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Original Article

Electrophoretic Patterns of Human Rotavirus among Adults in Mymensingh

Samsoon Nahar,[1] Sabiha Monowar,[2] Habiba Begum,[3] Jannatul Fardows,[4]
Sayed Ahmed Abdullah[5]

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Abstract

Introduction: *Rotavirus gastroenteritis is a major cause of severe dehydrating diarrhea in children worldwide. Group A rotavirus causes approximately 3-14% of hospitalization for diarrhea in adults. The aim of our study was to assess the occurrence of Human Rotavirus (HRV) electropherotypes.*

Methods: *Polyacrylamide gel electrophoresis (PAGE) and silver staining was applied to detect rotavirus dsRNA from acute diarrheic stool of 364 hospitalized adults. The study was conducted in MMCH, Bangladesh from January, 2013 to December, 2014.*

Results: *Among 364 stool specimens 34 (9.3%) were rotavirus positive in adults by PAGE. The rate of infection was highest in 26-35 years of age. Males were affected slightly higher than females and infection rate was more occurs in winter. Among 34 positive samples, 20 were positive for group A and 14 were positive for group B rotaviruses. RNA profiles of the analyzed specimens, 20 reaveled short electropherotype of group A, there were no long, mixed and no electropherotype detected.* ***Conclusion:*** *Electropherotyping technique can be applied as and excellent method for studying genomic variation, tracing mixed infections, detecting atypical rotaviruses.*

Key words: *Rotavirus, PAGE, Reverse transcriptase polymerase chain reaction*

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1. Assistant Professor, Department of Microbiology, North Bengal Medical College, Sirajganj
2. Assistant Professor, Department of Microbiology, Marks Medical College, Dhaka
3. Assistant Professor, Department of Microbiology, Delta Medical College, Dhaka
4. Assistant Professor, Department of Microbiology, Kumudini Womens Medical College, Tangail
5. Lecturer, Department of Microbiology , Mymensingh Medical College, Mymensingh

Correspondence *Samsoon Nahar, Email: joly.hasan@yahoo.com*

Introduction

Worldwide diarrheal illness rank as one of the top six causes of all death from an infectious disease.[1] Human Rotavirus (HRV) is the leading cause of severe diarrhoea in children under five years of age and causing approximately 527,000 each year.[1] It is responsible for an estimated 30% to 50% cases of hospitalization for acute gastroenteritis.[2] In Bangladesh it causes 6,000-14,000 deaths each year in infants and young under 5 year children.[3] Bishop and his colleagues discovered the rotavirus and its association with severe endemic diarrhea in infants and young children. On the basis of antigenic groups, Rotaviruses are classified into groups A to G.[1]

Group A rotavirus causes gastroenteritis also in adult, though a low frequency, which has been described as epidemic outbreaks, travel related gastroenteritis and endemic cases.[2] In some reports G2 and G3 was described as the common cause of outbreaks in adult.[4] Group A rotavirus causes approximately 3-14% of hospitalization for diarrhea in adults. The prevalence of ICDDRB, of group B rotaviruses is 2.4% which is not higher than group A.[13]

The rotavirus genome contains 11 segments of dsRNA, which fall into four classes is evident by PAGE of RNA. These RNA segments are numbered in order of migration during PAGE. The slowest RNA segment designated as gene 1 and so on.[1] Electropherotyping of rotavirus RNA has been shown to be an excellent method for studying genomic variation, tracing mixed infections, detecting atypical rotaviruses. Genomic variation and atypical rotaviruses cannot be detected by ELISA or Latex agglutination test. PAGE may be used to detect rotaviruses of different species and groups.

The HRV genome can be detected by the PAGE and Reverse transcriptase polymerase chain reaction (RT-PCR), both of which have proven most useful for taxonomic and molecular epidemiologic studies. Although HRV can be cultivated in cell culture system, it is not a useful diagnostic test.

In this study, we detected the occurrence of HRV electropherotypes among adult with acute gastroenteritis at Mymensingh Medical college Hospital (MMCH).

