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Symptomatic and Asymptomatic Rotavirus Infections Among Urban Population of Early Childhood: A Cross-Sectional Study

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ABSTRACT

The infections caused by rotavirus are given paramount attention worldwide as they are often implicated with significant morbidity and mortality of childhood population. The present study attempted to explore the prevalence of symptomatic and asymptomatic rotavirus infections among the children dwelling from Chennai city, India. Two hundred children below the age of 5, belonging to two categories, were screened for the rotavirus infection by means of detection of viral antigen from stool samples by Enzyme Linked Immunosorbent Assay. The overall prevalence of rotavirus infection was 34% which encompassed 53.3% and 22.4% symptomatic and asymptomatic cases. Within the symptomatic subjects, 48.7% and 58.3% of male and female children were positive for the infection. The rates of positivity to the viral infection among the male and female asymptomatic cases respectively were 25.9% and 19.4%. Attributable sociodemographic and medical factors identified for rotavirus infection among the symptomatic and asymptomatic cases respectively were, domicile of low scale area (77.5 and 71.4%), low education level of parents (80.0 and 60.7%) and lack of vaccination (92.5 and 82.1%). Breast feeding showed better protective impact on symptomatic cases (47.5) than the asymptomatic cases (32.1%). Need for further investigations on virus strains implicated with asymptomatic infections and vaccine failure has been suggested for ensuring sustainable public health of young population.

Keywords: *Rotavirus, prevalence, diarrhea, symptomatic, asymptomatic, vaccine*

1. INTRODUCTION

Infections leading to acute diarrheal diseases significantly contribute to serious public health problems around the world. The gastroenteritis infections targeting the children and the associated morbidity and mortality are the major threats to the health care in both developing and developed countries [1]. Rotavirus is viewed as the most common pathogen implicated with gastro enteric infections of the children worldwide. Rotavirus, even with a minimal infective dose (ID_{50} of 10 ffu), can affect the susceptible individuals and lead to a severe and life threatening disease conditions [2]. Children afflicted with the rotavirus infections often belong to the age group of below five years.

The Human Rotaviruses belong to Reoviridae family and comprise of 11 segments of double stranded RNA. The capsid proteins of the virus determine the major antigenic properties and designate it into group, subgroup, and serotype. There are seven major groups viz., A-G of the virus recognized thus far. Human infection is often caused by group-A viruses with the subtypes 1-4 are considered clinically important. Occasionally, the associations of group B and C viruses with the outbreaks are reported [3].

Rotavirus infections are classically present with three common symptoms such as vomiting and fever followed by acute diarrhea. The main clinical feature of rotavirus gastroenteritis is dehydration as an outcome of watery diarrhea. In severe cases, it may be profuse with >10 stools/day and last for 3-6 days and in immunocompromised patients even longer. Occasionally, concurrent bacterial infections together with the symptoms of dysentery and tenesmus may occur [4]. The infection is highly contagious during the symptomatic phase and may continue till 8th day even after the symptoms disappear.

In severe cases, rapid dehydration can lead to renal shutdown and death. The first onset of rotavirus infection usually occurs during early childhood of ages 6 months to 2 years. Nosocomial infections are often reported for the neonates to acquire the virus. Owing to its widespread, notably in developing countries, peak incidence is reported during 6-11 months with almost 80% of children infected by the first year of life. Reports indicate that almost all the children are victimized to the rotavirus infection at least once between the ages of 3-5 years [5].

Epidemiological studies report that the main mode of transmission of rotavirus is the feco-oral route [2,6]. However, the robust spread of rotavirus across the geographical areas and poor documentation about the etiology in many outbreaks point that there are additional possible routes of transmission. Since the patients excrete the virus to the extent of 10^{11} particles per mL of stool, substantial environmental contamination occurs during the acute phase of the disease. Poor sanitation and inadequate personal hygiene significantly contribute to eventual contamination of foods and water for further spread of the virus. Involvement of droplets generated from vomiting also has been speculated for the transmission [7]. Some researchers have reported the interspecies transmission of rotaviruses from animals such as pigs, cattle, dogs and cats. Nevertheless, the magnitude of zoonoses caused by this virus remains uncovered [8-11].

