Original Article

DOI: 10.7860/NJLM/2017/25466:2261

Microbiology Section

Prevalence of Rotavirus Diarrhoea in Children in Davangere, Karnataka

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ABSTRACT

Introduction: Rotavirus was described 40 years back, yet it is recognized as the leading cause of diarrhoeal morbidity and mortality, particularly in children in developing countries. In India, rotavirus infection accounts for ~26% of all childhood diarrhoea-related hospitalizations. Hence, it is important to know the prevalence of rotavirus diarrhoea in children.

Aim: To detect the presence of rotavirus antigen in the stool samples of suspected cases of rotavirus diarrhoea and to know its prevalence.

Materials and Methods: The study was conducted in the Department of Microbiology, JJM Medical College, Davangere, from June 2010 to May 2011 and was comprised of 100 stool samples. The study included 100 stool samples from pediatric patients from six months to five years of age. The stool samples were screened for the presence of rotavirus antigen by using monoclonal antibody based ELISA kit (Rotaclone).

Results: Out of the 100 samples, rotavirus antigen was detected in 20 samples by ELISA. Maximum cases were seen in the age group of six months to two years with a male preponderance. Rotavirus diarrhoea occurred throughout the year with a distinct peak in winter (October to March).

Conclusion: Rotavirus infection was seen in a considerable proportion of children in the present study. It's important to detect rotavirus diarrhoea as early as possible to curtail the unnecessary use of antibiotics.

Keywords: ELISA test, Gastroenteritis, Infection

INTRODUCTION

Diarrhoea remains one of the most common illnesses of children worldwide [1,2]. In developing countries, it is the third most common cause of death with an estimated 2 million deaths per year or 17% of all deaths in children younger than 5 years [3]. Even the developed part of the world is not spared from diarrhoea, as it is the second commonest cause of hospitalization in the developed countries [3]. Several micro-organisms cause diarrhoea. The frequently encountered causative agent is rotavirus in children lesser than five years of age [3]. Rotavirus causes 25-55% of all hospital admissions for diarrhoea and more than 0.6 million deaths every year [1]. Still many parents do not take their child, who is suffering from diarrhoea, to clinicians. Sometimes the healthcare provider doesn't ask for a stool sample for rotavirus testing. Hence, the exact prevalence of rotavirus is still underrated [2].

The first lead in the discovery of rotavirus was given by Bishop and Colleagues in 1973 [4]. Rotaviruses belong to seven Groups (A to G) based on a common group antigen [5]. Only Group A to C have been associated with human diseases. Most common causative agent is Group A [6]. The genome of the rotavirus consists of double standard RNA which is divided into 11 segments [7]. Rotavirus has six structural viral proteins (VP1-4, VP6, and VP7) and six non-structural proteins (NSP1-6). The complete particles resemble a wheel with short spokes and a well defined, smooth outer rim, hence, the name 'Rotavirus' (from the Latin word Rota meaning "wheel") [6].

Rotaviruses are transmitted by the fecal-oral route [8]. Rotavirus diarrhoea can occur with a small infective dose (<100 virus particles) [7] and incubation period of less than 48 hours. Diarrhoea accompanied by vomiting can quickly lead to dehydration in infants and young children. There is not much information about rotavirus infection in children in Davangere. Hence, this study was carried out to know the prevalence of rotavirus diarrhoea in children less than five years of age.

MATERIALS AND METHODS

This cross-sectional, hospital based descriptive study was conducted in the Department of Microbiology, J.J.M. Medical College, Davangere, Karnataka, India, during the period

between June 2010 to May 2011. One hundred Patients of pediatric age group (six months to five years) having diarrhoea were included in this study. Patients having watery greenish, foul smelling loose stools were enrolled in this study. Those patients with bloody tinged stools were excluded from the study.

Informed consent was taken from all patients' parents/ guardians, prior to the study.

Specimen collection: Once the patient gets admitted to the pediatric diarrhoea ward, the parents were given a clean container to collect the stool sample. After collection the sample was transported to the Microbiology laboratory at the earliest. Samples were stored at -20° C till further processing.

Specimen preparation: 1 ml of sample diluent (buffered saline with 0.02% thimerosal as preservative) was added in a tube with a micropipette. Then the stool sample was added to the diluent with the help of a transfer pipette.

ELISA method- Stool samples were examined for the presence of rotavirus antigen by ELISA. 100 µl of positive control (inactivated simian rotavirus SA-11 in buffered saline with 0.02% thimerosal as a preservative), negative control (sample diluents i.e., buffered saline with 0.02% thimerosal as preservative) and diluted fecal samples, were added to separate wells. About 100 µl of enzyme conjugate (Horseradish peroxidase) was added to each well. Contents in well were mixed and were incubated at room temperature for 60 ± 5 minutes. Wells were washed five times with distilled water. 100 µl of each substrate A (urea peroxide) and B (tetra methyl benzidine) solution was added to each well. Later incubated for 10 minutes at room temperature and 100 µl of stop solution (1 N H2SO4) was added. Absorbance value for each well was read at 450 nm using a > 600 nm reference filter against an air blank within 60 minutes.

Specimens with absorbance units (A450) greater than 0.150 were considered positive. Specimens with absorbance value equal or less than 0.150 were considered negative.

RESULTS

Total 100 cases of diarrhoea were included in the study, out of these 64 were male children and 36 were female children. Rotavirus antigen was detected in 20 stool samples by ELISA. Thirteen male children and seven female children were detected positive for rotavirus. Maximum numbers of cases were seen in the age group of six months to two years. There was a seasonal peak with more cases (15) seen during winter i.e., October to February. [Table/Fig-1] shows the distribution of diarrhoea cases and rotavirus positive cases according to age and sex. [Table/Fig-2] shows month wise distribution of diarrhoea cases and rotavirus positive children.

