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Prevelance of Rotavirus Genotypes among Children below 5 Years of Age with Acute Gastroenteritis in Western Uttar Pradesh

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Abstract

Introduction: Rotavirus is the most important non-bacterial cause of acute gastroenteritis (AGE) in young children worldwide, which may results in mortality for population at risk such as children and immunocompromised patients. Nosocomial transmission of Rotavirus is often a costly and difficult problem to resolve hence the need for inclusion of Rotavirus vaccine in the National Immunization Program, which can play a key role in significant reduction of Rotavirus associated mortality and hospitalization.

Material &Methods: 625 stool samples were collected from children below 5 years of age suffering from Acute gastroenteritis (AGE) and further processed for Rotavirus detection by ELISA and rt PCR followed by their Genotyping by using conventional PCR.

Results: Of the 625samples 180 (28.8%) and 188 (30.08%) samples were positive for Rotavirus by using Elisa and rt-PCR respectively. The most commonly isolated genotype was G1P [8].

Conclusion: rt- PCR for Rotavirus was found to be a more sensitive method compare to ELISA, Rotavirus G1P [8] genotype is one of the major cause of acute gastroenteritis in children below 5 years of age. Administration of Rotavirus vaccine introduction can lead to significant reduction in AGE morbidity and mortality.

Keywords: Acute gastroenteritis (AGE), Rotavirus, Rotavirus Vaccine, Genotype, G1P[8]

Introduction

Acute diarrhoeal diseases are a major cause of childhood morbidity and mortality all over the world.[1] Acute-gastroenteritis (AGE) remains a leading cause of post-neonatal under-five mortality in India contributing to about 13% of under-five mortality. [2, 3] Studies in the last decade estimate the annual mortality due to rotavirus estimated to be between 90,000 and 1,53,000 in India. [4, 5, 6] Rotavirus infection ranges from asymptomatic infection to severe life threatening gastroenteritis.

It has been estimated that 29% of all diarrhoeal deaths in children <5 years of age is due to rotavirus and about 23% of rotavirus deaths are in the Indian subcontinent.[7] India has estimated annual burden of 2.0-3.4 billion cases attributable to rotavirus.[8] A rising trend in proportion of rotavirus cases in hospitalized children has been reported; 26.1% before 2000 to 38.3% after

2005. A recent multi-centric surveillance study in India reported 39% prevalence of rotavirus in children below five years of age hospitalized for acute diarrhoea.[9] WHO estimated that states of Uttar Pradesh in India accounts for 32% of diarrheal deaths due to rotavirus infection among Indian children younger than five years. [10]

Numerous pathogenic agents like bacteria, parasites and viruses have been associated with AGE but viruses alone represents more than 75 % as etiological agents for AGE.[11] Among viruses, Rotavirus A (RVA) of family Reoviridae has been established as the leading agent of severe diarrhoea in children in both developing and developed countries. It consists of double stranded RNA genome and has been classified into 27G and 37P types on the basis of VP7 (G Glycoprotein) and VP4 (P Protease Sensitive) protein.[12]

Other viruses causing AGE include Norovirus (NoV), Adenovirus (AdV), Sapovirus (SaV) and Human Astrovirus (AstV). Rotavirus vaccines are expected to have highest impact on AGE mortality and morbidity. The present study aims to estimate the prevalence of Rotavirus associated AGE and their genotypic distribution in children below 5 years of age. The knowledge of viral agent and their genotypic distribution over the year can be essential for development of effective ways to control their better the cost effective treatment on a large scale.

Material and Methods

This was a cross-sectional study conducted in the Department of Microbiology & Paediatrics, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh. Ethical clearance was obtained from the Institutional Ethical Committee, reference no. SU/2015/793/(13). A total of 625 Children of age group less than 5 years with acute gastroenteritis or diarrhoea were included in this study. Written informed consent was taken from parents / guardian at enrolment.

Specimen Collection

10-15 ml of freshly passed stool samples from 625 symptomatic paediatric patients were collected in sterile wide mouth containers (Hi-Media®, Mumbai). Collected stools sample were stored at a temperature of - 20°C for Rotavirus antigen detection.

Specimen Preparation

Samples were taken by transfer pipettes provided with kit up to a given mark and 1ml of sample diluent was added to the properly marked tube using transfer pipette/precision pipette.

Rotavirus Detection

Procedure: For detection of (Group A) Rotavirus antigen in stools sample Premier (Rotaclone®, Meridian Diagnostics) Enzyme Immunoassay (EIA) based on monoclonal antibodies in a solid phase sandwich type EIA was used and samples were processed according to manufactures instructions**.** (Rotaclone®, Meridian Diagnostics)

Antigen Detection by ELISA: Microtiter plates were taken and their wells were marked for positive, negative along with sample numbers.100µl of diluted samples, positive control and negative control were added in the appropriate wells. 100µl of enzyme conjugate was added to each well and mixed it gently and incubated at room temperature for one hour. All the wells were washed with distilled water thoroughly for five times. 100µl of substrate (A) & (B) solution was added to each well and incubated at room temperature for 10 minutes. After 10 minutes visual determination of all the wells was noted for colour change. 100µl of stop solution were added to each well for spectrophotometric determination. Absorbance of each well was read at 450 nm by using ELISA reader.

