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SEROTYPE PREVALENCE OF ROTAVIRUS DIARRHOEA IN HOSPITALIZED PATIENT IN EASTERN ODISHA- A HOSPITAL BASED STUDY

Paediatrics

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ABSTRACT

Introduction- Diarrhea is a two Greek word 'dia' and 'rhein' meaning 'through' and 'to flow' respectively. Hippocrates (460-370 B.C.) gave his clinical and epidemiological description of the entity of diarrhea.1

According to World Health Organization diarrhea is defined as having three or more loose or liquid stools per day, or as having more stools than is normal for that person. Acute diarrhea is defined as sudden change in consistency towards loose water stools > 3 times/ day in 24 hours. Acute diarrhea may persist for > 2weeks in 5-15% of cases, which is labeled as persistent diarrhea. It should not be confused with chronic diarrhea which is loose stools for > 14days, insidious in onset and associated with features of malabsorption.

Rotavirus and diarrhoeagenic Ecoli are the most common pathogens responsible for acute diarrhea in children; Shigella, Salmonella, Campylobacter jejuni / Ecoli, Vibrio cholerae, Aeromonas, and Pseudomonas, occur more commonly in poorer areas and infections caused by protozoa and helminths occur mainly in areas where environmental sanitation is significantly deteriorated. Purpose of the study is to assess the serotype prevalence of different rotavirus diarrhoea in children under 5 years in hospitalized patient.

Methods: A hospital based observational study was done with 400 patients to determine the prevalence of rotavirus infection along with epidemiology and their genotype in children below 5 years with acute Diarrhea.

Four hundred children under 5 rotavirus positive(by IgM ELISA) children were selected for study . Background information, details of acute diarrhoea and treatment modalities were obtained from parents of the under 5 children. Any child having acute diarrhoea at the time of hospitalizations with rotavirus positive by IgM ELISA are taken for this study to find out prevalence of diferrent rotavirus serotype. This is a hospital based study conducted at a tertiary care hospital IMS & SUM HOSPITAL, Bhubaneswar the capital city of odisha, from 1stMay 2016 to 30st August 2018.

Conclusion : Majority of the patients (43.8%) were in the age group of 12-23 months followed by 40.7% between 0-11 months, 10% between 24-35 months, 4% between 36-47 months and 1.5% betwwn 48-60 months. The mean age of patients was 15.40 ± 9.33 months.224 (56%) patients lived in nuclear family while 176 (44%) patients lived in joint family. Rotavirus infection was predominantly detected in the winter season (62.5%) followed by monsoon (25%) and summer (12.5%).Out of 400 samples, both G and P genotypes were identified in a total of 390 samples. In 2 samples, G genotype was determined while P genotype was untypeable. In 8 samples both G and P genotypes were untypeable.Among G genotypes, G3 was the most frequently detected (n=238, 59.5%), followed by G1 (n=104, 26%), G2 (n=16, 4%) and G9 (n=34, 8.5%). The predominant P genotype was P[8] (n=340, 85.4%) followed by P[4] (n=48, 12%) and P[6] (n=2, 0.5%). Four common G types (G1, G2, G3, and G9) and two common P types (P[8] and P[4]) accounted for 91% and 97% of the samples respectively.G3P[8] was the most common G/P combination found in 51% of the samples followed by G1P[8] (21%), G9P[4] (6.5%), G3G12P[8] (4.5%), G2P[4] (3.5%), G1G3P[8] (2.5%) and G9P[8] (2%).It was observed that G3P[8] genotype was significantly associated with duration of diarrhea and frequency of Stool over 24 hours ($p<0.05$).

KEYWORDS

Diarrhoea,Serotypes, Intervention

Introduction

Diarrhea is a two Greek word 'dia' and 'rhein' meaning 'through' and 'to flow' respectively. Hippocrates (460-370 B.C.) gave his clinical and epidemiological description of the entity of diarrhea.[1]

According to World Health Organization diarrhea is defined as having three or more loose or liquid stools per day, or as having more stools than is normal for that person. Acute diarrhea is defined as sudden change in consistency towards loose water stools > 3 times/ day in 24 hours. Acute diarrhea may persist for > 2weeks in 5-15% of cases, which is labeled as persistent diarrhea. It should not be confused with chronic diarrhea which is loose stools for > 14days, insidious in onset and associated with features of malabsorption.

