Prevalence of Rotavirus diarrhea among under-5 hospitalized children in a Government Tertiary Hospital, Tirupati

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ABSTRACT

Context: Rotavirus is the most common cause of severe diarrhea requiring hospitalization among infants and young children worldwide. The prevalence of rotavirus diarrhea in India has been found to vary in the range 5-71% in hospitalized children aged under 5 years with acute gastroenteritis. The seasonal variation of rotavirus diarrhea in India varies across different geographical regions, with high incidence in the winter months at low relative humidity in northern India.

Aim: This study aimed to estimate the prevalence of rotavirus diarrhea among hospitalized children aged under 5 years and to learn about the genotypic distribution of rotaviruses causing diarrhea.


Materials and Methods: Study units: children under 5 years of age presenting with diarrhea. Stool specimens from all hospitalized children aged under 5 years who had presented with acute watery diarrhea were collected and tested for rotavirus by the enzyme-linked immunosorbent assay (ELISA). Positive samples were tested for G and P typing by the reverse transcription-polymerase chain reaction (RT-PCR) technique.

Statistical Analysis Used: Percentage and chi-square analysis. Results: Among the study sample, 68.7% of children were in the age group between 1-12 months and 25.6% children showed positive result for rotavirus by ELISA. Of the rotavirus positives, 50% were G1P8 viruses.

Conclusion: Rotavirus is an important cause of diarrhea in hospitalized children.

Key words: Diarrhea, enzyme-linked immunosorbent assay (ELISA), genotyping, rotavirus, Tirupati

INTRODUCTION

Rotavirus is the most common cause of severe diarrhea requiring hospitalization among infants and young children worldwide.[1] Very few studies on the prevalence of rotavirus among children have been conducted in Andhra Pradesh. Rotavirus infections spread easily through multiple modes of transmission. Rotavirus infections also spread in settings where many children are together, such as day-care centers and nurseries. Marked seasonality is seen in temperate or cooler climates, where outbreaks usually occur in the winter and early spring, between about November and April. Rotaviruses are classified based on the most abundant protein VP6, into groups A-G, with groups A-C infecting humans. Among them, Group A rotavirus is the most important human pathogen.[2] Diarrhea caused by rotaviruses may be due to impaired sodium and glucose absorption, as damaged cells on villi are replaced by nonabsorbing immature crypt cells.[3]
Exposure to infection occurs in early life. By the age of 3 years, 90% of children have serum antibodies against one or more types of rotavirus, indicating high levels of exposure. Several studies on rotavirus epidemiology have been carried out in different parts of India. Previous studies in the Indian Rotavirus Strain Surveillance Network have confirmed that rotavirus accounts for 39% of acute diarrheal hospitalizations. This study aimed to identify the proportion of children with acute gastroenteritis infected with rotavirus through systematic sampling over a 1-year period in a tertiary care Government hospital in Andhra Pradesh.

MATERIALS AND METHODS

The study was conducted at a Government tertiary care hospital in Tirupati, Chittoor district, Andhra Pradesh, India from September 20, 2012 to September 19, 2013. This study included all hospitalized children of aged under 5 years who had presented with acute watery diarrhea. Informed consent was obtained for each child from the respective parent/guardian after explaining the purpose of the study. A case of diarrhea was defined as increased stool frequency, compared with the usual pattern occurring in a child aged under 5 years for whom the parents sought care for treatment of diarrhea. Clinical details including age, sex, duration of illness, number of stools, associated vomiting and fever, degree of dehydration, and concomitant illness were recorded on a standardized case reporting form. This study is part of an Indian Council of Medical Research (ICMR)-funded project for the National Hospital Based Rotavirus Surveillance Network. Our Government tertiary care hospital in Tirupati is one of the study sites of the multicentric project, and we obtained Institutional Ethical Committee approval prior to commencement of the study.

Stool specimens from all hospitalized children aged under 5 years who had presented with acute watery diarrhea were collected and stored in the refrigerator at 4°C and later transported to the base hospital in iceboxes. All the stool samples were sent to the testing laboratory at the Christian Medical College (CMC), Vellore, Tamil Nadu at 4°C. Samples were tested for the presence of rotavirus using a commercially available antigen detection apparatus, the enzyme-linked immunosorbent assay (ELISA) (PremierTM Rotaclone®, Meridian Biosciences, Cincinnati, Ohio, USA), as per kit protocol. Samples showing an optical density value of ≥0.150 were reported as positive. An internal control was included in all runs, and the run was repeated if the internal control did not fall in the expected range.

