

Original Research Article

Prevalence of Norovirus and epidemiology of acute gastroenteritis in children

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Abstract

Pediatric gastroenteritis is a major cause of childhood mortality and morbidity worldwide, especially in developing countries. Diarrhoea can be caused by a variety of different pathogens including bacteria, viruses and parasites. Among the viruses; Rotavirus has been extensively studied and is responsible for 44% of GE cases. As the Rotavirus vaccination coverage improves, the causative agent's shift may be more towards the Calciviruses (Norovirus, Sappovirus) and other similar viruses, and consequently the investigations should focus on these viruses in future. This study was conducted in a Teaching hospital, Hyderabad, Telangana State included 118 cases of Gastroenteritis of which 6 cases were positive for Norovirus (NoV) i.e.; 5% of cases were NoV positive by RIDASCREEN EIA and RIDA QUICK, the rapid test for NoV virus. These 6 cases were children between 7 months and one and half year old. The age profile showed a fall in the number of diarrhoea cases as the child's age increases. 63 (53.3%) were male children and 55 (46.6%) were female children. In children < 2 years (n=83), 22 (26.5%) were breast fed, 30 (36.1%) were bottle fed and 7 (8.4%) were on mixed

feeds. Among mothers 25.42% never washed their hands with soap, 60.16% used soap occasionally and only 14.4% always used soap. 43.2 % presented with no dehydration, 27.96% presented with some dehydration and 28.8% presented with severe dehydration. 70.3% of mothers continued to feed during diarrhoea. Regarding the treatment used for diarrhoea before admission in hospital, 42.37% used ORS, 27.11% used both ORS and antibiotics, 55.08% were on antibiotics and 11% took no treatment at all.

Key words

Norovirus (NoV), Sappovirus (SaV), Calcivirus, Rotavirus, Gastroenteritis, Diarrhoea, Breast-Feeding, ORS, Prevalence, RIDA QUICK, RIDA SCREEN EIA, Molecular Diagnostic Assay.

Introduction

Though Noroviruses (NoV) was identified in 1972, the inability to cultivate these viruses in routine culture and the consequent challenges in developing sensitive molecular diagnostic assays hindered initial efforts to define the epidemiology and assess the impact of disease due to NoV.

Since the application of molecular assays, NoVs have been well documented as the leading cause of epidemic Gastroenteritis in all age groups [1]. Recent studies have demonstrated that NoVs may also fill the diagnostic gap in severe sporadic GEs in all age groups.

Role of Noroviruses is sporadic Gastroenteritis by Manish M Patel, et al. [2]. NoVs accounted for 12% of severe gastroenteritis among children < 5 years of age and 12% of mild and moderate diarrhoea cases among persons of all ages.

In a report from Hansman, et al., Thailand, 12% of stool specimens were positive for NoVs [3]. Reports from Malawi, China, Vietnam, Mongolia and India have shown NoVs associated with 6-25% of acute gastroenteritis cases.

In a study on the prevalence of Calciviruses among children hospitalized for acute GE in United States, by Zintzc, et al. [4] 8.5% were human Calciviruses positive. Among them 7.1% were NoV positive and 1.4% were SaV.

The studies done in India at Pune by Preeti Chabra, et al. [5] showed 11.9% positivity for

NoV. There was a predominance of GII4 along with co circulation of GII -1, GII -2, G – Iib. Clinical severity score showed severe disease in 70.8% and moderate disease in 29.2%.

Study conducted in Vellore, South India showed 19.4% positivity for human Calciviruses in hospitalized children, 15.1% were positive for NOVs and 5% for SaVs GII3 was the most frequently indentified genotype followed by GII -1 and GII -4 in equal proportion [6].

Hence, this study was undertaken to determine the Prevalence of Noroviruses and Epidemiological Data of Acute gastroenteritis in children in Hyderabad.

Materials and methods

This is a descriptive study, conducted upon 118 (n=118) children who were admitted with acute gastroenteritis in Niloufer Hospital, Hyderabad, Telangana State. The age group was 2 months to 12 yr and the study was conducted from the month of July to September 2012. Children with dysentery, persistent diarrhoea, age <2 months and >12 years were excluded from study. Before commencing the study the institutional Ethics Committee clearance was taken. Informed consent from the parent or guardian of each and every child enrolled in this study was obtained.