Contemporary research studies have demonstrated that the shedding of virus may also occur without diarrhea or other symptoms especially in neonates and adults [12,13]. These studies have reported that the asymptomatic infections are caused by some rotavirus strains with unique characteristics. Epidemiological investigations of maternities have explored certain resident strains that can be implicated with asymptomatic neonatal infections [4]**.** These findings necessitate a comprehensive research on asymptomatic infections of rotavirus which pose greatest risk for outbreaks in both community and healthcare settings requiring hospitalization.

Studies focusing on the immunity to rotavirus infer that single episode of previous infection with this virus does not confer complete protection. Development of immunological defense requires cumulative and multi-episodic infections. Despite the efforts on improving the sanitation and public health, the prevention and control of rotavirus is still a challenging task for the healthcare sector. This circumstance warranted the inclusion of rotavirus vaccine in the National Immunization Program of all the countries in compliance with the recommendation of World Health Organization in 2009 [14]. Currently, two types of rotavirus vaccines viz., monovalent (RV1) and pentavalent (RV5) are enrolled in the vaccination schedules of many countries. However, in recent years, there are increasing number of reports on the failure of rotavirus vaccines in rendering protective immunity especially in low-income countries where mortality rates are high [6,15-18].

Global annual disease burden of the diarrheal illness has been estimated to be 1.7 billion hospitalized cases and approximately 0.5 million deaths of childhood patients under the age of 5 which accounts to 8% of overall deaths [19]. Epidemiological data infer that India records an annual death toll of 1.1 lakh due to diarrheal diseases which include the casualties of approximately 10% of children <5 years old [5].

It is generally acknowledged that grooming young population to be the productive individuals would be a key strategy for a country to achieve its creditable development. Hence, it is necessary to strengthen the nation's health care services to protect its young population from preventable fatal diseases such as childhood diarrhea. This can be accomplished by a comprehensive investigation on the changing trends of the etiology, epidemiological features and associated risk factors of the disease. In view of this, the present research work was carried out to explore the current trend of prevalence of rotavirus infections among the pediatric population and the factors challenging its prevention and control.

2. MATERIALS AND METHODS

2.1 Study design

A total of 200 children under 5 years old dwelling in Chennai city, Tamil Nadu state, India, were enrolled with due informed consent of the parents. The study population encompassed three age-wise categorized groups of subjects, viz., infants (1-12 months), toddlers (1>3 years) and children (3≤5 years). The investigation was carried out using a cross-sectional study for a period of three months from January to March 2013.

Among the study population, 75 children, who were presented with the symptoms of gastroenteritis and seeking medical care at the institute of social pediatrics, Government Stanley medical college and hospital, were designated as symptomatic cases. Another group comprising 125 children of asymptomatic cases, who were apparently healthy and had no demonstrable symptoms were inducted from five different areas of Chennai. The details of the enrolment of study population is presented in table 1. The study design was approved by the ethical review committee of P.G. and Research Department of Microbiology, Asan Memorial college of Arts and Science, Chennai.

Table 1. Study population enrolled for the research

Study group	Symptomatic cases		Asymptomatic cases		Section total
	Male	Female	Male	Female	
Infants	7	5	8	14	34
Toddlers	13	8	17	23	61
Children	19	23	33	30	105
Total nos.	39	36	58	67	200

2.2 Collection of specimens and background data

From each child enrolled in the study, fresh stool sample of not less than 5g (5mL) was collected in a wide mouthed, sterile, leak-proof container. Then, the samples were labelled and transported aseptically within 1-2 hours to the laboratory. The samples were suspended in 1x phosphate buffer saline (PBS) and stored at 2-8ºC until further use.

