ORIGINAL ARTICLE

Rotavirus diarrhoea in under five population in Northern India

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Abstract

Background: Infant and childhood diarrhoea due to Rotavirus causes over half a million deaths globally of which an estimated one fifth occur in India alone. Vaccination is being recommended by WHO in most parts of the world. Objective: This study aims at providing estimates of disease burden in the under-five pediatric population and the demographic factors associated with the population affected. **Methods:** Stool specimens were collected from children suffering from acute diarrhoea and aged between one to five years. Acute diarrhoea was defined as passage of three or more liquid or loose stools per day (or more frequent passage than is normal for the individual, WHO fact sheet). Demographic and clinical information about the patient was collected using a precoded questionnaire. ELISA was used to test stool specimens for presence of antigen of Group A Rotavirus. Chi-square test was used to analyze data. Univariate and multivariate logistic regression models were used. **Results**: Of the 256 children whose samples were collected over two years 150 were hospitalized and 106 were treated in the community. Rotavirus prevalence was 26% in the hospitalized population and 5.6% in the community. Overall 17.6% (95%CI=12.9-22.3) were positive by ELISA. **Conclusion:** The risk of rotavirus infection was significantly higher in underweight children (z scores weight for age less than -2 SD (68.9%, OR= 4.76 95%CI=(2.38-9.53), <0.0001) , were severely dehydrated (4.36%, OR=4.36, 95%CI=2.13-8.92, p<0.0001) and had vomiting (40%, OR=0.28, 95%CI=0.15-0.55), p<0.0001).

Key Words

Rotavirus; Diarrhoea; Prevalence; EIA

Introduction

Group A Rotaviruses are a significant etiological agent in the pathogenesis of watery diarrhoea and gastroenteritis in infants and young children under five years of age. Over half a million deaths and 2 million admissions globally (1, 2) can be attributed to it. Rotavirus is a double stranded RNA virus belonging to the family Reoviridae. Mature viral particles are nonenveloped, 100nm in diameter with icosahedral symmetry. A triple layered capsid surrounds a genome of 11 segments of double stranded RNA. (3) Prevalence of rotavirus infection among children has been found to vary between 6% and 56 % globally. (4, 5, 6) In a review (7) the average prevalence in hospitalized children in India has been stated to be 20.8%. In temperate countries of Europe and Americas more cases are reported in the winter months but in tropical countries like India seasonal variation or winter peak has not been conspicuous. Researchers have found demographic factors like age, nutritional status, exposure to contacts, dehydration and maternal age and smoking to be variably associated with rotavirus diarrhoea. (8, 9) WHO has recently recommended rotavirus vaccines to be included in the universal immunization programmes of all countries. ELISA has been found to be a cost effective method for detection of rotavirus antigen in stool samples. RT-PCR assays based on structural and non-structural genes further

confirm diagnosis and are useful tools for molecular surveillance.

Aims & Objectives

To estimate the disease burden of rotavirus in children suffering from acute watery diarrhoea in both hospitalized and community populations.

Material and Methods

Study Design: Cross sectional study. Prior permission for undertaking the study was taken from the Institutional Ethics Committee. **Study population:** Infants and children in the age group 0 - 5 years suffering from acute watery diarrhoea referred to Emergency unit of department of Pediatrics, K.G. Medical University from Lucknow and adjoining areas enrolled. Besides these some community acquired cases treated in the community were also enrolled. Acute diarrhoea was defined as passage of three or more liquid or loose stools per day (or more frequent passage than is normal for the individual). (10) Patients with dysentery (diarrhoea with blood in stools with or without mucus), persistent diarrhoea (duration more than 2 weeks) and cases where parents did not give consent were excluded from the study. **Sample Size**: Sample size calculation has been done on the basis of a previous study which had reported a prevalence of 19.2% (11) Thus taking P(population proportion) as 19.2% and E (precision) as .05% in the formula: N (sample size) = P.Q. (Z α/2)2 / E2 sample size has been calculated to be 256.