O' Ryam et al.[3] observed more than one billion diarrhea episodes occur every year among children younger than 5 years of age in developing countries causing 2 to 2.5 million deaths. More than twenty viral, bacterial, and parasitic enteropathogens are currently associated with acute diarrhea. Rotavirus and diarrhoeagenic Ecoli are the most common pathogens responsible for acute diarrhea in children; Shigella, Salmonella, Campylobacter jejuni / Ecoli, Vibrio cholerae, Aeromonas, and Pseudomonas, occur more commonly in poorer areas and infections caused by protozoa and helminths occur mainly in areas where environmental sanitation is significantly deteriorated.

The rotaviruses are the single most important etiological agent of severe diarrheal illness of the infants and young children worldwide. Each year, the rotaviruses cause approximately 111 million episodes of the gastroenteritis requiring only home care, 25 million clinical visits, two million hospitalizations, and 352,000 – 592,000 deaths in the children of less than five years old worldwide. The children in the developing countries account for 82% of the rotavirus deaths.[4]

The rotaviruses are members of the Reoviridae family and the complete virus particle is often described as smooth, measures about 100nm in diameter and possesses a triple-layered icosahedral protein capsid composed of an outer layer (VP7 and Vp4), an intermediate layer (VP6), and an inner core layer (VP2). The viral genome consists of 11 double-stranded RNA (dsRNA).[5] Group and subgroup specificities are mediated mainly by VP6, which is the major intermediate capsid protein. To date, seven groups, designated as A-G, have been distinguished in the rotavirus strains from the humans and animals. The groups A, B, and C have been described in the humans and among them the group A is the most common cause of the acute gastroenteritis in the young children.[6]

The two outer capsid proteins, VP7 and VP4, are the basis of a binary rotaviruses classification system in the G (glycoprotein) and P (protease sensitive) types, respectively. So far, 15 different G and 25 different P genotypes have been reported.[7,8] Because the VP4 and VP7 are coded by different RNA segments, various combinations of the G and P types can be observed. Most G types were serologically confirmed as serotypes, but for the P types due to the lack of appropriate antibody reagents, a dual P typing system has been used. The G types G1, G2, G3, and G4 constitute more than 80% of all the human G genotypes detected worldwide. In humans, P[8] is the most common P genotype, followed by P[4] and P[6]. Studies of the genotyping have indicated that the P[8] G1, P[4] G2, P[8] G3, and P[8] G4 genotypes are the most frequently observed, followed by the P[8] G9 and P[6] G9.

While the rates of the morbidity related to the rotavirus diarrhea are similar in both the industrialized and developing countries, the disease is often severe and fatal among the infants and young children living in the developing countries, where inadequate sanitation conditions and mostly malnutrition may be an additional hazard.[6]

However, G1 has remained the predominant strain in India, while G9 and G12 are emerging as new strains.[10,11] Interestingly, recent studies have indicated that the G9 strain is mainly confined to the eastern part of India. Current available data indicates fluctuations in rotavirus G/P-type that also vary by geography and season, as well as the emergence of unusual types, such as G5, G6, G8, G9, G12, and P[6] for reasons that are poorly understood. This makes accurately predicting serotype circulation and understanding the clinical implications of the serotype changes a challenge.[9] The success of a prevention program based on the use of the vaccines is dependent on the previous knowledge of the most frequent rotavirus genotypes found in the population, particularly in the developing regions of the world.

Hence the present study was done at our tertiary care center to assess the prevalence, clinical and epidemiological profile of patient suffering from rotavirus diarrhea, to detect the most common genotype prevalent and the epidemiological profile of the commonest genotype of the rotavirus in our hospital.

MATERIALS AND METHODS:

A hospital based observational study carried out to assess the **"Serotype prevalence of rotavirus diarrhoea in hospitalized patient in eastern Odisha** at IMS & SUM HOSPITAL Bhubaneswar capital city of odisha over a period of 24months (1stMay2016-30thAugust 2018).

STUDY POPULATION:

Children visiting the paediatric department of our hospital with symptoms of acute diarrhea between the age of 0-5 years.Children between age group of 0 to 60 months having acute gastro enteritis and who were admitted to ward/cabin/ICU were enrolled and patient/parents were informed about the study in details and consent were taken for being enrolled in study.

SAMPLE SIZE:

400 patients Considering a confidence level of 95% and confidence interval of 5 the number of patients in our study to achieve statistical significance is 384. This was calculated by Survey System (http://www.surveysystem.com/sscalc.htm#one). The Survey System ignores the population size when it is "large" or unknown. Population size is only likely to be a factor when you work with a relatively small and known group of people (e.g., the members of an association). Hence a sample size of 400 was considered adequate for our study.

METHODOLOGY:

After approval by IMS &SUM Hospital ethics committee. Informed and written consent were taken from the cases after explaining the basis of the study. Confidentiality was ensured and participants were informed about the right to discontinue participation.Patients demographic including medical history, socio-economic details, area of residence, annual family income, family type (either nuclear or joint family), mothers education, breast milk feeding status and immunisation status were noted.

