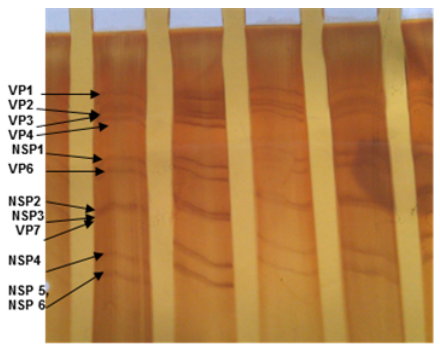


Figure 3: Electropherotyping of rotavirus isolated from clinical samples

Disease severity compared between two groups shows interesting findings as expected (Figure 4). Rotaviral diarrhea: 2/22 (9.1%) had mild disease, 5/22 (22.7%) had moderately severe disease and 15/22 (68.2%) had severe disease. Non rotaviral diarrhea: 64/185 (34.6%) had mild disease, 85/185(45.9%) had moderately severe disease while 36/ 185 (19.5%) had severe disease.

Figure 4: Disease severity compared amongst rotavirus and non-rotavirus cases

Figure from left shows disease severity for rotavirus cases whereas figure from right shows disease severity for non-rotavirus cases. Rotavirus cases positive cases were found to show maximum severity of the diarrhoea.

Age distribution

Age in study population ranged from 6- 48 months and mean age was 19.26 months. Rotavirus positivity was highest in 13-24 months age group (50 %) followed by in 25 - 36 months age group whereas it was lowest in >36 months (Figure 5a).

Mean age (in months) in rotaviral diarrhea cases was 19.2 months while it was 24 months in non rotaviral diarrhea cases (Figure 5). P value was 0.04.

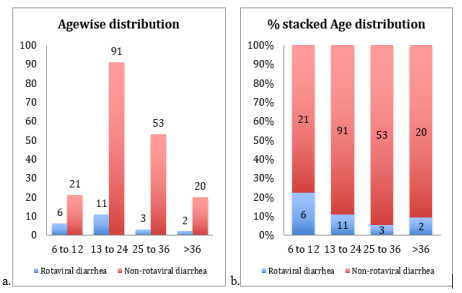


Figure 5: Agewise distribution

Bar diagram and percent stacked bar diagram showing agewise distribution of the diarrhoea cases. on x –axis age groups have been plotted in months while on y axis number of cases. a. Graph shows that more cases of viral diarrhoea were observed in age group 13-24 months. b. Graph clearly shows that frequency of rotavirus diarrhoea is in age group of 6-11 months.

In the present study, the majority of infections causing pediatric diarrhoea were observed in 6- 12 months age group (70.9%). This was similar to that reported by Sheriff et al. Looking at percent stacked graph 5b it can be interpreted that although more number of rotavirus positive cases were in 13 – 24 age group (Figure 5a), incidence was more in 6 – 12 months age group.

Rotavirus positive stools were in 22.22% children of 6 – 12 months; 10.78% for 13 – 24 months; 5.35% for 25 – 36 months and 9.09% for children above 36 months.

Sexwise distribution of rotavirus infection

There was no significant difference in proportion of males and females among the rotavirus diarrhoea cases. Percent Doughnut charts explaining sexwise distribution of diarrhoea cases involved in this study. Prevalence of rotavirus infection was not very different among boys or girls (Figure 6). There was no significant difference in sex ratio.

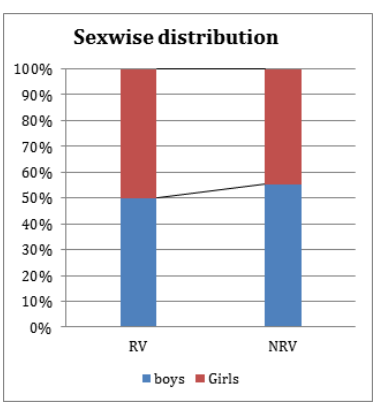


Figure 6: Percent stacked chart explaining sexwise distribution of diarrhoea cases involved in this study. For rotavirus cases there

was no significant difference in sex ratio but for non-rotavirus cases frequency of girls was lower than boys.

Height and Weight Distribution of Rotavirus Infected and Non-infected Patients

Weight was measured in Kilogram unit and height in centimeter unit for each case. Depending on the age, there was no difference in weight and height of the study cohort which proves that malnourishment was not the reason for the infection. The weight and height though lower for rotavirus positive cases, in a parallel way age was also lower (Figure 7). Hence demographical difference in rotavirus positive and negative cases is difficult to be interpreted whether it is age related or infection associated.