Variables for data collection: Data was collected using a pre-coded questionnaire after obtaining written informed consent. Variables included child's age, gender, weight, height or length, dietary practices with emphasis on exclusive breast feeding, birth weight, immunization status, rural or urban dwelling, symptoms and signs, associated complications, degree of dehydration among other factors. We also obtained information regarding the parent's age, gender, education level and occupation. **Sampling and laboratory methods:** Participants were consecutively enrolled until the required sample size was obtained. Stool samples were collected in clean screw capped containers, labelled properly and transported at 4o C (Cold packs) to the laboratory. All samples were stored at 4o C. **Enzyme immunoassay (EIA):** The presence of rotavirus antigen in the stool sample was tested using Enzyme Immunoassay kit, RIDASCREEN rotavirus EIA (R- Biopharm, Germany). **Statistical methods:** All the categorical variables were compared using Chi-square test. Both univariate and multivariate logistic regression analysis models were used. Statistics software SPSS 21.0 (IBM Corp., Armonk, New York, USA) was used for the calculations.

Results

From January 2010 to Feb 2012 a total of 280 subjects were enrolled for the study. Out of these stool sample could not be obtained for 20 while 3 did not give consent. Eventually samples were processed for 257 subjects. Out of these 150 were hospitalized while 106 were being treated by primary health care workers in the community. The prevalence of rotavirus infection was 26% in the hospitalized children and 5.6% in the community. The overall prevalence was 17.6 % (95%CI=12.9-22.3). The seasonal prevalence is represented in Fig 1. Majority (79.3%) of the children were below ≤12 months and were males (59.4%). The prevalence was insignificantly higher in children aged less than 12 months (86.7%) and of male sex (60%). The prevalence was insignificantly higher in those children who were on exclusive breastfeeding. The prevalence was significantly higher in children with weight for age less than - 2 SD of normal and those presenting with severe dehydration and vomiting. However, the prevalence was also lower in rural children as compared to urban (Table-1).

The multivariate logistic regression analysis revealed that place of residence, associated vomiting, weight for age less than -2SD and severe dehydration were significantly associated with rotavirus positivity (Table-2).

11 out of 45(24%) Rotavirus positive cases had been hospitalized previously for some other complaint and had developed diarrhoea after more than 48 hr of admission. 2 were admitted in neonatal period for congenital anomalies, 2 for pneumonia, 3 were preterm infant with sepsis, 1 each for Tubercular meningitis, nephrotic syndrome, hepatic encephalopathy and Down's syndrome.

Discussion

Since its discovery by Bishop et al in 1973(12) rotavirus has been shown to be the leading cause of mortality associated with infantile and childhood gastroenteritis. Initially electron microscopy was the only means to screen stool samples for the wheel shaped appearance of RV and also to differentiate it with other small round structured viruses (SRSV) implicated in the pathogenesis of diarrhoea. Later on

ELISA for antigen detection in stool has proved to be a cost effective alternative for diagnosing rotavirus infection. In this study we have also used ELISA to test all stool specimens for RV antigen and found a prevalence of 17.6% in samples collected over a period of two years. Comparing the two subset of population prevalence was markedly higher in children needing hospitalization (26%) compared to children in the community. This observation highlights a well-known fact that rotavirus diarrhoea in the non-immune child leads to severe watery diarrhoea requiring hospitalization and fluid replacement. (13) The reason for the high mortality extracted by the virus is the delay or non-availability of health services in the developing world. The Indian Rotavirus Strain Surveillance Network have reported that rotavirus can account for as high as 39% of acute diarrheal hospitalizations. (14)