CLINICAL DATA:

Date of onset of symptoms (diarrhea, vomiting and fever) number of days for which symptoms continued, maximum frequency of INCLUSION CRITERIA for the study are Rotaviral diarrhea (GENOTYPE PROVEN <5 YEARS),Duration of diarrhea <14 DAYS and Patients/parents giving consent to be included in study. EXCLUSION CRITERIA for our study are Age >5 YEARS,Duration of duration> 14 DAYS ,Dysentry and People not giving consent. stool, vomiting episodes per day, maximum temperature recorded and hydration status were recorded.

STOOL SAMPLE COLLECTION AND LABORATORY ANALYSIS:

Stool sample of a minimum of 5 grams from each subject was collected within 14 days from onset of symptom. Sample was stored between 2 to 8 degree Celsius.

Stored sample was sent to CMC vellore for genotyping. Our hospital has a tie up with CMC vellore for Rota viral detection and genotyping.

Detailed history was taken from parents of all patients and anthropometric and clinical examination was performed as per the study proforma. Details regarding nutrition, feeding practices and environmental factors were also noted. An evaluation of degree of dehydration was done in all patients as per WHO guidelines.

RESULTS:

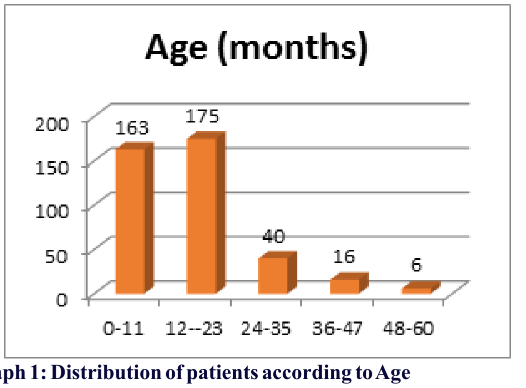
A hospital based observational study was done with 400 patients to determine the prevalence of genotypes of rotavirus diarrhea in children below 5 years of age.

Distribution of patients according to Age:

Majority of the patients (43.8%) were in the age group of 12-23 months followed by 40.7% in the age group of 0-11 months, 10% in the age group of 24-35 months, 4% in the age group of 36-47 months and 1.5% in the age group of 48-60 months. The mean age of patients was 15.40 ± 9.33 months(Table 1/ Graph 1).

Table 1: Distribution of patients according to Age

Age (months)	N	%
0-11	163	40.7%
12-23	175	43.8%
24-35	40	10%
36-47	16	4%
48-60	6	1.5%
Total	400	100%
Mean ± SD	15.40 ± 9.33	



Graph 1: Distribution of patients according to Age

Distribution of patients according to Sex:

There was male preponderance (66.30%) in the study while female patients constituted 33.7% of the study group.(Table 2)

Table 2: Distribution of patients according to Sex

Sex	N	%
Male	265	66.3%
Female	135	33.7%
Total	400	100%

Distribution of patients according to Type of Family:

224 (56%) patients lived in nuclear family while 176 (44%) patients lived in joint family.(Table 3)

Table 3: Distribution of patients according to Type of Family

Type of Family	N	%
Nuclear	224	56%
Joint	176	44%
Total	400	100%

Distribution of patients according to Mother's Education :

85 (21.2%) patient's mothers were educated upto primary level while 134 (33.6%) and 73 (18.2%) patient's mothers studied till SSC and

HSC respectively. 60 (15%) and 48 (12%) patient's mothers were graduates and uneducated respectively (Table 4)

Table 4: Distribution of patients according to Mother's Education

Mother's Education	N	%
Primary	85	21.2%
SSC	134	33.6%
HSC	73	18.2%
Graduation	60	15%
Uneducated	48	12%
Total	400	100%

Distribution of patients according to Residence:
125 (31.3%) patients resided in rural areas while 275 (68.7%) patients lived in urban areas.(Table 5)

Table 5: Distribution of patients according to Residence

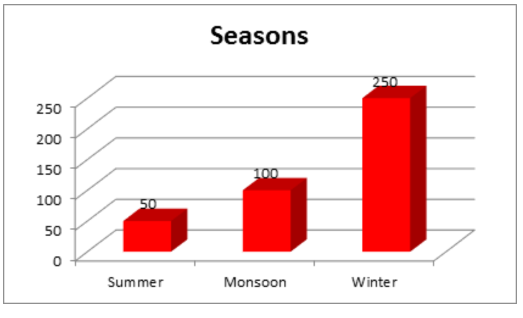
Residence	N	%
Rural	125	31.3%
Urban	275	68.7%
Total	400	100%