Study Procedures

Stool sample from children >2 months and < 12 years with acute gastroenteritis were collected into a sterile wide mouthed container within 24 hours of reporting to hospital. The stool samples

were made homogenous by vortex of liquid or by drop by drop dilution with water. Two aliquots of 1 ml were placed in CRYO. S screw cap tubes and the remainder in the wide mouthed plastic container. The aliquots and remainder of homogenized sample were frozen at -20⁰ C until further need.

Samples were prepared for the assay as specified by manufactures instructions. 100 ml of liquid stool was suspended in 1 ml of dilution buffer; these were homogenized using vortex and let to settle for 6 minutes. The supernatant was used in the assay.

RIDA SCREEN EIA

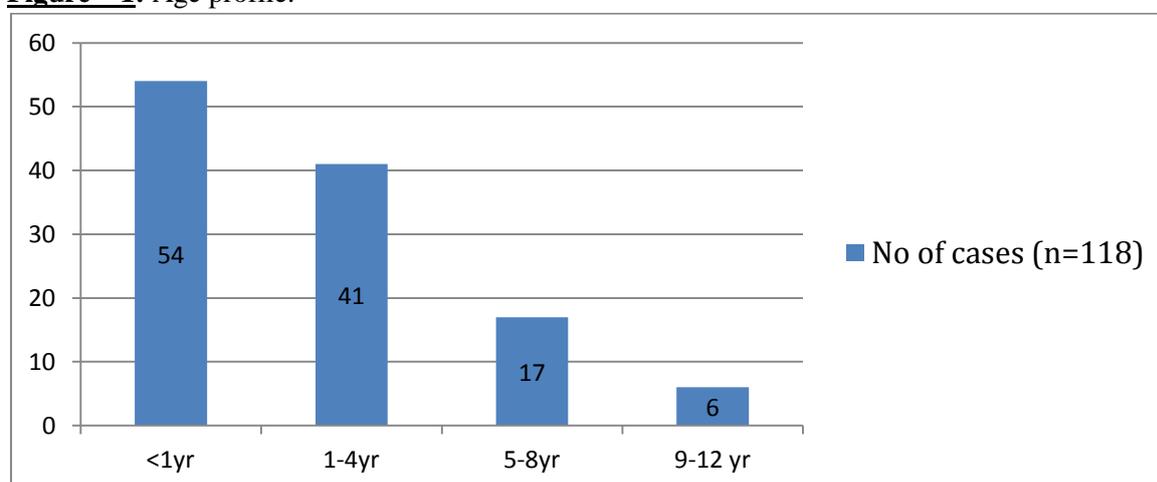
For NoV Ag detection samples were processed using the steps in manufactures protocol, processing 30 samples at a time and allowing for replication. Then following further steps, the plate was available for OD 450nm reading after 3 incubations, i.e. about 105 min.

Samples was considered positive if OD 450nm value is 10% of more above the cut off point and negative if OD 450 is reading will fall 10% or more below out off point.

Table – 1: Age Profile (n=118).

Age	< 1 year	1-4 years	5-8 years	9-12 years
No. of cases	54	41	17	6
%	45.7 %	34.7%	14.4%	5.08%

Figure – 1: Age profile.



RIDA Quick -Rapid test for Norovirus

The samples for this assay were similar to the samples prepared for RIDASCREEN; the assay was conducted as described in manufactures protocol using sample supernatant. The Cassette with sample was incubated for 15 min at room temperature. Results were read within first 3 minutes of adding the substrate to the reaction window.

Cassettes with only blue line on C side visible were NoV negative and the cassettes with a blue line on the C side and T side were NoV positive.

Results

Out of 118 cases, 6 (5%) cases were Noroviruses positive. These 6 cases were between 7 months to 1½ year of age. 3 of them were bottle fed and 3 were breast fed. 4 children had no dehydration and 2 had moderate dehydration.

Age profile

The age profile showed a fall in the number of diarrhoea cases as the child's age increases (**Table – 1** and **Figure – 1**).