Genotyping was performed on the antigen-positive samples. Ribonucleic acid (RNA) was extracted using the QIAamp Viral RNA Mini Kit (Qiagen N.V., Venlo, Netherlands). Complementary deoxyribonucleic acid (DNA) was synthesized using random primers [Pd(N)6 hexamers; Pharmacia Biotech, Amersham Pharmacia Biotech, Piscataway, New Jersey, USA] and 400 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and was used as the template for VP7 and VP4 (G and P) typing in PCRs using published oligonucleotide primers and protocols to detect the VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12, and the VP4 genotypes P[4], P[6], P[8], P[9], P[10], and P[11].

Data were collected and entered into Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) software that were later analyzed using SPSS version 16.0 (IBM Corporation, Armonk, New York, USA). Appropriate statistical tests, i.e., percentage calculation and chi-square analysis, were performed.

RESULTS

For the 1-year period, a total of 187 children were included in the study group. Among the study subjects, a majority of them were male children [107 (57.2%)], and 80 (42.8%) were female children. The stool samples of 48 (25.67%) children were positive for rotavirus by ELISA [Table 1]. Among 187 cases, a majority [110 (58.8%)] were in the age group of 1-12 months. Similarly, out of 48 ELISA-positive cases, 33 (17.7%) were found to be in the same age group. There were no rotavirus-infected cases among neonates and in the age group of 48-60 months [Table 2]. Applying chi-square analysis, it was found that there was no statistical significant difference in ELISA reactivity between different age groups of cases. Of 48 ELISA positives, 24 (50%) belonged to the G1P8 type [Table 3]. Out of the 48 ELISA-positive cases, 27 belong to the G1 type, followed by G12 and G2 [Table 4]. Most (32) of the rotaviruses belonged to the P8 type [Table 5].
DISCUSSION

In the present study, it was found that 25.66% of the children aged under 5 years, hospitalized with the complaint of diarrhea are due to rotavirus. The Indian Rotavirus Strain Surveillance Network carried out a multicentric study in seven different regions of India and reported that rotavirus was detected in the stools of 39% children aged under 5 years. \cite{4,5} Studies in other parts of Asia have shown a much higher prevalence rate, perhaps due to the absence or lower rates of other causes of acute gastroenteritis. \cite{6} A few studies done in Indian outpatient facilities and in the community revealed that 30% of cases were under 6 months of age. \cite{7,8}

In Chandigarh, rotavirus was detected in 16-19% of instances of acute gastroenteritis in children under 5 years of age, \cite{9-11} while in Aligarh it was detected in 19% of cases of acute diarrhea. \cite{12} In the eastern states of India and in Pune, rotavirus was detected in 28-30% of children aged under 5 years with acute diarrhea. \cite{13,14} In Kolkata, the incidence of rotavirus-associated diarrhea varied 5-22%, \cite{15,16} but in Manipur, the incidence was as high as 41%. \cite{17} However, it is important to note that these studies did not use similar methods and therefore a direct comparison of results is difficult.

Regarding seasonality, some studies in India have found no association between rotavirus infection and the time of year. \cite{8,18} Other studies have observed an increase in rotavirus-associated diarrhea during the winter months, October-February, throughout the country. \cite{19-21} Rotavirus was markedly seasonal in northern India but was less seasonal in southern locations with a more tropical climate. \cite{22,23} In the present study, although the number of cases increased from December to April, there was uneven distribution throughout the year [Figure 1].

In the present study, 56.25% of the rotaviruses were of G1P [8] genotype, followed by G12P [6] (14.5%) and G12 [P8] (12.5%). In a study from Kolkata, the predominant genotype was G1P [8] (20%), followed by G2P [4] (15%) and G4P [8] (6%). A number of uncommon genotypes, G1P [4] (4%), G2P [8]...
handwashing practices of family members. Should also be given to the personal hygiene and vaccination against rotavirus. Due importance preventive measures in terms of compulsory years are due to rotavirus, which calls for stringent diarrheal disorders among children aged under 5 years are due to rotavirus, which calls for stringent measures in terms of compulsory vaccination against rotavirus. Due importance should also be given to the personal hygiene and handwashing practices of family members.

REFERENCES


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