Socioeconomic StatusN%Upper class307.5%Upper middle class10426%Lower middle class19548.8%Upper lower class5012.5%Lower class215.2%Total400100%Distribution of patients according to Socioeconomic Status :30 (7.5%) patients were from upper class while 104 (26%) and 195 (48.8%) patients were from upper middle class and lower middle class respectively. 50 (12.5%) and 21 (5.2%) patients were from upper lower class and lower class respectively.(Table 6)

Table 6: Distribution of patients according to Socioeconomic Status

Socioeconomic Status	N	%
Upper class	30	7.5%
Upper middle class	104	26%
Lower middle class	195	48.8%
Upper lower class	50	12.5%
Lower class	21	5.2%
Total	400	100%

Distribution of patients according to Seasons :
Rotavirus infection was predominantly detected in the winter season (62.5%) followed by monsoon (25%) and summer (12.5%) .(Graph 2)



Graph 2: Distribution of patients according to Seasons

Distribution of patients according to rotavirus vaccination Status :386 (96.5%) patients were immunized including roravirus vaccine while 14 (3.5%) patients were not immunized.(Table 7)

Table 7: Distribution of patients according to Immunization Status

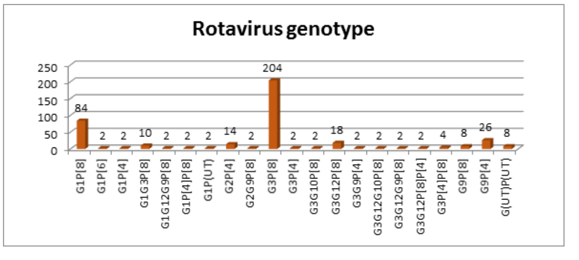
Immunization Status	N	%
Yes	386	96.5%
No	14	3.5%
Total	400	100%

Distribution of patients according to G and P genotype combinations :
Out of 400 samples, both G and P genotypes were identified in a total of 390 samples. In 2 samples, G genotype was determined while P genotype was untypeable. In 8 samples both G and P genotypes were untypeable.Among G genotypes, G3 was the most frequently detected (n=238, 59.5%), followed by G1 (n=104, 26%), G2 (n=16, 4%) and G9 (n=34, 8.5%). The predominant P genotype was P[8] (n=340, 85.4%) followed by P[4] (n=48, 12%) and P[6] (n=2, 0.5%). Four common G types (G1, G2, G3, and G9) and two common P types (P[8] and P[4]) accounted for 91% and 97% of the samples respectively.G3P[8] was the most common G/P combination found in 51% of the samples followed by G1P[8] (21%), G9P[4] (6.5%), G3G12P[8] (4.5%), G2P[4] (3.5%), G1G3P[8] (2.5%) and G9P[8] (2%).(Table 8/Graph 3)

Table 8: Distribution of patients according to G and P genotype combinations

Rotavirus genotype	N	%
G1P[8]	84	21%
G1P[6]	2	0.5%
G1P[4]	2	0.5%
G1G3P[8]	10	2.5%
G1G12G9P[8]	2	0.5%
G1P[4]P[8]	2	0.5%
G1P(UT)	2	0.5%
G2P[4]	14	3.5%
G2G9P[8]	2	0.5%
G3P[8]	204	51%
G3P[4]	2	0.5%
G3G10P[8]	2	0.5%
G3G12P[8]	18	4.5%
G3G9P[4]	2	0.5%
G3G12G10P[8]	2	0.5%
G3G12G9P[8]	2	0.5%
G3G12P[8]P[4]	2	0.5%
G3P[4]P[8]	4	1%
G9P[8]	8	2%
G9P[4]	26	6.5%
G(UT)P(UT)	8	2%
Total	400	100%

UT – Untypeable

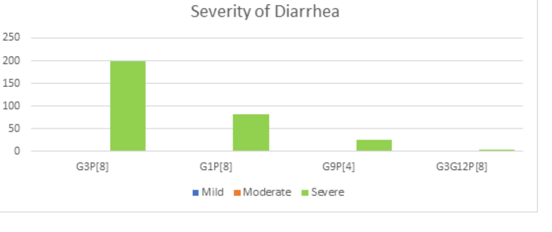


Graph 3: Distribution of patients according to G and P genotype combinations

Distribution of patients according to severity of diarrhea for most common G and P genotype combinations :
Vesikari scoring was done for every rotavirus positive patients but there were no difference in severity of diarrhea with different genotype combination. (Table 9/ Graph 4)

