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Original Research Article

Study on incidence and genotypic prevalence of rotaviral diarrhoea in children below 2 years of age in a tertiary care hospital of eastern Odisha, India

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Abstract: Diarrhoeal diseases are the leading cause of significant morbidity and mortality in children. Rotavirus continues to be the commonest cause of acute gastroenteritis across the world. Currently available rotavirus vaccines are effective in reducing the disease burden, but correct understanding of local epidemiology of rotavirus infection is important for rotavirus immunization. The aim of this study was to estimate the incidence of rotaviral diarrhoea and the prevalent rotavirus genotypes causing diarrhoea among children below 2 years of age.138children below 2years of age admitted to our hospital for acute diarrhoea were enrolled in our study. Stool samples were collected from all patients having watery diarrhoea and sent for rotavirus assay by ELISA. Genotyping was done in all positive cases by reverse transcription polymerase chain reaction (RTPCR). Results of the lab analysis were recorded. Data were entered in the Microsoft Excel work sheet and were analysed using SPSS version17. P value of <0.05 was considered significant. Out of 138 cases of acute diarrhoea 46(33.33%) cases was positive for rotavirus. Incidence of rotaviral diarrhoea was 8.40% of all admissions in children below 2years of age. Rotavirus positivity was significantly higher in children between 6 to 18 months of age.G3P8was the most prevalent strain followed by G1P8.Rotavirus is the most common cause of diarrhoea among children more so in infants. G3P8was the most prevalent genotype in this part of the state followed byG1P8.
Keywords: diarrhoea, ELISA, genotype, rotavirus

INTRODUCTION

Rotaviral diarrhoea is a leading cause of morbidity and mortality in fewer than five children in India. It accounts for about one third of diarrhoeal deaths in fewer than five children. As per WHO (April 2016),globally 215000 children died due to rotaviral diarrhoea in the year 2013. India, Nigeria, Pakistan and Kango tops the list and account for 49% rotaviral diarrhoeal deaths in 2013.India contributes 22% of global rotaviral deaths. The burden of rotavirus gastroenteritis is highest in very young children and decreases rapidly thereafter. Rotaviral diarrhoea causes 4.4% and 3.2% of deaths worldwide in children below 1year and between 1-4 years of age respectively [1]. India has estimated annual burden of 2.0-3.4 billion cases of diarrhoea attributable to rotavirus. Recent estimates have shown that about 872,000 hospitalizations and 78,500 deaths occur due to rotavirus infections annually in India [2]. Over the years there is a rising trend in the proportion of rotaviral cases in hospitalized children, that is 26.01% before 2000 to 38.03% after 2005 [3]. Prevalence of rotavirus positivity in gastroenteritis was 38.1% among children below 1year of age; whereas the highest rotaviral disease burden was seen among children between 12 to 24 months [4]. There is circulation of a diverse range of rotavirus strains in various parts of India. Expanded National Rotavirus surveillance Network highlights the high prevalence (39.6%) of rotavirus disease burden in fewer than five children across the country.G1 is the most common G type followed by G2 and G untypable with distinct regional variations across the country [5]. Similarly P4 is the most prevalent P type followed by P6 and P untypable, with recent emergence of G 12 strains particularly G12P6 strains in both western and northern region[6]. Though G1P8 is the most commonly prevalent strain in India there have been emergence of other strains like G9,G12[7] from time to time. There is resurgence of uncommon genotypes at the same place at different periods like emergence of G3P8 in 35-40% of cases in 2012-2013[8] in Brazil. A study by Hera Nirwati *et al.;*[9] from Indonesia showed

G1P6 was the predominant strain in2006 and 2009 where as in 2010, G1P6 was just a minor strain Though acute diarrhoea is an easily treatable condition, unfortunately lack of awareness, inappropriate use of ORS, rapid progression of the disease in young infants amounts to severe dehydration and death in children. As our hospital had recently been included in multicentre surveillance, the present study was done to estimate the rotaviral disease burden in children below 24months of age, their clinical profile along with the prevalent genotype in this region.

METHOD

This was a prospective observational study conducted in a tertiary care hospital, Odisha from March 2016 to October 2016.All children in the age group 0 to 24 months admitted with acute watery diarrhoea as per WHO case definition were included in the study. Those children with other associated systemic illnesses, those with dysentery were excluded from the study. Out of a total of 544 children (below 24 months of age) admitted with various diseases during the study period, 138 babies (25.36 %) were of acute watery diarrhoea. After obtaining IEC clearance and written informed consent from parents of enrolled children detail history, clinical examination was done. Treatment was as per WHO guidelines [7]. Children were classified as those with no dehydration, some dehydration and severe dehydration. Severity was scored as per Vesikari scale. Maximum number of loose stools in 24 hours, nutritional status, and presence of vomiting, fever were recorded. Sociodemographic data, source of drinking water, need for admission to intensive care unit was recorded as per the preformed performa. Vaccination status, course during hospital stay and outcome were recorded. Details of vaccination including administration of rotavirus vaccine were obtained from immunization card. This study was a part of national rotavirus surveillance network programme. 5ml of stool sample of all participants were collected in sterile screw cap containers within 24hours of admission and stored in the freezer compartment of refrigerator. Samples were transported every monthly in boxes with ice packs to the testing laboratory. Stool samples were screened by using a commercial enzyme immunoassay (premier Rota clone, Meridian Biosciences) and genotyping for VP7 (G typing) and VP 4(Ptyping) was done with the help of reverse transcription polymerase chain reaction(RT-PCR)[8]. Results of the lab analysis were recorded. Results were entered in the Microsoft Excel work sheet and were analysed using SPSS version17.0. P value of <0.05 was considered significant.