Along with the specimen collection, the parents / guardians of the study population were interviewed with a structured questionnaire to collect the background data of the subjects necessary for clinical reasoning and interpretation of study output. Some of the highlights of the details gathered were demographic (gender, age, and nutrition), socioeconomic (domicile and level of education of parents), and medical history (vaccination status, past infections, and clinical symptoms). Children suffering with likelihood clinical features of rotavirus infection such as vomiting, abdominal pain and acute watery diarrhea were categorized as symptomatic cases. Any single episode of symptomatic gastroenteritis experienced by the child during the last 6 months was presumed as past infection.

2.3 Laboratory investigation

Screening of stool specimens for the presence of rotavirus was performed using rapid enzyme linked immunosorbent assay (ELISA). Commercial ELISA kit intended for the detection of rotavirus group specific antigen VP6 was procured from ALPCO, USA (Cat. no. 86-ROTHU-E01). Before the test, 1g of specimen was suspended in 9mL PBS and centrifuged at 2000 rpm for 15 minutes. Then, the supernatant was collected in a sterile vial. Qualitative determination of rotavirus antigens from this supernatant was carried out by adopting the indirect sandwich ELISA technique. The assays for all the samples were performed according the manufacturer's user manual given along with the testing kit.

2.4 Statistical analysis

All the data obtained from the study were analyzed by simple statistics and Chi-square test of association. Significance of association between the variables was determined at 95% confidence interval and a *p*-value of ≤ 0.05. Categorical variables of the data are presented in terms of absolute numbers and percentage of proportion. Analysis of laboratory data considered the specificity, sensitivity and control values of the assays.

3. RESULTS

3.1 Overall prevalence of Rotavirus

Compilation of laboratory data obtained from the assays of all the specimens indicated an overall prevalence of 34% of rotavirus infections across the categories of children (Table 2). The prevalence of the virus was twofold higher among the symptomatic cases than the asymptomatic cases of the study population.

Table 2. Overall prevalence of Rotavirus among study population

Study group	Symptomatic cases		Asymptomatic cases		Section total
	Male	Female	Male	Female	
Infants	5	2	4	3	14
Toddlers	7	5	3	5	20
Children	7	14	8	5	34
Total nos.	19	21	15	13	68
Overall %	53.33 [40/75; *p* 0.153]		22.4 [28/125; *p* 0.041]		34.0 [68/200]

3.2 Category-wise prevalence of Rotavirus

Results of screening of specimens of symptomatic and asymptomatic cases for the presence of rotavirus antigens are presented respectively in tables 3 and 4. Among the symptomatic cases, the female subjects showed a 10% higher prevalence of the infection than that of the males. However, individual analysis indicated that the male infants had a substantial positivity to the virus followed by the toddlers and children categories of female subjects (Table 3).

Table 3. Results of screening of Symptomatic cases for Rotavirus infection

Study group	Male		Female	
	No. Positive	% of positivity	No. Positive	% of positivity
Infants	5	71.42	2	40.0
Toddlers	7	53.84	5	62.5
Children	7	36.84	14	60.86
Overall %	48.72 [19/39; *p* 0.092]		58.33 [21/36; *p* 0.054]	

In contrast to the symptomatic cases, the asymptomatic male subjects depicted 30% higher prevalence of the virus than their counterparts. Similar to the data of the symptomatic cases, the half of the asymptomatic male infants were found infected which was twofold higher than that of any category of subjects of this group (Table 4).

Table 4. Results of screening of Asymptomatic cases for Rotavirus infection

Study group	Male		Female	
	No. Positive	% of positivity	No. Positive	% of positivity
Infants	4	50.0	3	21.43
Toddlers	3	17.65	5	21.73
Children	8	24.24	5	16.67
Overall %	25.86 [15/58; *p* 0.013]		19.4 [13/67; *p* 0.046]	

3.3 Attributable factors of Rotavirus infection

Figures 1 and 2 represent the data of socioeconomic, demographic and medical factors associated respectively with the symptomatic and asymptomatic cases of rotavirus infections. In both the cases, more than 2/3 of the infected subjects were observed to be dwelling from low scale areas. Low level of education of parents and abstinence from vaccination significantly contributed higher percentage of infections in symptomatic cases. Nearly 3/4 of the subjects of this category were newly infected with the rotavirus (Fig.1).