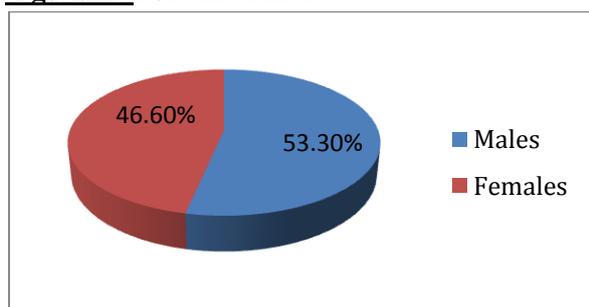
Sex incidence

63 (53.3%) were male children and 55 (46.6%) were female children (**Table – 2** and **Figure – 2**).

Table – 2: Sex incidence (n=118).

Sex	No. of cases	Percentage
Females	55	46.6%
Males	63	53.3%

Figure – 2: Sex incidence.



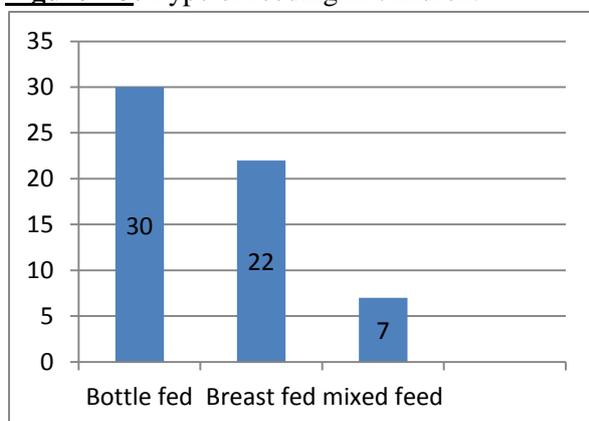
Type of feeding

In children < 2 years (n=83), 22 (26.5%) were breast fed, 30 (36.1%) were bottle fed and 7 (8.4%) were on mixed feeds (**Table – 3** and **Figure – 3**).

Table – 3: Type of Feeding in children <2 years (n=83).

Type of feeding	No of cases	Percentage
Breast fed	22	26.5%
Bottle fed	30	36.1%
Mixed feed	7	8.4%

Figure – 3: Type of feeding in children.



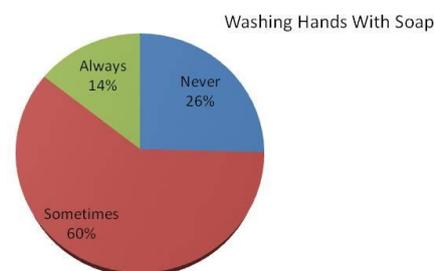
Washing hands with soap

Regarding the knowledge of hygiene and hand washing with soap before preparing food, before feeding children, after work etc, among mothers 25.42% never washed their hands with soap, 60.16% used soap occasionally and only 14.4% always used soap (**Table – 4** and **Figure – 4**).

Table – 4: Habit of washing hands with soap.

Washing hands with soap	No. of cases	Percentage
Never	30	25.42%
Sometimes	71	60.16%
Always	17	14.4%

Figure – 4: Habit of washing hands with soap.



Clinical presentation

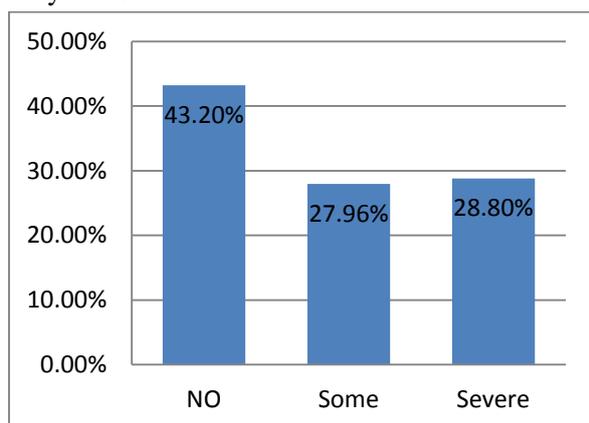
43.2% presented with no dehydration, 27.96% presented with some dehydration and 28.8% presented with severe dehydration (**Table – 5** and **Figure – 5**).

Table – 5: Regarding the severity of dehydration.