Table no 9

Genotype	N	Mild	Moderate	Severe
G3P8]	204	0.98%	0.98%	98%
G1P[8]	84	1.1%	1.1%	97.6%
G9P[4]	26	0	0	100%
G3G12P[8]	18	0%	0%	100%



Graph 4: Distribution of patients according to severity of diarrhea for most common G and P genotype combinations

Association of G3P[8] genotype with various factors :
It was observed that G3P[8] genotype was significantly associated with duration of diarrhea and frequency of Stool over 24 hours

(p<0.05). (Table 10)

Table 10: Association of G3P[8] genotype with various factors

Parameters	G3P[8] (n=204)		Total		p Value
	N	%	N	%	
Age (Mean±SD)	15.29	9.09	15.40	9.33	>0.05
Sex					
Male	139	68.1%	265	66.3%	>0.05
Female	65	31.9%	135	33.7%	
Type of Family					
Nuclear	142	69.6%	224	56%	>0.05
Joint	62	30.4%	176	44%	
Mother's Education					
Primary	39	19.1%	85	21.2%	>0.05
SSC	70	34.3%	134	33.6%	
HSC	45	22.1%	73	18.2%	
Graduation	42	20.6%	60	15%	
Uneducated	8	3.9%	48	12%	
Residence					
Rural	62	30.4%	125	31.3%	>0.05
Urban	142	69.6%	275	68.7%	
Socioeconomic status					
Higher class	14	6.9%	30	7.5%	>0.05
Upper middle class	48	23.5%	104	26%	
Lower middle class	106	52%	195	48.8%	
Upper lower class	25	12.2%	50	12.5%	
Lower class	11	5.4%	21	5.2%	
Seasons					
Summer	27	13.2%	50	12.5%	>0.05
Monsoon	53	25.9%	100	25%	
Winter	124	60.9%	250	62.5%	
Immunization Status					
Yes	197	96.6%	386	96.5%	>0.05
No	7	3.4%	14	3.5%	

Correlation of G and P genotype combinations with risk factors :
The logistic regression analysis showed that G and P genotype combinations frequency of Stool over 24 hours (p<0.05).(Table 11)

Table 11: Correlation of G and P genotype combinations with risk factor

Parameters	p Value	95% C.I.	
		Lower	Upper
Age	>0.05	0.544	3.059
Sex	>0.05	0.785	4.231
Type of Family	>0.05	0.137	3.173
Mother's Education	>0.05	0.166	3.918
Residence	>0.05	0.418	1.624
Socioeconomic Status	>0.05	0.048	4.723
Seasons	>0.05	0.067	3.184
Immunization Status	>0.05	0.984	1.024
Duration of Vomiting	>0.05	0.565	4.012
Dehydration Status	>0.05	0.375	1.671
Type of Diarrhea	>0.05	0.791	4.792
Duration of Diarrhea	>0.05	0.997	1.004
Frequency of Stool over 24 hours	<0.05*	1.004	3.806
Duration of Hospital Stay	>0.05	0.674	2.168

DISCUSSION:

A hospital based observational study was done with 400 patients to determine the prevalence of rotavirus infection along with epidemiology and their genotype in children below 5 years with acute Diarrhea.Rotavirus infection is common cause of pediatric acute diarrhea especially in developing countries[4] and the most common cause of non-bacterial gastroenteritis in children not only in developing countries but also in developed countries. The positive rate for rotavirus varies between countries and regions, and the different detection rates may be explained by different study conditions, such as the season of the year or sampling methods.Assuming a similarity in identification techniques, the difference in results may indicate that genotype prevalence in circulating rotaviruses may change periodically subject to natural fluctuations as reported by Gentsch et al[14]. It is recommended that rotavirus vaccination programs conduct surveillance on an ongoing basis, including before and after implementation of vaccination programs. Tate JE et al[15] suggested that the genotypes found to be circulating before the vaccination period should be used as a reference for the composition of the rotavirus vaccine, in order to produce vaccines with high efficacy. Monitoring of genotypic variability after rotavirus vaccination makes it possible to identify genotypes that evolve in response to the selection pressure of the vaccine to emerge as new phenotypes that are immune to older vaccines. The emergence of immune genotypes has to be watched for and accommodated in the design of the next vaccines[16,28].Babji S et al[11] observed that Rotavirus was responsible for 32% of all diarrheal admission to the hospital. G2P[4] was the predominant strain in the initial years and was gradually replaced by G1P[8]. The emergence of G9P[4] replacing G9P[8], and the detection of G12 strains over several years were documented.