Materials and Methods

Stool specimens were collected from inpatients and outpatients at SK Hospital, Mymensingh, during two year period between January 2013 to December 2014. A total of 364 faecal specimens were collected from adult patients. All stool samples were stored at 20^0C before examination for rotavirus infection. Laboratory work was done in the department of Microbiology, Mymensingh Medical College, Mymensingh.

Extraction of RNA from stool

The presence of rotavirus in stool specimens was determined by detection of migration pattern of dsRNA segments of rotavirus following polyacrylamide gel electrophoresis.[14] Faecal suspension was prepared with phosphate buffer saline in the ratio of 10%. The suspension was vortexed for few seconds and centrifused at 12,000 rpm for 10 minutes at 4^0C (Digi System VM-2000). Four hundred µl of supernatant was mixed with 60 µl disrupting solution in a 1.5 ml micro-centrifuge tube and incubated at room temperature for 30 minutes. The deprotenisation was done with 0.5 ml saturated phenol. The tubes were vortexed for 30 seconds and then centrifuged at 12,000 rpm for 2 minutes. The supernatant was poured in another 1.5 ml micro-centrifuge tube. Then the RNA was precipitated by adding 0.8 ml of chilled ethanol and 10 µl of 5M NaCl and vortexed for 5 seconds. The tubes were then incubated at 20^0C for overnight. After thawing, the tubes were centrifuged at 12,000 rpm for 10 minutes and the supernatant was discarded by pipetting. Then the pellets were dried up under table lamp for one hour. The RNA pellets at the bottom of the tubes were re-suspended with 10 µl simple buffer. Then half of the suspension was used for electrophoresis in polyacrylamide gel and remaining portion kept in 20^0C freezers for further use.

Polyacrylamide gel electrophoresis for rotavirus dsRNA

For routine detection of RNA, a 90 mm x 80 mm dimension 10% polyacrylamide gel was casted using in 1mm thick spacer. A separation gel containing 10% polyacrylamide (30% acrylamide and 0.8% bisacrylamide) was prepared by mixing reagent in a conical flask. The separation gel was poured into glass sandwich. A 1 mm thick and 18 well comb was inserted into the gel and gel was allowed to polymerize for 1 hour. After that the comb was removed gently and the wells were flushed several times using distilled water. There after, the spacer was removed from the gel and placed in buffer chamber containing running buffer. The buffer chamber was filled with running buffer. Extracted RNA 15 µl was mixed with 6 µl of loading buffer and loaded into each well. Then it was allowed to run for 3.5 hours at room temperature at constant current of 20 mA per gel using electrophoresis power supply in vertical gel electrophoresis apparatus.

Silver Nitrate staining of the gel and visualization of dsRNA

Rotavirus RNA segments were detected by silver staining of polyacrylamide gels as described by Herring AJ et al.[15] with some modifications. Briefly, following electrophoresis, gels were removed and the upper comb portion of the gels were trimmed away and discarded. The upper

right corner of the gels was cut away for identification of lanes in chronology. Gel were placed in 50ml of silver nitrate solution in a transparent tray and rocked for one hour at room temp and drained off. The gels were rinsed briefly three times with double distilled water and drained off. Then, 50 ml developer solution was added and gels were rocked until the RNA bands were satisfactorily visible in about 5-10 minutes. The developer solution was discarded and replace with sufficient amount of 5% acetic acid. Gels were examined on a white view box and photographed (ATTO, PRINTGRAPH Bio-instrument, AE-0905H Image Saver HR, Japan).

Results

A total 364 samples screened, 34 (9.3%) were positive for group A rotaviruses. The positive specimens were examined by PAGE, all showed clearly stained electrophoretic patterns of viral RNA which enabled their classification into different electropherotypes. Among the positive samples 58% (20/34) were positive for group A and 42% (14/34) were positive for group B rotaviruses. RNA profiles of the analyzed specimens of group A, 20 reaveled short electropherotype, there were no long, mixed and no electropherotype detected. Remaining 14 were group B.

Table I. Detection of Group A and Group B Rotaviruses among adults

Group	Positive in adult
A	20 (58.8%)
B	14 (41.2%)

Out of 34 cases, Group A positive was 20 (58.8%) and Group B was 14 (14.2%).