Majority of the children affected were male less than 1year old. Rotavirus is known to affect children primarily before their first birthday. Infection does not induce lifelong immunity but subsequent infections are known to be less severe. (15) It is not clear why males were affected more than females. Exclusive breast feeding has been shown to confer protection against bacterial agents of gastroenteritis but the observation so far is that there is no difference in the incidence of viral gastroenteritis between breastfed and top fed children (16) though in one study it was noted that the disease was less severe in the former. (17) We have in fact found a higher incidence (not statistically significant) of rotavirus infection in breastfed children as compared to top fed and mix fed children. Children residing in urban areas were affected more than those of rural origin. Studies done in the developed world have shown that improved hygiene and provision of safe drinking water as seen in urban localities have not helped in reducing the incidence of rotavirus diarrhoea. (18) Reasons put forward are many. The non-enveloped hardy virus from clinical and subclinical cases can be shed in the stool from one week to one month (19) and can survive for long periods at ambient temperature. As the infective dose is low the infection follows a fomite borne route. (20) Further evidence is provided by the fact that of all the admitted rotavirus patients about 27% in developed and 32% in developing world, the infection was nosocomially acquired. (21) In our study the figure was 24%. One interesting finding is that many children with rotavirus diarrhoea also exhibit respiratory symptoms. This has led to the speculation that the virus could be airborne. Some workers have experimentally induced infection intra-nasaly in gnotobiotic piglets but the issue is largely unresolved. (22) Other factors which were found to be significantly associated with rotavirus infection were malnutrition (wt for age less than -2SD) and severe dehydration. Malnutrition is a known to predispose children to infections in general and as such increases the severity of diarrhoeal symptoms. (23) According to WHO estimates about half of the mortality in the under-five age group (of which diarrhoeal deaths account for 18%) can be attributed to underlying malnutrition. (24) Most of the positive cases had been admitted with symptoms and signs of severe dehydration requiring active resuscitation with intravenous fluids and electrolytes. All recovered except one 18 month boy who was admitted with a one day history of diarrhoea and vomiting leading to seizures and unconsciousness and despite intensive efforts could not be saved.

Conclusion

To sum up Rotavirus produces severe dehydration requiring hospitalization in young infants and children who are under nourished. Breast feeding is not protective and the infection can be nosocomially acquired. All hands should join in the implementation of effective vaccines to save precious lives lost in all parts of the globe

Authors Contribution

All authors have contributed equally.

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References

1. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, WHO-coordinated Global Rotavirus Surveillance Network. Lancet Infect. Dis. 12, 136–141 (2012)
2. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhoea. Emerg Infect Dis 2006; 12:304–6.
3. Kapikian AZ, Hoshino Y, Chanock RM. Rotaviruses. In: Knipe H, DM, editor. Fields virology. 4th edition. Philadelphia: Lippin-cott-Raven Publishers; 2001. p. 1787-1833.H
4. Odimayo MS, Olanrewaju WI, Omilabu SA, Adegboro B. Prevalence of rotavirus-induced diarrhea among children