There was no clear seasonality of disease.In the present study, majority of the patients (43.8%) were in the age group of 12-23 months followed by 40.7% in the age group of 0-11 months, 10% in the age group of 24-35 months, 4% in the age group of 36-47 months and 1.5% in the age group of 48-60 months. The mean age of patients was 15.40 ± 9.33 months. There was male preponderance (66.30%) in the study while female patients constituted 33.7% of the study group. This is similar to the studies of Sudarmo SM et al[17], Ali Z et al[18] and Durmaz R et al[19].Sudarmo SM et al[17,29,30,31,32] cross-sectional study correlating between rotavirus genotypes, clinical factors and degree of severity of acute diarrhea in children under 5 years old reported 51 (58%) were male and 37 (42%) were female. The mean age of the patients (months) was 14.6 ± 11.0.Ali Z et al[18] prospective, multicenter, hospital-based surveillance study estimating the rate of RVGE hospitalization, identifying RV genotypes, determining the seasonal and geographical variations found age-specific rates of the patients with GE rose sharply from age zero to 7 months (25.6%), peaked in the 7–12 months age group (32.6%), and subsequently dropped to (25.7%) for those aged 13–24 months and (16.1%) for those aged 25–60 months with no significant difference in age-specific rates between the RV-positive and RV-negative groups. In both groups, 83% of the patients were younger than 2 years of age.Durmaz R et al[19] sentinel surveillance study obtaining baseline data on the rotavirus G and P genotypes found age of rotavirus RNA positive patients ranged from 15 days to 59 months with 138 children (8.4%) in the age group of 0 to 12 months, 637 (38.7%) in the age group of 13 to 24 months, 466 (28.3%) in the age group of 25 to 36 months, 176 (10.7%) in the age group of 37 to 48 months, and 227 (13.8%) in the age group of 49 to 59 months. The prevalence of rotavirus infection was significantly higher in the age group of 13 to 24 months than the other age groups (p,0.05). Of the 1644 children, 721 (43.9%) were female and the remaining 923 (56.1%) were male. Rotavirus positivity did not differ significantly between females and males.In our study, 224 (56%) patients lived in nuclear family while 176 (44%) patients lived in joint family. 85 (21.2%) patient's mothers were educated upto primary level while 134 (33.6%) and 73 (18.2%) patient's mothers studied till SSC and HSC respectively. 60 (15%) and 48 (12%) patient's mothers were graduates and uneducated respectively. 125 (31.3%) patients resided in rural areas while 275 (68.7%) patients lived in urban areas**.** *No such recent comparision of RVGE with family type and education is reported in any recent lirerature.*30 (7.5%) patients were from upper class while 104 (26%) and 195 (48.8%) patients were from upper middle class and lower middle class respectively. 50 (12.5%) and 21 (5.2%) patients were from upper lower class and lower class respectively. Rotavirus infection was predominantly detected in the winter season (62.5%) followed by monsoon (25%) and summer (12.5%).Ali Z et al[18] reported RV genotype predominance differed significantly across seasons. As such, G9P[8] was predominant in seasons winter and monsoon, while G1P[8] was the major genotype in season summer (P<0.001). As for G2P[4], it was more frequent in season summer compared to seasons winter and monsoon (P<0.001), and G4P[8] was more encountered in seasons winter and summer rather than season monsoon (P =0.004). All genotypes appeared equally in all age-groups examined. *The Vesikari score did not differ significantly between the different genotypes.*.386 (96.5%) patients in our study were immunized while 14 (3.5%) patients were not immunized.Ali Z et al[18] estimating the rate of RVGE hospitalization, identifying RV genotypes, determining the seasonal and geographical variations reported only 18.1% (n = 255) of the children hospitalized for GE were vaccinated against rotavirus. Sudarmo SM et al[17] correlated between rotavirus genotypes, clinical factors and degree of severity of acute diarrhea in children under 5 years old reported frequency of diarrhea was 6.22 ± 3.85 times a day.In the present study, majority of the patients (56.1%) had diarrhea for 49-96 hours followed by 25-48 hours (29.7%) and 1-24 hours (14.2%).