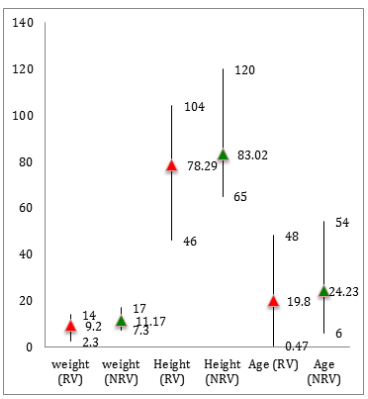


Figure 7: Demographic parameters of diarrhoea cases.

Graphs showing difference in Rotavirus positive and negative cases with regards to weight and height of child. Rotavirus infected group was at lower range of weight and height compared to NRV group which goes parallel with age differecne in two groups.

Stool consistency

Watery stools were associated with 18/22 (81.8%) of cases of rotaviral diarrhea as compared from 76/ 185 (41.1%) of cases of non-rotaviral diarrhea (Figure 8). P value is <0.001, which is significant.

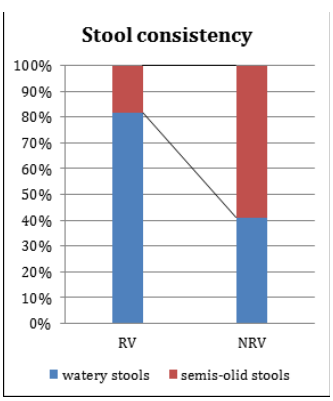


Figure 8: Stool consistency

comparison between rotavirus positive and rotavirus negative groups

Incidence of Fever & Respiratory symptoms

Fever was present in 3/22 (13.6%) of rotaviral diarrhea as compared from 25/185 (13.5%) of cases of non- rotaviral diarrhoea (Figure 9). P value is NS. This shows that any type of viral diarrhoea may show symptom of fever.

Cough and coryza were present in 18.2% (4/22) of rotaviral diarrhoea cases as compared from 23/185 (12.4%) of cases of non-rotaviral diarrhoea (Figure 9). There was no significant difference in two groups.

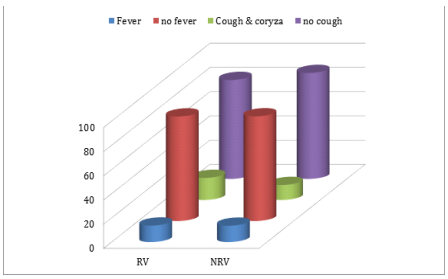


Figure 9: Fever & Respiratory symptoms

There was no significant difference in RV and NRV cases

Physiological parameters of study groups were distinct.

Lethargy: Higher percentage of rotavirus positive cases were found to be lethargic than rotavirus negative cases (Figure 10). Rotavirus cases due to severe dehydration were highly lethargic.

Perianal excoriation: It was found to be associated with 2/22 (9.1%) of rotaviral diarrhoea cases as compared from non-rotaviral diarrhoea where it was associated with 5/185 (2.7%) of cases (Figure 10). P value is NS.

Vitamin deficiency: Rotavirus positive kids were highly deficient for vitamins and nutrients (Figure 10). This data shows importance of vitamins in GUT health and further study has to be carried out to investigate association of vital nutrients with rotavirus infection.

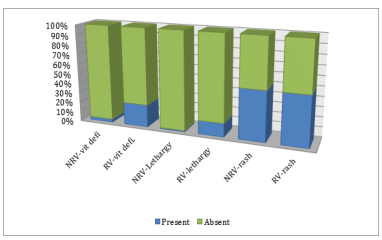


Figure 10: Physiological parameters included in study

Perianal excoriation due to severe diarrhoea can be seen by rashes formed. Rotavirus positive as well as negative cases were found to present with severe rashes. Lethargy and vitamin deficieny parameters were significantly differnt in two groups of rotavirus positive (RV) and rotavirus negative (NRV).

Treatment: Need for hospitalization

Rotavirus positive patients mostly had to be hospitalized due to severe dehydration and diarrhoea symptoms. The difference in rotavirus and non-rotavirus diarrhoea was statistically significant (Figure 11).

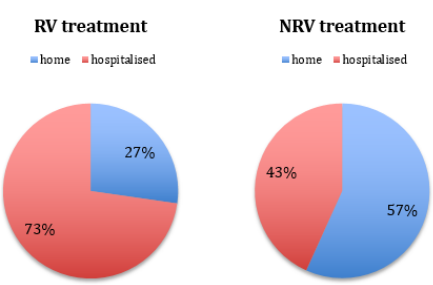


Figure 11: Requirement of hospitalization in rotavirus and non-rotavirus cases

Environmental Factor: In seasonal variation winter season was not found to be a significant factor for induction of rotavirus diarrhoea.