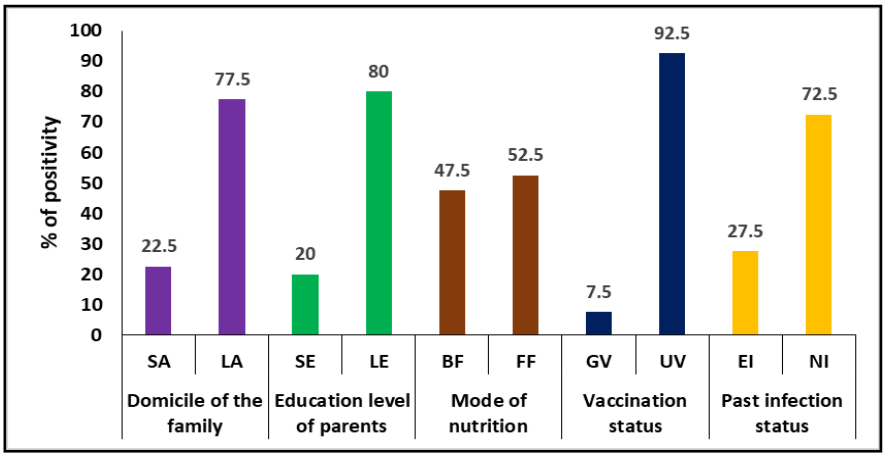

Figure 1. Association of sociodemographic and medical factors with symptomatic rotavirus infections

[SA-Standard area; LA-Low scale area; SE-Standard education; LE-Low education; BF-breast fed; FF- Formula fed; GV-Vaccination given; UV- Unvaccinated; EI-Episodic infection; NI-New infection]

The factors identified to contribute more for the asymptomatic rotavirus infections were, low education level of parents and formula feeding. The trend of past infection among the asymptomatic cases aligned with that of the symptomatic cases. Notably, 18% of vaccinated subjects were positive for rotavirus infections despite not showing any apparent symptoms (Fig. 2).

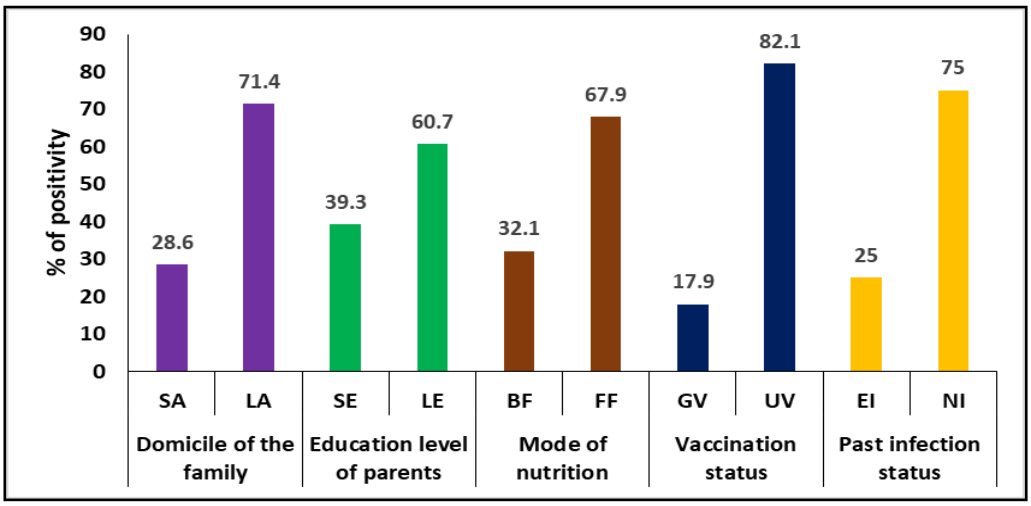

Figure 2. Association of sociodemographic and medical factors with asymptomatic rotavirus infections