In adults the highest incidence was found 26-35 years age group 7.1% (8/113), followed by 36-45 years age group, 6.3% (6/96).

Table II: Age Distribution of Rotavirus in Adult

Age in years	No. of Diarrheal cases	PAGE Positive (%)
16-25years	68	3 (4.4%)
26-35years	113	8 (7.1%)
36-45years	96	6 (6.3%)
>45years	47	3 (6.4%)

The incidence of rotavirus infection was slightly higher in males than female, though the difference was not statistically significant. The rate of detection is highest in winter 62%, decreasing in summer and lowest in March-April (18%).

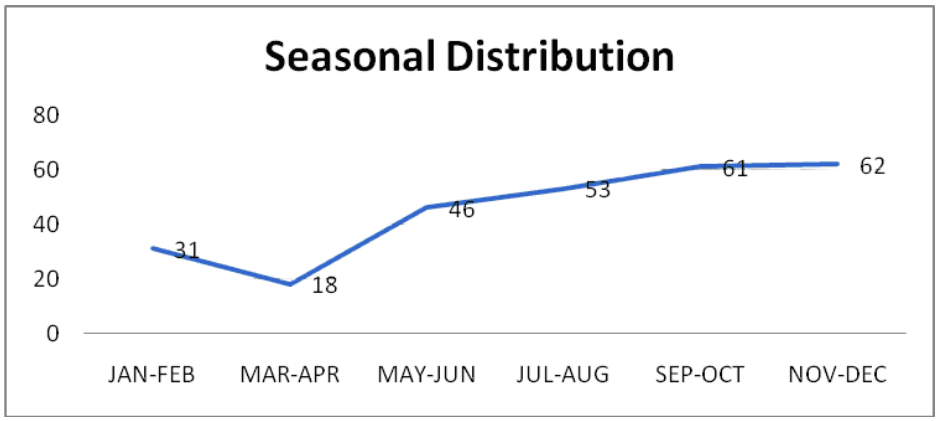

Figure 1 Percentile of Seasonal Distribution of PAGE Positive Rotavirus Strains

The electropherotypes of group A were assigned into short types from Lane 1 to 12 and lane 14-15

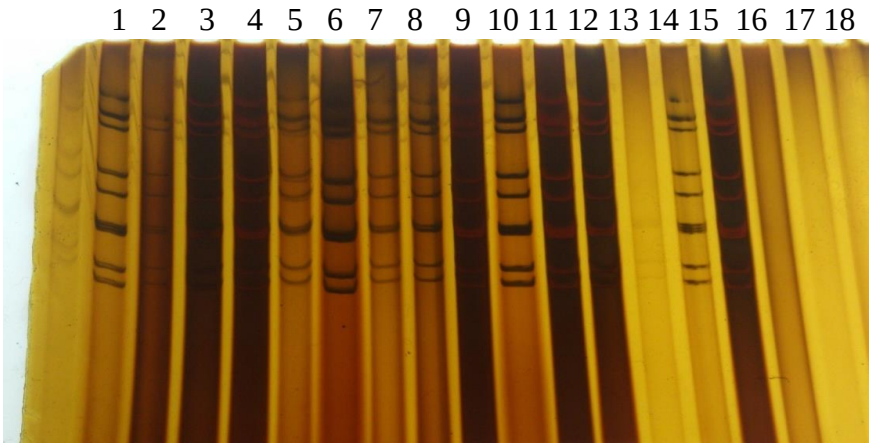

Figure 2 : Short Electropherotype of Group A Rotavirus Strain isolated in this Study