Severity of dehydration	Percentage
No	43.2%
Some	27.96%
Severe	28.8%

Diarrhoea is associated with loss of nutrients. So there is a need for continuous feeding during diarrhoea. 70.3% of mothers continued to feed during diarrhoea. Regarding the treatment used for diarrhoea before admission in hospital, 42.37% used ORS, 27.11% used both ORS and antibiotics, 55.08% were on antibiotics and 11% took no treatment at all.

Figure – 5: Regarding the severity of dehydration.



Discussion

The age profile showed a fall in the number of diarrhoea cases as the child's age increases which is explained by improved personal hygiene and less susceptibility to infection as age increases. This is supported by the study done by Yehuda, et al. [7] which showed the age of the child was the most important risk factor, with mean no. of episodes of acute diarrhoea per 12 child months was 2.28 in 0-2 years, 0.44 in 2-6 years, 0.12 in 6-13 years and 0.03 in 13-18 years old. More number of boys was affected than girls.

Among children <2 years with GE, it was found that 36.1 were bottle fed, 26.5% were breast fed and 8.4% were given mixed feeds. Bottle feeding is an important risk factor for GE.

A breast fed baby is 14.2 times less likely to die of diarrhoea (IAP) as it contains a number of protective factors which include immunoglobulin, mainly secretory Ig A, macrophages, lymphocytes, lysozyme, bifidus factor, interferon and other protective substances. In a study by V Kumar, et al. [8], the morbidity from diarrhoea was higher in bovine, bottle fed group than breast fed. In a study by Cesar et al. Brazil, the risk for infants who did not receive breast milk was 14.25 times higher and those who received mixed feeding the risk was 4.2 times higher [9].

Good environmental sanitation and hygiene are important in preventing diarrhoea. There 3Cs: clean hands, clean container and clean environment are the key messages in preventing diarrhoea.

In this study, it was found that only 14.5% use soap to wash hands regularly before preparing food, before feeding etc.

60.16% used soap occasionally for hand washing and 25.42% never washed their hands with soap, so there is a need for emphasis on hands washing. In a RCT on effect of intensive hand washing on childhood diarrhoea in Pakistan by Luby, et al. in 2004, the incidence of diarrhoea in older children is decreased by 53% compared to controls and by 39% in infants [10].

Diarrhoea is associated with nutrient losses. Food is often withheld from the child by the mother because of an erroneous belief that rest to the bowel promotes early recovery. Since children with diarrhoea may develop protein energy malnutrition it is desirable to continue feeding in acute diarrhoea and the diet should be digestible and nutritionally balanced. Presence of nutrients in the gut hastens recovery of intestinal epithelium because food in the intestine stimulates rapid cell turnover and renewal of intestinal lining.

In this study, 70.3% of mothers continued to feed their children during diarrhoea.

About the treatment given to the child before admission to hospital, it was found that 55.08% were started on antibiotics only, 42.37% received ORS, 27.11% received both ORS and antibiotics and 11% received no treatment at all. According to the diarrhoea disease control programme, ORS is the treatment protocol for diarrhoea. ORS is appropriate for both prevention and treatment of dehydration in diarrhoea. Only 42.37% used ORS in this study.

Antibiotics are not indicated in diarrhoea as a large majority of cases of diarrhoea are caused

by viruses or toxogenic bacteria and there is little evidence of inflammation of gut mucosa. Their indiscriminate use leads to emergency of resistant strains of harmful bacteria and eliminates resident flora which protects the guts.

In this study it was found that 55% of children were put on antibiotics before coming to hospital.

The National Family Health Survey (1992-93) revealed that 42.7% of mothers knew about ORS and 25.9% had ever used them. ORS use rates in children who suffered from diarrhoea varied from 8.3% in Rajasthan to 50.1% in West Bengal. This is a reminder that we still have to reach more than half of households [11].

In this study, the contribution of NoV to GE is 5%. Among those positive, all are under 1½ year of age. 4 has no dehydration, 2 has moderate dehydration.

However because these novel assays are not available outside of reference labs the true global prevalence and the potential economic impact of NoVs remains unrecognised.

Recommendations

The various interventions required are as below.

Environmental: Water can be treated with chlorine bleach 1000 ppm 1 part of bleach in 50 parts water, as NoV is relatively resistant to chlorine.

Phenolic compounds like Lysol, pine sol are also effective disinfectants but 2-4 fold higher concentration is required.