[SA-Standard area; LA-Low scale area; SE-Standard education; LE-Low education; BF-breast fed; FF- Formula fed; GV-Vaccination given; UV- Unvaccinated; EI-Episodic infection; NI- New infection]

4. DISCUSSION

Globally, the infection caused by rotavirus is viewed as a major threat to the public health as it is responsible for the life threatening diarrheal illness among the childhood population. Reports on the etiology of rotavirus diseases indicate that while the symptomatic infections account for 10-13% fatalities of affected individuals, it may reach as high as 30% in severe cases [20,21]. Prevalence of rotavirus usually occurs with high magnitude in circumstances where the barriers of feco-oral transmissions are breached.

It is generally acknowledged that the communicable diseases occur more commonly in places where the practices of personal hygiene and environmental sanitation are compromised. In order to verify and reaffirm this view with reference to the rotavirus infections, the present research investigated the prevalence of the virus across the study population of different socioeconomic backgrounds and domiciles. Among the study population, the subjects with clinical symptoms (37%) were enrolled from the health care set up and the remaining apparently asymptomatic cases (63%) represented the domicile of both standard and low scale areas of Chennai city (Table 1). Similar studies involving both symptomatic and asymptomatic cases have been conducted in Mexico [22], Iran [23], Argentina [24] Nigeria [25] and so on for obtaining insight on the epidemiology of rotavirus infections.

The overall prevalence of rotavirus infection among the children below 5 years old, based on the laboratory report of our study, was 34%. Individual study of study population revealed the prevalence percentages of 53.3% and 23.4% among symptomatic and asymptomatic cases respectively (Table 2). A previous study conducted by Saravanan et al. [26] in Chennai has reported an infection rate of 22.3% among children with acute diarrhea. A recent study conducted on the children hospitalized for acute diarrhea in another Indian city, Indore, has recorded the positivity to rotavirus infection in 74 out of 295 subjects screened (25%) [27]. Compared to these studies, our study reported 50-60% higher prevalence of rotavirus among symptomatic cases. This infers that, despite the efforts taken by the healthcare department to control the rotavirus infection, the disease has been on the increasing trend over the years.

Prevalence of asymptomatic rotavirus infections, as reported in various parts of the world, have been 5.6% in Serbia [21], 12.1% in Iran [23], 9.2% in Nigeria [12], 28.3% in Mexico [22]. The rate of prevalence of asymptomatic infections of the present study has been observed to be within the global range (5-28%) based on the previous reports (Table 2). However, a 11-year cohort study carried out in Bangalore city has recorded the prevalence of asymptomatic rotavirus infections accounting to 58% among the neonates [28]. Although the prevalence rate appears to be substantially higher than that of the present study, the earlier study has reported that the infections were caused by an atypical strain which had a limited

occurrence in the geographical location of the investigation.

Studies conducted earlier have mostly reported the collective prevalence of rotavirus infections in both male and female subjects together. Only few studies have demonstrated the distinct gender wise prevalence of the rotavirus. Individual study of the prevalence of rotavirus among the symptomatic cases of the current study indicated that the frequency of infection is higher among the female subjects than their male counterparts (Table 3). In contrast to our finding, Habib et al. [2] have reported 61.9% and 38.1% of prevalence of rotavirus among the male and female children admitted with acute diarrhea in Bangladesh. Another similar hospital based study conducted in Iraq has recorded 44.1% and 33.3% prevalence of symptomatic infections among the male and female subjects under study [29]. The female predominance of rotavirus infections in our study can be explained by the social practice of prioritized general care for male children than the female in Indian context. Besides, Dhiman et al. [30] explain that the females, by owing XX chromosome, tend to have more resistance to rotavirus infection. Nevertheless, the data brought out by the present study underscores the need for detailed study of rotavirus prevalence for better insight into its epidemiological pattern.