under 5 years in Ilorin, Nigeria. J Trop Pediatr. 2008 Oct;54(5):343-6. doi: 10.1093/tropej/fmn081. Epub 2008 Sep 11. PubMed PMID: 18786984.[PubMed]
5. de Wit MA, Koopmans MP, van der Blij JF, van Duynhoven YT. Hospital admissions for rotavirus infection in the Netherlands. Clin Infect Dis. 2000 Sep;31(3):698-704. Epub 2000 Oct 4. PubMed PMID: 11017818.[PubMed]
6. Sethi SK, Al-Nakib W, Khuffash FA, Majeed HA. Acute diarrhoea and rotavirus infections in young children in Kuwait. Ann Trop Paediatr. 1984 Jun;4(2):117-21. PubMed PMID: 6083746.[PubMed]
7. Ramani S, Kang G. Burden of disease & molecular epidemiology of group A rotavirus infections in India. Indian J Med Res. 2007 May;125(5):619-32. Review. PubMed PMID: 17642497; PubMed Central PMCID: PMC2474761.[PubMed]
8. Dennehy PH, Cortese MM, Bégué RE, Jaeger JL, Roberts NE, Zhang R, Rhodes P, Gentsch J, Ward R, Bernstein DI, Vitek C, Bresee JS, Staat MA. A case-control study to determine risk factors for hospitalization for rotavirus gastroenteritis in U.S. children. Pediatr Infect Dis J. 2006 Dec;25(12):1123-31. PubMed PMID: 17133157.[PubMed]
9. Sethi D, Cumberland P, Hudson MJ, Rodrigues LC, Wheeler JG, Roberts JA, Tompkins DS, Cowden JM, Roderick PJ. A study of infectious intestinal disease in England: risk factors associated with group A rotavirus in children. Epidemiol Infect. 2001 Feb;126(1):63-70. PubMed PMID: 11293683; PubMed Central PMCID: PMC2869674.[PubMed]
10. World Health Organization. Fact sheet N^ 330 August 2009.
11. Mishra V, Awasthi S, Nag VL,Tandon R. Genomic diversity of group A rota virus strains in patients aged 1-36 months admitted for acute watery diarrhea in northen India : a hospital based study. Clin Microbiol Infect 2009 ; 202-07
12. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet. 1973 Dec 8;2(7841):1281-3. PubMed PMID: 4127639.[PubMed]
13. Rodriguez WJ, Kim HW, Arrobio JO, Brandt CD, Chanock RM, Kapikian AZ, Wyatt RG, Parrott RH. Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children. J Pediatr. 1977 Aug;91(2):188-93. PubMed PMID: 195031.[PubMed]
14. Kang G, Arora R, Chitambar SD, Deshpande J, Gupte MD, Kulkarni M, Naik TN, Mukherji D, Venkatasubramaniam S, Gentsch JR, Glass RI, Parashar UD; Indian Rotavirus Strain Surveillance Network. Multicenter, hospital-based surveillance of rotavirus disease and strains among indian children aged <5 years. J Infect Dis. 2009 Nov 1;200 Suppl 1:S147-53. doi: 10.1086/605031. PubMed PMID: 19817593.[PubMed]
15. Bishop RF. Natural history of human rotavirus infection. Arch Virol Suppl. 1996;12:119-28. Review. PubMed PMID: 9015109.[PubMed]
16. Glass RI, Stoll BJ. The protective effect of human milk against diarrhea. A review of studies from Bangladesh. Acta Paediatr Scand Suppl. 1989;351:131-6. Review. PubMed PMID: 2692384.[PubMed]
17. Golding J, Emmett PM, Rogers IS. Gastroenteritis, diarrhoea and breast feeding. Early Hum Dev. 1997 Oct 29;49 Suppl:S83-103. Review. PubMed PMID: 9363419.[PubMed]
18. Bern C, Glass RI. Impact of diarrhoeal diseases worldwide. In: Kapikian AZ editor, Viral Infections of the gastrointestinal tract, 2nd Edn. New York:Marcel Dekker,1-26.
19. Richardson S, Grimwood K, Gorrell R, Palombo E, Barnes G, Bishop R. Extended excretion of rotavirus after severe diarrhoea in young children. Lancet. 1998 Jun 20;351(9119):1844-8. PubMed PMID: 9652668.[PubMed]
20. Wilde J, Van R, Pickering L, Eiden J, Yolken R. Detection of rotaviruses in the day care environment by reverse transcriptase polymerase chain reaction. J Infect Dis. 1992 Sep;166(3):507-11. PubMed PMID: 1380049.[PubMed]
21. Fischer TK, Bresee JS, Glass RI. Rotavirus vaccines and the prevention of hospital-acquired diarrhea in children. Vaccine. 2004 Dec 6;22 Suppl 1:S49-54. Review. PubMed PMID: 15576202.[PubMed]
22. Azevedo MS, Yuan L, Jeong KI, Gonzalez A, Nguyen TV, Pouly S, Gochnauer M, Zhang W, Azevedo A, Saif LJ. Viremia and nasal and rectal shedding of rotavirus in gnotobiotic pigs inoculated with Wa human rotavirus. J Virol. 2005 May;79(9):5428-36. PubMed PMID: 15827157; PubMed Central PMCID: PMC1082764.[PubMed]
23. Brown KH, Gilman RH, Gaffar A, et al. Infections associated with severe protein-calorie malnutrition in hospitalized infants and children. Nutr Res 1981;1:33â€“46.
24. Bryce J, Boschi-Pinto C, Shibuya K, Black RE; WHO Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. Lancet. 2005 Mar 26-Apr 1;365(9465):1147-52. PubMed PMID: 15794969.[PubMed]