Antigen Detection by rt-PCR

Rotavirus- A- Real Time PCR (HELINI Biomolecules, Chennai, India) Stool samples were processed with stool processing buffer and Helini pure fast viral mini spin prep kit was used for extraction of viral nucleic acid which utilizes exclusive silica based membrane technology in the form of a convenient spin column.

Isolated dsRNA's were then used for further downstream application. First cDNA strand synthesis was performed, using cDNA synthesis kit. The cDNA was stored at -20°C until further use. For detection of Rotavirus A, Real time PCR kit manufactured by (HELINI Biomolecules, Chennai, India) was used.

Genotyping of Rotavirus

50 rt-PCR positive samples were chosen by randomisation method for their Genotyping using conventional PCR Kit obtained from (HELINI Biomolecules, Chennai, India).G & P genotyping was done by amplification of variable sequences of VP7 &VP4 Genes by using G & P type specific primers.

Further the PCR product were analysed on 2% agarose gel as per standard method. Result in the form of bands was viewed in UV trans-illuminator and the band patterns were analysed. Data was analysed by using SPSS Version 22. P value of <0.005 was considered statistically significant.

Results

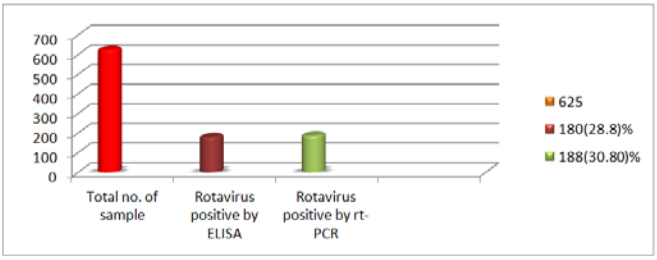
During this study 625 samples were included of which 360(57.6%) were male children and 265(42.4%) were female children and all were tested for Rotavirus detection by ELISA and PCR. Of the total enrolled cases more of the children were from rural background 370 (59.2%) while 255(40.8%) belonged to urban background. 180 (28.8%) samples were positive for Rotavirus antigen by using ELISA. However with Rotavirus rt-PCR 188(30.08%) samples were positive for Rotavirus antigen (Graph-1) while the remaining samples were negative for Rotavirus antigen.

Among Rotavirus ELISA positive cases male children were 102(56.66%) & female children were 78(43.33%). Among rt-PCR positive cases male children were 108(57.44%) and female children were 80(42.55%). Most of the cases were in the age group between 7 to 24 months (Table 1).

Rotavirus diarrhoea was more common during the months of November to April (Graph-2). Major clinical symptoms associated with disease severity in the present study were diarrhoea, vomiting, dehydration and fever.

PCR for the G (VP7) and P (VP4) genotypes, G-P type combination were found in our study strains (Graph-3).

Graph-1: Distribution of Positive Samples by Elisa & rt-PCR Method

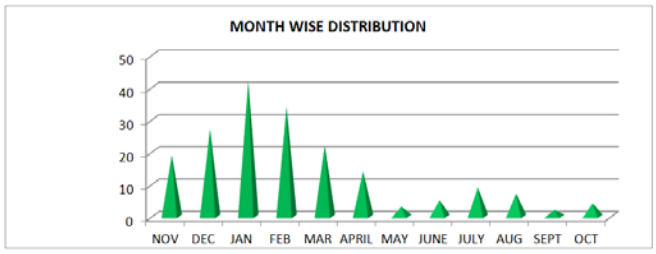


Higher positivity was seen with rt-PCR

Table-1: Distribution of Rotavirus Positive Cases based on Age & Sex by ELISA and rt-PCR Method

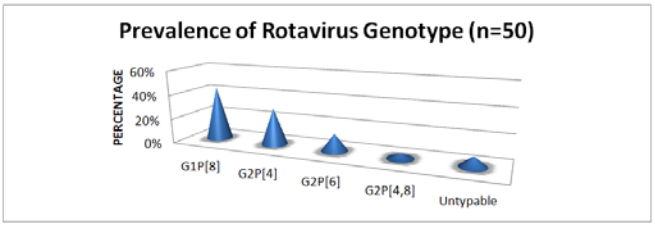
Children Age in Months	Positive for ELISA (%)	Total No. of Male Children (%)	Total No. of female Children (%)	Positive for rt- PCR (%)	Total No. of Male Children (%)	Total No. of female (%)
0-6	12 (6.66)	07 (03.88)	05 (02.77)	12 (06.38)	07 (03.72)	05 (02.65)
7-12	115 (63.88)	66 (36.66)	49 (27.22)	115(61.17)	66 (35.10)	49 (26.06)
13-24	40 (22.22)	21 (11.66)	19 (10.55)	46 (24.46)	26 (13.82)	20 (10.63)
25-60	13 (7.22)	08 (04.44)	05 (02.7)	15 (07.97)	09 (04.07)	06 (03.19)
TOTAL	180	102(56.66)	78(43.33)	188	108(57.44)	80 (42.55)

Graph-2: Seasonal Distribution of Rotavirus Positive Cases



Rotavirus was positive common between November to April.