Study of asymptomatic infections in the current research demonstrated the rotavirus prevalence of 25.9% and 19.4% respectively among the male and female subjects. These figures are in contrast with the studies of Okoh et al. [12], who reported 5.8% and 3.5% of asymptomatic infections among 260 male and female subjects. The reason for higher prevalence of asymptomatic infections in our study may be due to the low sample size amounting 125. It may be noted that the prevalence rates of rotavirus are fivefold higher than that of the previous study. Recently, Lee et al. [13] have demonstrated that the individuals with mutant *FUT2* gene which determines the specific histoblood group antigen (HBGA) often develop asymptomatic infections owing to their decreased susceptibility to rotavirus. Nevertheless, the occurrence of asymptomatic infections in children cannot be underestimated as there is a risk of disease complications in the event of compromise of their immature immune system.

The study of frequency of infection among different categories of study subjects indicated that there has been gradual decrease of prevalence in pace with increasing age of female group except the toddlers (Tables 3 and 4). This may be attributed to the higher possibility of exposure to the virus due to the active behavior of toddlers. Children at this stage tend to be more active, unhygienic and get in contact with contaminated surfaces such as floor, stairs, doors and so on, which facilitate frequent infections. This finding is agreement with the reports of previous studies which determined highest prevalence of rotavirus infections among children aged 0-12 months [19,25]. However, in contrast to this trend, the infants of symptomatic male and female and asymptomatic male subjects showed higher rates of positivity than their counterparts (Tables 3 and 4). A similar study conducted in Calicut city documented a substantial prevalence (75.1%) of rotavirus among infants [31]. The higher positivity among the infants to infection may be due to the attributable factors as described in the following section.

Study of attributable factors for the prevalence of rotavirus infection among the present study population provided significant information (Figures 1 and 2). In both the symptomatic and asymptomatic cases, the domicile of the family exhibited direct impact on the occurrence of rotavirus infection. For the purpose of identifying asymptomatic cases and for the comparative analysis, children appeared to be healthy and living in two categories of areas of Chennai were enrolled. These included standard areas (places with middle and high income people, better living conditions, proper environmental sanitation, cleanliness, improved sewage systems and clean water facilities) and low scale areas (places with compromised living conditions, densely populated, poor sanitation and cleanliness). These low scale areas, referred to as slums by the local slang, are probably the domiciles of low income people and mostly located on the bank of the cooum river which is often polluted with domestic sewage and municipal wastes. Owing to these factors, the people of low scale areas have high vulnerability for communicable diseases. The laboratory screening our study revealed that a substantial number of children showing positivity for the rotavirus infection (71-78%) were dwelling from the low scale areas. Concomitant findings of higher rates of rotavirus mediated acute diarrhea among the children of low socioeconomic level in Indonesia have been reported by Bishop et al. [32].

Another important factor analyzed in this study is the education level of parents of the study population. For the convenience of study, the levels of education were categorized into two viz., standard education (minimum of high school education) and low education (less than primary school education or nil). Besides the environmental sanitation measures, prevention of diseases depends on personal hygiene and awareness on basics of public health. It is generally acknowledged that the education level of the individuals is critical to understand the significance of personal hygiene in disease prevention. Due to the lack of awareness and poor personal hygiene, the feco-oral transmission of rotavirus occurs frequently in this group. As a testimony of this concept, our study recorded four times higher positivity of rotavirus infection among the children of the parents with low education level than those with standard education (80% vs. 20%) (Fig. 1). Consistent with our finding Bennett et al. [33] have attributed the probability of rotavirus infection to the care taker's educational level.

In order to assess the impact of type of nutrition on the rotavirus disease occurrence, the present study examined the effect of breast feed and formula feed on the health of the study population. As anticipated, there was a preponderance of rotavirus infection among the children nourished with formula food than those with breast feeding (Figures 1 and 2). Since the habitat