The mean frequency of stool over 24 hours was 6.11 ± 3.66.Ali Z et al[18] estimated the rate of RVGE hospitalization, identifying RV genotypes, determining the seasonal and geographical variations reported patients with RV-positive GE significantly differed from the RV-negative group by fever and vomiting, treatments during hospitalization and at discharge (oral rehydration and antibiotics), year of hospitalization, and RV season. As such, fewer RV-positive patients had fever compared to those who were RV-negative (62.1% versus 71.1%, P = 0.001); more RV-positive children (19.9%) were treated with oral rehydration while only 15.1% were offered the same treatment in the RV-negative group (P = 0.03) probably related to the increased incidence of vomiting in the former group (76.4% vs 57%, P <0.001). 53.2% of children with RV-negative GE were given antibiotics compared to 40.4% in the RV-positive group.In our study, majority of the patients (57%) were hospitalised for 4-7 days followed by 0-3 days (38%) and >7 days (5%). The mean duration of hospital stay of patients was 4.31 ± 1.97 days.Ali Z et al[108] estimated the rate of RVGE hospitalization, identifying RV genotypes, determining the seasonal and geographical variations reported mean duration of hospitalization was 4 days and ranged between 1 and 73 days with vast majority of GE cases (91.6%) being severe according to Vesikari score of 11 or above. The difference in the duration of hospitalization between the RV-positive and RV-negative children was not statistically significant.130 (32.3%) samples in our study were positive for rotavirus while 270 (67.5%) samples tested negative for rotavirus.It was observed in our study that out of 400 samples, both G and P genotypes were identified in a total of 390 samples. In 2 samples, G genotype was determined while P genotype was untypeable. In 8 samples both G and P genotypes were untypeable.Among G genotypes, G3 was the most frequently detected (n=238, 59.5%), followed by G1 (n=104, 26%), G2 (n=16, 4%) and G9 (n=34, 8.5%). The predominant P genotype was P[8] (n=340, 85.4%) followed by P[4] (n=48, 12%) and P[6] (n=2, 0.5%). Four common G types (G1, G2, G3, and G9) and two common P types (P[8] and P[4]) accounted for 91% and 97% of the samples respectively.G3P[8] was the most common G/P combination found in 51% of the samples followed by G1P[8] (21%), G9P[4] (6.5%), G3G12P[8] (4.5%), G2P[4] (3.5%), G1G3P[8] (2.5%) and G9P[8] (2%). This is comparable to the studies of Ali Z et al[18], Sudarmo SM et al[17], Durmaz R et al[19], Kang G et al[20] and Daga N et al.Ali Z et al[108] estimated the rate of RVGE hospitalization, identifying RV genotypes, determining the seasonal and geographical variations reported G and P genotypes of rotavirus in the 428 RV-positive stool specimens out of the total 1,414 samples (30.3%) by sequencing of the VP7 and VP4 genes, respectively. There was a predominance of G1P[8] RV accounting for 36% (n = 154) of specimens, G9P[8] RV was responsible for 26.4% (n = 113) of cases, G2P[4] for 17.8% (n = 76) and G4P[8] for 15.9% (n = 68).Sudarmo SM et al[107] cross-sectional study reported Rotavirus typing in VP7 and VP4 was classified as common (G1, G2, G3, G4 and G9 (VP7) and P[4], P[6] and P[8] (VP4)) and uncommon (others) typing. The predominant VP7 genotyping (G type) was G2 (31.8%), followed by G1 (29.5%), G9 (11.4%), G4G9 (8.0%), and G1G2 (4.6%). As to VP4 genotyping (P typing), P[4] was predominant (31.8%), followed by P[6] (27.3%), P[8] (26.1%), P[9] (4.5%) and P[6]P[8] (4.5%). The typing was confirmed by PCR. Combinations of G and P typing were classified as common (G1P[6], G1P[8], G2P[4], G2P[6], G3P[6], G4P[6] and G4P[8]) or uncommon types as well .Durmaz R et al[19] sentinel surveillance study obtaining baseline data on the rotavirus G and P genotypes reported among the 1644 rotavirus PCR positive samples, six were partially typed. Both G and P genotypes were identified in a total of 1638 samples. Among the G genotypes, G9 was the most frequently detected (n5800, 48.7%), followed by G1 (n5425, 25.9%), G2 (n5267, 16.2%), G3 (n571, 4.3%), G4 (n545, 2.7%), G8 (n515, 0.9%), G12 (n513, 0.8%), and G10 (n56, 0.4%). G12 was detected for the first time. The predominant P genotype was P[8] (n51305, 79.4%), followed by P[4] (n5320, 19.5%), P[9] (n57, 0.43%), P [6] (n55, 0.30%), P[11] (n52, 0.12%), and P[10] (n51, 0.06%). Four common G types (G1, G2, G3, and G9) and two common P types (P[8], P[4]) accounted for 95.1% and 98.8% of the strains, respectively. The most common G and P combinations in the samples tested were G9P[8] (n5666, 40.5%), followed by G1P[8] (n5355, 21.6%), G2P[8] (n5153, 9.3%), G2P[4] (n5107, 6.5%), G3P[8] (n557, 3.5%), and G4P[8] (n539, 3.4%). Kang G et al[20] meta-analysis on epidemiological Profile of Rotaviral Infection in India reported significant differences in the distribution of G types. Overall, G1 strains were the most common strains noted, except in western India. *Eastern India had the highest percentage of G1 strains (41% of strains) ,G2 strains were also seen in all parts of the country, and G2 was the single G type most often identified in northern India (29% of strains). but in our study Among G genotypes, G3 was the most frequently detected (n=238, 59.5%), followed by G1 (n=104, 26%), G2 (n=16, 4%) and G9 (n=34, 8.5%).* Daga N et al[25,26,27] in study determining the distribution of the G and P genotypes of rotavirus seasonal variation and to monitor if there was any emerging genotype or unusual strain circulating among children with diarrhoea, age less than 5 years reported G1P[8] (63.30%) was the most prevalent strain observed. Other genotypes observed were G1P[4], G1P[6], G2P[4], G9P[4], G9P[8], G12P[6], G12P[8] and some mixed infections were also observed with different rotavirus strains such as G1P[4]P[8] and G1G2P[6].The reports on G serotyping of rotaviruses from children with diarrhea in India have shown the presence of all four major serotypes, i.e., G1 to G4, with one or two serotypes being predominant at a particular peak of infection[21,22]. Sudarmo SM et al[17] reported G1 and G2 genotypes were the predominant types, after which came G3 and G4. The untypeable strains may belong to the G9 or G10 genotype, both of which have been reported in neonatal infections from India[23]. All P4 strains were associated with the G2 genotype, except for one strain that had multiple G types (G1 and G2), thus confirming the general association of G2 and P4 genotype specificity. Some of the samples had multiple G or P types that may be attributed to mixed infections. In previous studies, mixed G and mixed P types have been reported[24]. Coinfection with multiple rotavirus strains can lead to the emergence of reassortants during the course of natural infection.