Tables

TABLE 1: FACTORS ASSOCIATED WITH ROTAVIRUS INFECTION

Study factors	Rotavirus positive no. (%) [n=45]	Rotavirus negative no. (%) [n=211]	Unadjusted OR (95%CI), p-value
Age ≤12 months	39 (86.7)	164 (77.7)	1.86 (0.74-4.67), 0.18
Male	27 (60.0)	125 (59.2)	1.03 (0.54-1.99), 0.92
Exclusive breast feeding	25 (55.6)	97 (46.0)	1.47 (0.77-2.81), 0.24
Rural dwelling	21 (46.7)	148 (70.1)	0.37 (0.19-0.72), 0.003*
Low maternal literacy (Illiterate)	20 (44.4)	72 (34.1)	1.54 (0.80-2.97),0.19
Diarrhoea duration less than 5 days	20 (44.4)	104 (49.3)	0.82 (0.43-1.57), 0.56
Episodes of diarrhoea ≤15 per day	32 (71.1)	144 (68.2)	1.15 (0.57-2.32), 0.71
Associated vomiting	18 (40.0)	148 (70.1)	0.28 (0.15-0.55), <0.0001*
Weight for age less than -2 SD (z scores)	31 (68.9)	67 (31.8)	4.76 (2.38-9.53), <0.0001*

Severe dehydration	18 (40.0)	28 (13.3)	4.36 (2.13-8.92), <0.0001*
Occupation**			
Farmer	11 (24.4)	58 (27.5)	1.00
Unskilled labour	9 (20.0)	82 (38.9)	0.58
Skilled labour	9 (20.0)	22 (10.4)	2.16
Business	8 (17.8)	22 (10.4)	1.92
Office/Institution staff	5 (11.1)	19 (9.0)	1.39
Others	3 (6.7)	8 (3.8)	1.98

TABLE 2 MULTIVARIATE REGRESSION ANALYSIS OF FACTORS FOUND SIGNIFICANT IN UNIVARIATE ANALYSIS

Factors	Beta coefficient	Standard Error	Adjusted OR (95%CI)	p-value
Place of residence				
Rural	-1.185	0.574	0.30 (0.10-0.94)	0.03*
Urban	Ref.		1.00	
Associated vomiting				
Yes	-1.722	0.560	0.18 (0.06-0.54)	0.002*
No	Ref.		1.00	
Weight for age less than -2 SD (z scores)				
Yes	3.582	0.629	35.96 (10.49-123.27)	<0.0001*
No	Ref.		1.00	
Severe dehydration				
Yes	1.616	0.589	5.03 (1.59-15.97)	0.006*
No	Ref.		1.00	
Occupation				
Farmer	Ref.		1.00	
Unskilled labor	-0.655	0.595	0.52 (0.16-1.68)	0.27
Skilled labor	-0.384	0.766	0.68 (0.15-3.06)	0.62
Business	0.077	0.741	1.08 (0.25-4.62)	0.92
Office/Institution staff	-0.698	1.021	0.50 (0.7-3.68)	0.49
Others	0.042	1.103	1.04 0.12-9.07)	0.10
Constant	-0.815			