RESULTS

For the 8 months period, from March 2016 up to October 2016 a total of 544 children below 2 years age group were admitted for various ailments. Out of these 138 cases were for acute watery diarrhoea which was enrolled in the study group. The mean age of the study subjects was 12.27 months. The majority of the patients were male 99 (57.2%), and 39 (42.8%) were female children. The stool samples of 46 (25.67%) children were positive for rotavirus by ELISA (Table 1). Among 138 cases, a majority 84 (58.8%)] were in the age group of 1-12 months. Similarly, out of 46 ELISA positive case, 28 (17.7%) were found to be in the same age group. (Table 2). On further analysis it was found that maximum number of rotavirus positive cases35 (76.08 %) were seen between 6 to 18 months. Only 14 children(10.14 %) of the study group were vaccinated with atleast 1dose of available rota virus vaccine .10 stool samples out of the 14 vaccinated cases were negative for rotavirus antigen,2were positive for untypable strain and 1 each for G3P[8] and G9P[4]genotype. Applying chi square analysis, it was found that there was no statistical significant difference in ELISA reactivity between different age groups of cases. As shown in table 3 the month wise distribution suggests maximum number of rotavirus positive cases was in the month of March, October and September. Out of the total46rotavirus positive cases, 19(41.30%) belonged to the G3P8 type followed byG1P8(17.39 %) (Table 4) .Out of 46(21) belonged to the G3 type, followed by G1and G2 (Table 4]. Most (35) of the rotaviruses belonged to the P8 type [table 4].Most of the cases admitted had disease of moderate severity as per Vesikari's clinical severity score, with a score between 7 and 10.Majority of patients were with some dehydration but needed intravenous fluid due to frequent association of vomiting. Except for 1 case which needed admission to ICU for severe dehydration and shock all were treated in general ward. There was no death reported in our study. The mean frequency of maximum number of loose stools in 24hours, in rotavirus positive cases was 13.80 against10.91 in rotavirus negative cases which is statistically significant (P value-0.015).G3P [8] was the predominant genotype in the age group 6 to12 and 12 to 18 months whereas G1P[8] was relatively more common in children of 12 to 24 months of age.(Table 5).

Table 1: Age and gender distribution of diarrhoeal cases(n,%)

Gender	≤ 12 month(n,%)	12m to 24 m(n, %)	Total
male	58(69.04)	41(75.92)	99
female	26(30.95)	13(24.07)	39
Total	84(100)	54(100)	138

p-0.19 (1-tail value)

Table 2:Elisa reactivity in various age groups n(%)

Age group (in months)	ELISA		Total
	Negative n(%)	Positive n(%)	
≤6m	14(15.21)	7(15.21)	21
6- ≤12	42(45.65)	21(45.65)	63
12- ≤18m	23(25.0%)	14(30.4)	37
18 - ≤24m	13(14.13)	4(8.6)	17
Total	92(100)	46(100)	138

Chi square=1.073,degree of freedom-3, p-value=0.78

Table 3:Month wise distribution of rotavirus cases,n(%)

Month	Total cases	Rota positive cases n(%)
March	20	12(26.08)
April	15	4(8.6)
May	20	5(10.86)
June	16	3(6.5)
July	26	5(10.86)
August	11	1(2.1)
September	18	7(15.21)
October	12	9(19.56)
total	138	46(100)

P value=0.14

Table 4 Genotypic distribution of G and P Types of rotavirus

Genotype	P8	P4	P4+P8	untypable	Total
G_3	19	0	0	2	21
G1	8	1	1	0	10
G2	2	3	0	0	5
G9	2	0	0	0	2
G2G9	1	0	0	0	1
G3G12	1	0	0	0	1
G1G3	2	0	0	0	2
G untypable	0	0	0	4	4
Total	35	4	1	6	46

P= 0.0001

Distribution of G and P types of rotavirus among the 46 ELISA positive samples. 19 cases (41.30%) cases belonged to the G3P8 type and 8 cases (17.39%) belonged to G1P8 strain.