It was observed in our study that G3P[8] genotype was significantly associated with duration of diarrhea and frequency of Stool over 24 hours **(p<0.05)**.Saluja T et al[10] study examined the possible relationship between epidemiologic and clinical features of rotavirus gastroenteritis and G types. Reported among those with G1 rotavirus infection, very severe diarrhea (Vesikari score ≥ 16) was reported in 59 (33.9%) children, severe diarrhea (Vesikari score 11–15) in 104 (59.8%), moderate (Vesikari score 6–10) and mild diarrhea (Vesikari score 0–5) in 11 (6.3%). Among those with G2 infection, very severe diarrhea was reported in 26 (27.4%) children, severe diarrhea in 46 (48.4%), and moderate and mild diarrhea in 23 (24.2 %). Among those with G9 infection, very severe diarrhea was reported in 47 (54.5%) children, severe diarrhea in 29 (33.6%), and moderate and mild diarrhea in 10 (11.9%). Among those with G12 infection, very severe diarrhea was reported in 9 (40.9%) children and severe diarrhea in 13 (59.1%) , *but in our study there was no such association seen between rotavirus serotypes and severity of gastroenteritis.* In the present study, the logistic regression analysis showed regression in G and P genotype combinations frequency of Stool over 24 hours **(p<0.05)**. Similar observations were noted in the study of Sudarmo SM et al[107].Sudarmo SM et al[107] cross-sectional study reported G3, G4 and G9 were significantly correlated with severe diarrhea (p = 0.009) in multivariate analyses and with frequency of diarrhea (>10 times a day) (p = 0.045). in univariate analyses, but there was no significant correlation between P typing and severity of diarrhea. Regarding the combination of G and P typing, G2P[4] was significantly correlated with severe diarrhea in multivariate analyses (p = 0.029).

Conclusion:

The present study reported the current situation for acute diarrhea caused by rotavirus in infants or younger children at our tertiary care centre. Genotype G2P[4] has the highest prevalence, and G3, G4 and G9 and G2P[4] combination genotype were found to be associated with severe and frequent diarrhea. Further long-term studies as well as surveillance programs are necessary for overcoming rotaviral disease.The complex epidemiological profile of rotaviruses in India highlights the need for a unified protocol for surveillance of circulating strains by Indian laboratories, and a national rotavirus surveillance program is being developed with support from the Indian Council of Medical Research and the India Rotavirus Vaccine Project at the Program for Appropriate Technology in Health. With the rapid evolution of rotaviruses by the accumulation of point mutations and the generation of reassortant in multiple infections, both the application of existing methods and the frequent monitoring and updating of genotyping methods will be required on a national scale.Though in my study education and family type(nuclear/joint) is inversely related to incidence of rotaviral diarrhea so there should be adequate knowledge of personal hygiene , hand washing and increased awareness of vaccination for its prevention.

Conflict of Interest

The authors report no conflicts of interest.

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