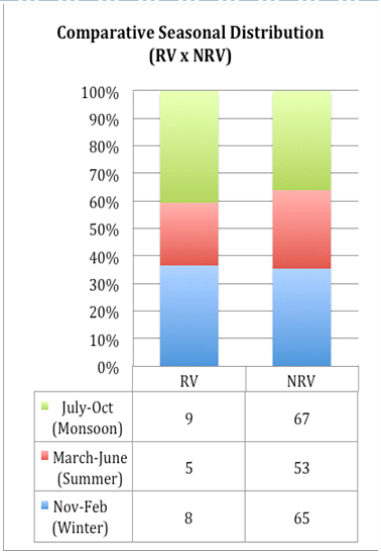


Figure 12: Seasonal Distribution in RV & NRV cases

Percent stacked bar diagram showing Seasonal distribution of cases comparing betweem test groups Rotavirus diarrhoea and non-rotavirus diarrhoea

The temporal distribution of rotavirus incidence was observed throughout the year. Higher incidence during rainy season (July-October) was observed. Maximum cases in this study i.e. 9 out of 76 (11.84%) cases were reported in July- October (rainy season), 8/73 (10.95%) reported in November to February (winters) while 5/58 (8.62%) cases in the summer (Figure 12).

In the US and Europe, rotavirus infection occurs primarily during the winter season. Some studies in India have found no association between rotavirus infection and time of year., But many other studies an increase in rotavirus-associated diarrhoea during the winter months, November to February, throughout the country has been reported. In the North India, in more temperate climate with low relative humidity may contribute to stronger seasonal variation. In Mumbai since past few years monsoon starts by july end. Specifically for Navi Mumbai, climate is considered to be Am according to the Köppen-Geiger climate classification, January is the driest month and May is the warmest. This study has found 3 peaks for rotavirus prevalence (Figure 13).

Some studies have found 2 peaks per year as in a study from Punjab where rotavirus infection have maximum occurrence in November and another peak in the hot and dry months of May. The maximum incidence in Pune occurred in winter and the minimum in the rainy season.[22]

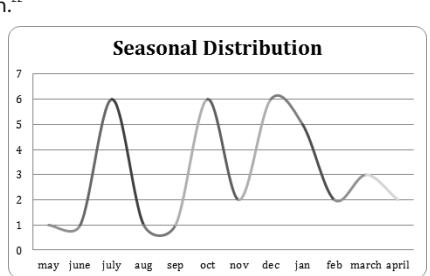


Figure 13: monthwise distribution of rotavirus infection for region under study

Nevertheless, studies in Kolkata, Pune and Chennai20 have observed seasonal effects despite their tropical climate, so the degree to which seasonality variation exists, remains yet unresolved.

Discussion

This is a prospective observational study to determine the association of rotavirus in with clinical symptoms other than vesikari scoring. A total of 207(both inpatient and outpatient) patients with suspected viral gastroenteritis from January, 2013 to Nov, 2014 were included in the study.

Of total 257 patients with diarrhea, 50 were excluded in view of various exclusion criteria. 207 patients were enrolled in the study.

Characteristics of the study group

Table 3: Baseline characteristics of all the cases included in the study: (n: 207)

	Range (minimum – maximum)	Mean	
Age (in months)	0.6 to 58 months	23.55	(±10.96)
Sex	115 boys, 92 girls	207	
Bottle fed	36/ 207	17.4%	
Weight / Age	67-105	86.76	(±7.45)
Height / Age	71-96	84.16	(±7.21)
Stool frequency	2-14	7.18	(±2.61)
Vesikari Score	4-17	8.6	(±3.12)

The proportion of diarrhea cases attributable to rotavirus is notably lower for outpatient and community in this study. Ramani et al found that rotavirus accounts for 16% of diarrheal outpatients. Two studies done in Pune and Vellore, found a mean proportion of 12% of rotaviral infection in all cases of suspected viral diarrhea., The higher prevalence of rotavirus among hospitalized persons suggests that rotavirus gastroenteritis is generally more severe than that of other etiologies as collaborated by Vellore cohort, where the proportion of diarrhea cases due to rotavirus increased with increasing disease severity, from 11.5% in the least severe cases to 67.4% in the most. In an Indian multi-centric study, one in seven different regions of India reported that rotavirus was detected in 39% of children aged less than 5 years with acute gastroenteritis.
Neonatal infections are commonly asymptomatic, with 69-95% not showing overt signs of gastroenteritis, . Viral shedding can begin as early as 2 days of age, generally peaks around 3-6 days and resolves by 2 weeks of age; the likelihood of acquiring an infection is related to length of stay inthehospital after birth. Neonatal infections maybe protective against future rotavirus diarrhea, although results are conflicting. In a cohort in New Delhi, infants with neonatal infections suffered 46% fewer episodes of rotavirus diarrhea and 22% fewer episodes of all-cause diarrhea in the first year of life. However, a larger study in Vellore did not find any association between neonatal infections and either incidence or severity of future rotavirus or all cause gastroenteritis. The Vellore studies limited the analyses to children infected with G10 P(11) in Vellore, making it difficult to compare results. The role that neonatal rotavirus infections play in disease epidemiology remains unclear, although given the high burden of rotavirus disease observed in India any protective effect seems likely to be minimal. In this study, we have found 2 cases of neonatal infections which had peculiar electrophoretic pattern (data not shown here). These were twins of 2 weeks. Both samples are processed further for strain typing.