Out of 20 rotavirus positive diarrhoea cases, maximum cases were seen during cooler months (Oct-March=16). The p-value < 0.03, shows significant association of rotavirus with cooler months than with hotter months.

Month	Number of Cases	Number of Rotavirus Positive Cases
June	6	0
July	9	0
August	6	1
September	8	1
October	11	2
November	8	3
December	10	3
January	14	4
February	8	3
March	8	1
April	6	2
May	6	0
Total	100	20

[Table/Fig-2]: Table shows month wise distribution of total cases and rotavirus positive cases.

DISCUSSION

The present study showed a prevalence rate of 20% of rotavirus diarrhoea in children ≤ five years of age. The result of our study supports other studies from India. The majority of the cases occurred in children younger than 24 months, which is the susceptible expected target age group. Above two years of age, rotavirus infections become less severe and asymptomatic because of development of local immunity due to previous infection. Varying rates of prevalence of rotavirus infections have been reported in literature from India and other countries. In India the prevalence range from 5% to 71% [9].

Age	Male Children	Rotavirus Positive Male Children	Female Children	Rotavirus Positive Female Children	Total No. of Children	Total No. of Rotavirus Positive Children
6-12 months	24	5	12	2	36	7
1-2 years	22	6	14	4	36	10
2-3 years	12	1	6	0	18	1
3-4 years	4	1	2	0	6	1
4-5 years	2	0	2	1	4	1
Total (6 months-5 years)	64	13	36	7	100	20

[Table/Fig-1]: Table shows Age and Sex Distribution of Diarrhoea Cases and rotavirus positive cases.

This wide range can be due to the differences in age group studied, detection methods employed, time of onset and the seasonal variation of rotavirus diarrhoea in different regions of the country.

ELISA has been used in most of the studies for the detection of rotavirus. The rapidity of ELISA in detection of rotavirus antigen has increased its use by diagnostic laboratories.

Rotavirus infection's rate varies from city to city in North India. A prevalence rate of 6-45% was observed in Delhi, Chandigarh and Aligarh. 28-30% prevalence rate was observed in the west India (i.e. in Pune), whereas Kolkata and Manipur in east India reported 5-22% and 41% as prevalence rate respectively. A prevalence rate of 18%, 20% and 16-22% was reported from Vellore, Chennai and Bangalore respectively, in south, whereas Calicut showed a very high rate of 71% [9].

Bahl R et al., found out rotavirus in 23.5% of stool sample of children suffering from diarrhoea [10]. The study done by Saravanan P et al., showed an overall infection rate of 22.55% among children with acute diarrhoea [11], whereas another study done at Vellore, found rotavirus in 27.4% of all diarrhoea cases in children aged up to five years [12]. Kang G et al., found a prevalence rate of 39% in their study [13]. Across the world too prevalence of rotavirus diarrhoea varies from country to country. The reveal study done by Damme PV et al., reported a prevalence rate of 40.6% across seven European countries [14]. Junaid SA et al., observed 13.8% of prevalence rate in Nigeria [7].

In present study, peak infection was observed in children of 6 to 24 months of age, constituting 83.3 % of total positive children. It has been stated that the maternal antibodies protect the infants below four months of age partly [11].

Saravanan P et al., reported 29.95% rotavirus positive children among age group of 7-12 months [11]. Banerjee I et al., observed the median age of detection of rotavirus in hospitalized children as 10 months (IQR 7.5 to 12.5) [12]. Raboni SM also reported that the majority of the cases of rotavirus diarrhoea occurred in children younger than 2 years of age [15]. Bahl R et al., in their study found that most (98%) of the children who were hospitalized with rotavirus diarrhoea were <2 years of age. They reported that the number of cases were less in the initial three months of life and increases slowly with a peak at 9-11 months which is followed by a decline after 18 months [10].

The present study shows that male children (13) were affected more than female children (7) in a ratio of 1.9:1. Banerjee I et al., found out that a larger proportion of children admitted in the hospital were male children (63.8%) [12]. In another study done by Ghazi HO et al., out of 48 patients infected with rotavirus, 30 (63%) were male and 18 (37%) were female, [16]. Whereas, Raboni SM et al., found that 54.6% of male children were positive for rotavirus [14]. Junaid SA et al., also found more number of male rotavirus positive patients than female rotavirus positive patient [7]. In contrast Saravanan P et al., could not find any male preponderance statistically [11].

We had observed more cases during cooler months than hotter months. There was a higher prevalence during October to March (16 out of 20). Efficient transmission of the human and animal rotaviruses is aided by the influence of low temperature on its stability. This is further facilitated by low relative humidity at home that increases the survival of rotavirus on surfaces [11]. From their study, Sarvanan P et al., anaylsed that the hotter months i.e., March to August, during the years 1996,1997 and 1998 had low rate of rotavirus infection (17.6, 17.0 and 14.3% respectively) than the cooler months i.e., September-February (21.8, 31.8 and 22.5 % respectively) [11]. Seasonal variation in rotavirus infection was also shown by Raboni SM et al. In their study, more number of cases were observed in the winter season [15]. In contrast many studies have shown rotavirus diarrhoea occur throughout the year without any seasonal variation [9].

LIMITATION

Different serotypes of rotavirus cannot be detected by this test. Also we have done the test to detect rotavirus only. The presence of other enteric pathogens cannot be excluded. Hence, other tests should be done to detect presence of organisms causing concurrent infection.

CONCLUSION

Early and correct diagnosis of Rotavirus antigen in the stool sample prevents the unnecessary use of antibiotics by the treating physicians. Various preventing measures ranging from simple improved hygiene to advanced vaccines are being explored to reduce the morbidity and mortality due to this infection.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Oct 01, 2017**