of rotavirus in the infected patients is the small intestine, the local immunity spearheaded by IgA antibodies render protection against the viral invasion. Due to the possession of immature immune system, the child might struggle to mediate active immune response against the virulent virus. Therefore, the breast feeding plays a pivotal role by providing passive immunity by way of administering antimicrobial peptides and secretory antibodies along with the mother's milk [3,7,19]. The formula food lacks these essential immune factors and thus remains inefficient in offering natural or acquired immunity. However, the infants, who are supposedly breast fed in general, were found infected with the virus to the extent of 21-71% in both symptomatic and asymptomatic cases (Tables 3 and 4). It can be explained that most of these infected infants have been formula fed which could have eventually made them susceptible to the virus. In consonance with our finding Kurugöl et al. [20] and Ojobor et al. [25] have independently demonstrated 50% higher infections among the bottle-fed children than the breast fed. It may also be noted that there is a gradual decrease in the magnitude of infection in toddlers and the children which may be due to the development of acquired immunity subsequent exposure to the virus though contact with the contaminated surfaces and unhygienic activities.

Study on the implication of vaccination status on the rotavirus infection brought out salient information in the present research. It is pertinent to note that the rotavirus vaccine Rotavac® was inducted in the public immunization system of India during 2016 and in the following year the vaccination was implemented by the Government of Tamil Nadu [34]. The present study was conducted three years before this process, during which period the children were immunized with commercial vaccines available in private healthcare organization upon parent's voluntary requests. As can be seen the figures 1 and 2, the rates of infection in unvaccinated subjects during the research were 45-120% higher than the vaccinated counterparts in both symptomatic and asymptomatic cases. Abstinence from vaccination and eventual lack of protective active immunity can be attributed for the substantial prevalence of rotavirus in this section of study population. However, the positivity of vaccinated subjects in both symptomatic (8%) and asymptomatic cases (18%) cannot be voided from interpretation (Figures 1 and 2). This observation can be explained by the diminishing effectiveness of vaccine in inducing rotavirus specific IgA antibodies. Similar studies conducted elsewhere [18,24,33] have implicated the wild-type rotavirus for the infections in vaccinated children which is antigenically different from the strain employed in the vaccine preparation. Thus the findings of our study necessitates reviewing the effectiveness of present vaccines and to focus on developing improved vaccines using the prevailing candidate virus strains.

The medical history of symptomatic and asymptomatic cases indicated that approximately 1/3 of the subjects had episodic clinical infections in the past (Figures 1 and 2). The data of these past infections were based on the answers of the parents to the questionnaire of the study. It may be noted that past infections in this context refer to the gastroenteritis and diarrheal symptoms whose cause is unknown. Since these symptoms are also associated with the clinical features of infections caused by other enteric pathogens such as bacteria, protozoan and helminthic parasites, the present infection of the study population need not be considered as recurrence of past rotavirus infections. Consistent with our finding, Bagali et al. [19] have demonstrated the association of Adenovirus with the episodes of acute gastroenteritis prior to the rotavirus infection. Nevertheless, these reported past gastroenteritis associated symptomatic infections can be viewed as index outcome of poor hygiene and sanitary practices in the family of the children under study.

5. CONCLUSIONS

Study of prevalence of symptomatic and asymptomatic infections of the rotavirus enables the clear insight into the epidemiological trend of the disease caused by the virus. Prevalence of >50% of symptomatic cases among the infected individuals necessitates formidable measures to control the disease. The female subjects tend to have higher preponderance of symptomatic infections than the male which might be due to natural genetic makeup. The rate of asymptomatic rotavirus infections in the geographical area of the study is within the global range (5-28%) of the disease. Most often, the asymptomatic infections are implicated with atypical viral strains. Owing to the mutation in histoblood group antigen coding gene, the infected individuals might develop asymptomatic infections of rotavirus. Even though the disease prevalence decreases with the advancing age, the infants develop the disease due to the attributable sociodemographic and medical factors. Standard living conditions, awareness par with the education level and breast feeding constitute the critical factors for prevention and control of rotavirus infections. Occurrence of symptomatic and asymptomatic infections in 8-18% of vaccinated children might be due to poor induction of IgA antibodies and the etiology of wild strain different from that of the vaccine. Other microbial agents of gastroenteritis can be associated with the previous diarrheal episodes resembling rotavirus clinical infection.

COMPETING INTERESTS

The author has declared that no competing interests exist.

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