Most rotavirus disease in India occurs in the first two years of life. In hospital-based studies, 87% of all rotavirus cases in children under 5 years occurred by 18 months of age, . Additionally, rotavirus gastroenteritis is uncommon in the youngest children; only 13% (ISV: 10-25%) of rotavirus cases in hospital studies were in children younger than 6 months old. The difference in age distribution between settings is likely largely a function of severity: in young children, infection with rotavirus may be attenuated by the persistence of maternal antibodies and thus, severe disease is less common.

A cohort study in an urban slum population in New Delhi, the annual incidence of rotavirus hospitalizations in children <5 years of age was 337/100,000; incidence for 1-year-olds was 1,270/ 100,000 with low incidence in the first 3 months of life; incidence for 2-year-olds was 534/100,000; and incidence for 3-5 year olds was 12/100,000. This study highlights the importance of young age in severe rotavirus infections, but because the study looked at incidence of hospitalization and not disease, these numbers do not represent the true incidence of rotavirus disease in India.

In present study, the highest (50%) incidence of Rotavirus diarrhea was seen among 13-24 months of the age. Neonates were excluded from this study as acute gastroenteritis can be just one of the manifestations of myriad of underlying conditions in them. Various antenatal and postnatal factors can contribute, which are not under the preview of our study. Sheriff et al 18 in his study had 56/160 in the age group 6-11 months, 52/160 in 12- 24 months age group. The majority of infections were in 6- 24 months age group (70.9%) which is the same as reported by Sheriff et al.

In our study, out of 22 patients, 11 were males and 11 were female. Male to female ratio was 1:1.In the study by nafi et al, rotavirus was detected at a higher rate in the stools of male than female patients with a male to female ratio of 1.95:1.

Table 4: Comparison of Rotaviral from Non-rotaviral diarrhea

Sr. No.	Study Parameters	P value
1.	Age less than 2 years	Significant
2.	Sex	Not significant
3.	Weight / Age	Not significant
4.	Weight/ Height	Not significant
5.	Stool consistency	Significant
6.	Fever	Not significant
7.	Cough	Not significant
8.	Lethargy	Significant
9.	Vitamin deficiency	Significant
10.	Perianal excoriation	Not significant
11.	Treatment by hospitalization	Significant

While some studies in India have found no association between rotavirus infection and time of year27, most have observed an increase in rotavirus-associated diarrhea during the winter months, October to February, throughout the country 20, 36, 24,. The observed proportion of rotavirus cases occurring in the cooler season has ranged from 59% to 72%, with a median of 64%.

For this region association of parameters considered for vesikari scoring and disease severity with that of the pediatric diarrhoea and specifically with rotavirus induced diarrhoea has been shown earlier., This study for the first time has tried to consider demographical and environmental factor for rotavirus infection.

Conclusion:

It was found that the proportion of cases of rotavirus diarrhea in clinical profiling study group (n=207) was 10.6%. In the study population, there were 115 male infants and 92 female infants with mean age of 23.5 months. In present study, rotaviral diarrhoea does not seem to have any sex preference. None of the children included belonged to severely malnourished category. 17.4% children were bottle-fed. Exclusive breastfeeding has been found to show decreased incidence of infection. Duration of illness was significantly more in rotaviral diarrhea. No significant difference was observed between rotaviral and non-rotaviral diarrhea cases for symptoms such as Fever, Perianal rash and Respiratory symptoms as cough and coryza. Watery stools, lethargy and vitamin deficiency was statistically significant in association with rotavirus infection. Vesikari scoring and disease severity are inevitable for pediatric diarrhoea cases but other than this age, physical appearance in terms of malnutrition (vitamin deficiency) and weakness (lethargy) can be considered by clinicians for early diagnosis of rotavirus induced diarrhoea.

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