Quaternary ammonium compounds are not useful as they act by disrupting the viral envelopes and NoVs are not enveloped.

At a time, pasteurization at 60⁰ C has been suggested.

Health education: Education of mother regarding hand washing with soap after using toilet, before preparing food, disposal of waste in dustbin etc. is an important step towards diarrhoea control.

The importance of ORS in the treatment of gastroenteritis is also to be impressed upon the mothers. This can be achieved by training the community health workers, Anganwadi workers who in turn will train the mothers; Sheth, et al. [12].

Encouraging breast feeding: Bottle feeding is to be strongly discouraged and the importance of breast feeding is to be propagated.

Conclusion

As incidence of diarrhoea due to Rotavirus is reducing due to improved vaccine coverage, incidence due to other Calciviruses has increased worldwide. Norovirus is one of the contributors, emerging recently as a cause of gastroenteritis in children < 12 years of age in Hyderabad. Various studies from China and Sub-African countries have shown increasing incidence and indicating an impending epidemic in future. Vaccine development against Norovirus is on the anvil [13, 14]. There is a need for larger and long term studies in future. Further studies will fill the gap of undiagnosed aetiology of diarrhoea.

References

1. Widdowson MA, Monroe SS, Glass RI. Are Noroviruses emerging? *Emerg. Infect. Dis.*, 2005; 11: 735 – 7.
2. Manish M Patel, Mare – Alin Widdowson, Roger I Glass, Kenichiro Akazawa, Jan Vinje, Umesh D Parashar. Systematic Literature Review of Role of Noroviruses in Sporadic gastroenteritis. *Emerging Infectious Diseases*, 2008; 14(8).
3. Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamrin P, Tonusin S, Okitsu S, Nishio O, Takeda N, Ushijima H. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. *J. Clin. Microbiol.*, 2004a; 42: 1305 – 1307.
4. Zintz C, Bo K, Parada E, Barnes – Eley M, Berke T, Sturat MA, Azimi P, Jiang

- X, Matson DO. Study of prevalence of Caliciviruses among children hospitalized for acute GE in the United States, 2004.
5. Preeti Chhabra, Shoba D. Chitamber. Norovirus genotype G IIB associated acute gastroenteritis in India. *Jr. of Clin. Virology*, 2008; 42: 429 – 432.
 6. Bindhu Monica, Sasirekha Ramani, Indrani Banerjee, Beryl Primrose, Miren Iturriza, et al. Human Caliciviruses in Symptomatic and Asymptomatic infections in Villore, South India. *J. Med. Virol.*, 2007; 78(5): 544-551.
 7. Yehuda Lerman. Epidemiology of acute diarrhoeal diseases in children in a high standard of living rural settlement in Isreal. *Pediatr infectious diseases Journal*, 1994; 13: 116 – 122.
 8. V. Kumar, S. Sharma, P. Khanna, K. Vanaja. Breast VS Bottle feeding impact on urban infants, Chandigarh, IJP, 1981.
 9. Cesar G Victoria, Peter G smith, et al. Departmentode Medicina social, Faculdade de Medicina, Universidade Federal de Pelotas, Pelotas, Brazil. *American Journal of Epidemiology*, 1989; 129(t): 1032 – 1041.
 10. Luby SP, Agboat Walla M, painter J, Altaf A, Billhimer WL, Hoektva RM. Randomized control trial of effect of Intensive handwashing promotion on childhood diarrhea in high risk communities in Pakistan. *JAMA*, 2004; 291(21): 2547-54.
 11. National Family Health Survey data. *Ind. Jr. of Pediatrics*, 1999.
 12. Sheth M, Obrah M. Diarrhoea Prevention through: food safety education. *Ind. J. Paedtr.*, 2004; 71: 878 – 882.
 13. Frank Kowalzik, Margarita Riera-Montes, Thomas Verstraeten, Fred Zepp. The Burden of Norovirus Disease in Children in the European Union. *Pediatr Infect Dis J.*, 2015; 34(3): 229–234.
 14. Samuel Munalula Munjita. Current Status of Norovirus Infections in Children in Sub-Saharan Africa. *Journal of Tropical Medicine*, 2015, Article ID 309648, 7 pages, 2015.