Graph-3: Prevalence of Rotavirus Genotypes



G1P[8] was most common isolates with 22(44%) isolates followed by G2[P4]15 (30%),G2[P6] 7(14%), Mixed strain with G2[P4P8] was 2(4%) while 4(8%) isolates were untypable strain.

Discussion

Rotavirus infection is single most important cause of gastroenteritis in children below 5 years of age & associated with high morbidity and mortality in developing countries. Typically 50-60 % of acute gastroenteritis in hospitalized is caused by Rotavirus throughout the world.

The virus mainly spreads via faecal-oral route, contaminated environmental surfaces and fomites[13, 14]. Symptomatic infections are most common in children between the ages of 6 months to 2 year with a peak incidence at 9-12 months [15-17] and male children are more frequently affected than females[17]. In this study also we have observed that male children were more affected than female children however this could be due to less no of female children admitted. Rotavirus infection was mostly seen between age group 7 to 12 months; similar results were also found by Rajiv Bahl et al [18]. Rotavirus infections in large numbers were observed in rural population and this could be due to vaccination, health awareness and hygiene conditions. In temperate climates Rotavirus gastroenteritis has higher prevalence during winter month [16].

In present study it was observed that most of the positive cases were seen during November to April month. In certain studies higher incidences have been reported during the rainy season while some studies also show no seasonal variation[19].Rotavirus detection prevalence was found to be 30.08%. A number of studies have been conducted on the prevalence of childhood rotavirus diarrhoea in various parts of the country in which rotavirus was detected in 5-71 % of the hospitalized children less than 5 years of age with acute gastroenteritis [20,- 24]. This variation may be due to the period of the study, number of cases and the seasonal variation of rotavirus diarrhoea in different regions of the country.

The prevalence of rotavirus in the north Indian cities of Delhi, Chandigarh and Aligarh has been reported to vary from 6-45%. Some studies, revealed rotavirus infection rates from 15-18 %, 24 %, 32 % and 45% in cases with diarrhoea. In Chandigarh, rotavirus was detected in 16-19% of instances of acute gastroenteritis in children < 5 year of age. [25-27] In Aligarh it was detected in 19% of cases of acute diarrhoea.[28] In the western states of India, Pune, rotavirus was detected in 28-30 % of children ≤5 year of age with acute diarrhoea.[29, 30] In eastern India, Kolkata the incidence of rotavirus associated diarrhoea varied from 5-22 %. [31, 32] on the other hand, in Manipur the incidence was as high as 41%.[33]

Rotavirus was found to be positive by both ELISA (28.8%) & by rt-PCR (30.08%), Greater sensitivity of

PCR assay allows viral identification in specimen containing few copies of viral genes. In a studies conducted by L.Pang, et al. [34] rt- PCR was found to be more sensitive and lower chances of cross-contamination. However our study shows Rotavirus detection rate was more by rt- PCR compared to ELISA which co-relates study conducted by Buesa et al. Another study conducted by (Widal et al.1990, Slovis et al. 2014).[35] also shows that rt-PCR is the best confirmatory detection method for group A Rotavirus in faecal specimens.

Rotavirus group A is most commonly associated with human disease, our study shows that G1P [8] strains was most common (44%) followed by G2 [P4] 30%, G2 [P6] 14%, 4% were mixed strains G2 [P4, P8] while 8% strains were untypable. This study is also consistent with the result from National Rotavirus surveillance in India showing that the G1P [8] was two of the most common strains. In another study conducted by Sharma et.al (2008) G type G1, G2 and G9 observed, among the P type P4, P6 &P8 to be widely circulating genotypes in Delhi. Some earlier studies shows similar trends in India with G1 as the most predominant type followed by G2 stain (Ramani & Kang,2007; Samajdar et.al. 2008).

Conclusion

Our study had few limitations as this was a hospital based study and hence the results are unlikely to be a correct reflection of the disease burden in the community, however Rotavirus prevalence of 30.08% makes an important public health issue .Rotavirus infection does not have any specific treatment and repeated infection is commonly seen in children. In case of Rotavirus infection when vomiting and diarrhoea is treated with oral rehydration solution it makes not much significant recovery and only immunization is the possible prevention towards control of Rotavirus infection. Further studies of Rotavirus genotype with different stains G1P[8], G2P[4], G2P[6] should be accounted for selection of vaccine strains and introduction of Rotavirus vaccine in National Immunization Programs. Vaccination on a large scale does have a great impact on disease but mixed Rotavirus strains suggest continuous strains surveillance is essential to increase the effectiveness of vaccination in India.

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