Table 5: Common Genotypes of Rotavirus among Different Age Groups

Age group (in months)	G3P8	G1P8	Untypable
≤6 m	3(15.78)	1(25)	1(25)
6 ≤12m	9(47.36)	1(25)	2(50)
12≤18m	5(26.31)	4(50)	0
18≤24	2(10.52)	2(25)	1(25)
Total	19(100)	8(100)	4(100)

P-value=0.474

DISCUSSION

Diarrhoeal diseases are one of the commonest causes of death in children in developing countries and rotavirus has been consistently identified as the commonest pathogen associated with severe diarrhoea. In the present study 25.36% of hospitalization in children below 2years was due to acute watery diarrhoea. In all age groups males outnumbered females though statistically not significant; it is similar to other studies[5]. In our study 84 (60.86 %) children were

below 12months of age.54 (39.13 %) children were between12-24months age. Maximum number of rota positive cases was between 6 to 18 months of age which is similar to other studies [10, 11]. Out of the total 138 study population,46(33.33%) cases were positive for rotaviral antigen by ELISA which is similar to various other studies.

In Chandigarh and north India, rotavirus was detected in 16-19% of instances of acute gastroenteritis in children under 5 years of age [15, 16]. While in Kerala in it was detected in 35.9% of cases of acute diarrhoea [12]. In the eastern states of India and in Pune, rotavirus was detected in 28-30% of children aged less than 5 years with acute diarrhoea [11,12,21]. In Kolkata, the incidence of rotavirus associated diarrhoea varied 5-22%[19].

Mehendale *et al.;* in a multi-state 28 hospital based surveillance study of 11898 children between 2012-2014 found that 39.6% of cases were positive for rota virus. In their study highest percentage of positive cases were found in Tanda and Bhubaneswar i e (60.4%) with lowest prevalence in Nalanda (3.6%) [22]. Though the rotaviral positivity in our city was quite high in their study in 2012-2014, in this present study the lower rate may be attributable to increasing number of children being vaccinated in both private and Govt health centres especially after introduction of rotavirus vaccine in national immunization schedule

Many studies have observed an increase in rotavirus-associated diarrhoea during the winter months[5, 17, 25] particularly in October to February, throughout the country. Rotavirus was markedly seasonal in northern India but was less seasonal in southern locations with a more tropical climate [14]. In the present study, although the relative number of cases was more in March, September and October there was uneven distribution throughout the year and it was not statistically significant. Jain *et al.;* in their study suggested decline in rotavirus positivity in rotavirus-vaccinated children hospitalized for acute gastroenteritis and high prevalence of G1P [8] and non-rotaviral co infections in Pune, India [21]. In our study 10.14% of total diarrhoeal cases were vaccinated with at least 1dose of rota vaccine against only 3.3% in in their study.in our study 70.14% of the vaccinated children was negative for rotavirus. This may suggest higher efficacy of the vaccines in preventing rotaviral diarrhoea.

In this study G3P[8](41.30%) was the most common genotype isolated which is different from many Indian studies [5, 23, 25, 27,29,30]as well as various other studies from across the globe[9,26,]In all these above studies G1 was the most common genotype in various combination with P8,P4 or P6.G3P8 was a minor strain of 4.3% and 4.4% of cases in a study by Chitamber *et al.;*[25] and Aly *et al.;*[26] respectively.G1P6was the commonest strain in a study by Jain *et al.;*[28]. In our study G3 (45.65%) was the most common G serotype followed by G1 (21.73%) and G2 (10.86%).P8 was the most prevalent (76.08%) P type followed by P untypable (13.04%) and P4 (8.6%) which is in agreement with other studies[8, 24]. where G3P8 was the most prevalent strain.In view of current introduction of rotavirus vaccine in immunization schedule and increasing awareness of rotavirus vaccination in private settings it may point towards a changing trend in the prevalent serotype. Earlier reports from other countries have well described rise in circulation of strains other than the vaccine strains after introduction of the mono and pentavalent vaccine[31]. The increasing numbers of untypable strains may be a result of genetic reassortment and pose a real threat for universal vaccination against rotavirus in future. So tracking of the prevalent strains is important to understand the epidemiology of the disease and to monitor changes following the introduction of vaccine.

There were a few limitations of present study. As this was a hospital-based study and the sample sizewas small, the prevalence of rotaviral diarrhoea might have been different from the actual prevalence in the community. As our patients were not considered it may not truly reflect the actual disease burden in the community.

CONCLUSION

This study shows that rotavirus accounts for one third of the total cases of acute diarrhoea in children below 2 years of age. Diverse range of rotavirus strains have been identified including several uncommon genotypes with high proportion of untypable strains in this part of eastern India. Regional variation and serodiversity of rotavirus requires a polyvalent vaccine which covers all major serotypes prevalent in a country. Emergence of newer serotypes over a period of time requires large population based studies and continuous surveillance, which will help the policy makers.

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