The electropherotypes of group B were assigned into Lane 2, 6, 8, & 10

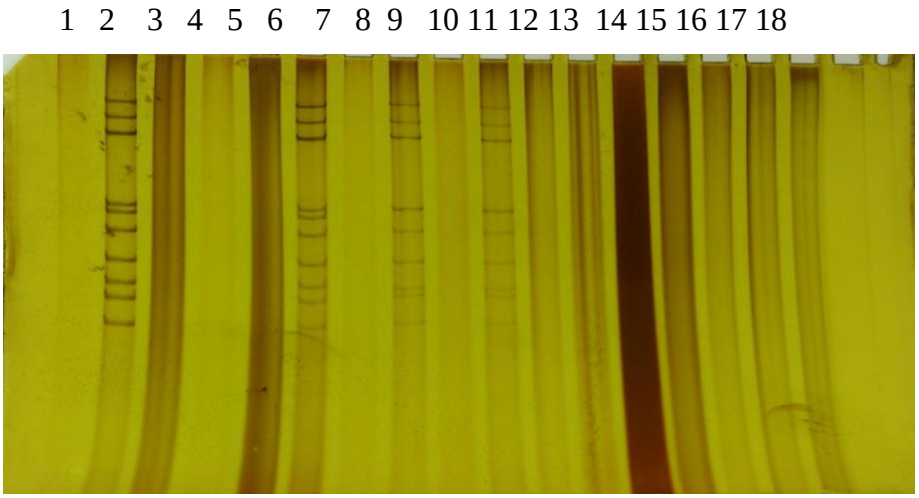

Figure 3 : Group B Rotavirus Strain from Adult isolated in this study

Discussion

Group A rotaviruses are the single most important cause of severe acute gastroenteritis in infants and young children in both developed and developing countries worldwide.[1] The high annual morbidity in developed countries and high mortality in developing countries necessitates development of effective rotavirus vaccine. Rotavirus diarrhea in adult populations has been noted mostly as sporadic cases and occasionally as outbreaks. Polyacrylamide gel electrophoresis of the RNA genome of rotavirus is a valuable tool for identification, group detection as well as strain differentiation in epidemiological studies. Several studies conducted with this technique have yielded important information on the molecular epidemiology of rotavirus infection. The detection rate of rotavirus in sporadic cases of diarrhea in adults has been described as 2-17% in most reports of different countries in the world.[1-5] In Bangladesh the adults rotavirus detection rate was 10.1% (99/895).[2] In the present study, the rotavirus positive rate in adult cases were 9.2% (both group A and group B rotavirus) which may be comparable with the epidemiologic study of rotavirus in Wuhan, China where the detection rate was 9.0% [4] and 7.6% [5] in adult population. Electropherotyping of rotavirus RNA has been shown to be an excellent method for studying the genomic variation, tracing mixed infection, detecting atypical rotaviruses with lacking group antigen and characterizing virus strains in any outbreak.

In the present study, 20 (58%) of 34 rotavirus RNA positive specimens showed long patterns and no short, mixed electropherotype, remaining group B strains revealed short patterns. In present study specimens from (1.8%) patients revealed mixed infection. Several studies have reported the presence of mixed rotavirus electropherotypes in diarrheic patients.[9, 10] The report from Kenya long electropherotypes accounted for 60% and short electropherotype accounted for 40% in adult.[12.] Rotavirus disease has been reported to have a seasonal pattern in many parts of the world. In temperate climate, a definite peak of disease occurs during the winter due to low humidity, but in monsoon it is not common. In tropical climates, there is no consistent seasonal pattern, rotavirus infections frequently occur throughout the year. In the America, Canada, Spain, Poland, Japan, Vietnam and Europe, rotavirus infection occurs primarily during the winter. A few studies from Indian researchers suggested the disease occurs year-round there. The peak of infection occurs during the winter (October-January).[8,11] In the present study, it was observed that the number of rotavirus

associated diarrhea was relatively high in November-December and relatively low in March- April. Seasonal shifts of group A rotavirus strains may be a possible mechanism of persistence of strains in human population. In the present study, rotaviruses were detected throughout the year. Our study is consistent with the study conducted in Mymensingh in 2008 by paul SK et al.[6]

In the present study it was observed that the percentage of rotavirus associated diarrhoea was higher in males than females but the difference was not statistically significant ($p > 0.05$).[6, 7]

Conclusion

It has been concluded that Rotavirus associated diarrhoea in adult population has been noticed mostly as sporadic cases and occasionally as epidemic outbreaks.

Contribution of the Authors

First author was the principal researcher for this study. Others helped in data collection and statistical analysis.

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