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Original Research Article

Incidence of Rotavirus Infection and Associated Risk Factors among Children Under 5 Years in Eritrea

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ABSTRACT

Background: Rotavirus is the leading cause of severe and fatal diarrhoea in African children. The main objective of the study was to determine the incidence of rotavirus among children less than 5 years old attending Orotta Pediatric Referral and Mendefera Hospitals in Eritrea and determine the possible associated risk factors that could expose them to rotavirus infection.
Methods: This cross-sectional study was conducted between January to June 2015.Out of 82 children who satisfied the inclusion criteria, 45(54.9%) were male and the remaining 37(45.1%) were female with the ratio of 1.2:1. Stool samples collected from the children were subject for detection of rotavirus antigen using enzyme linked immune sorbent assay. A standardized researcher-administered questionnaire for demographic data collection, clinical manifestation and associated risk factors for rotavirus infection was used.
Results: Out of 82 subjects 8(9.8%) were positive while the remaining 74(90.2%) were negative. The male: female ratio was 1.2:1 with largest proportion of cases among children greater than 6 months old. Possible risk factors from all the participants and caretakers were assessed and associated with the results. The association of risk factors with ELISA results indicated that there is no statistically significant association with a *p-value* of greater than 0.05. Out of 8 rotavirus diarrhea cases, 3 (3.7%) showed vomiting only while remaining 5(6%) had vomiting along with fever and severe dehydration. None of them showed upper respiratory infection.
Conclusion: Rotavirus was found to be an etiologic agent of acute watery diarrhea in children in this study even though vaccine has been introduced in mid-2014 as preventative measure in controlling rotavirus infection.

Key words: Rotavirus, diarrhea, children, ELISA, Eritrea.

INTRODUCTION

Gastroenteritis caused by microorganisms is common in infants and young children worldwide. Rotavirus infection remains the commonest cause of severe dehydrating diarrhea among children worldwide. The infection is associated with high rate of morbidity throughout the world and a high rate of mortality in developing countries. It accounts for more than 800,000 child deaths per year due to poor nutrition and health care in developing countries. An estimated 527,000 children aged less than 5 years died from rotavirus infection in 2004 with > 85% of these deaths in South-East Asia and sub-Saharan Africa (WHO, 2013). In developing countries children aged 6-12 months and in developed countries children aged 12-18 months are severely infected with rotavirus. [1] It is estimated that 28 percent of all under-five diarrheal disease hospitalizations in Eritrea are caused by

rotavirus. [2] In Eritrea there is no national data indicating the magnitude of rotavirus but studies conducted in other Sub-Saharan African countries indicated nearly a quarter of a million children die from the dehydrating diarrhea caused by rotavirus infection every year. This accounts for more than 50% of the global total of rotavirus deaths. [1,3] Based on this evidence Eritrea introduced rotavirus vaccination into its national immunization program in mid-2014.

Asymptomatic infections are more common with successive reinfection. Local immune factors, such as secretory IgA or interferon, may be important in protection against rotavirus infection. Asymptomatic infections are common in infants before age 6 months, the time during which protective maternal antibody acquired passively by newborns should be present. Such neonatal infection does not prevent reinfection but it does protect against the development of severe disease during reinfection. [4] Despite the similarity in the prevalence of rotavirus diarrhea between the developed and developing countries, there is a variation in the factors associated with rotavirus diarrhea. Factors associated with rotavirus diarrhea in sub-Saharan Africa include; nutritional status, dehydration, episodes occurring in the dry season and age under 2 years. Other studies have found contact with diarrhea outside the household, poor food hygiene, dehydration, education level, and accommodation with less than five rooms, bottle-feeding, low birth weight, male gender, maternal smoking, and maternal age < 20 years to increase the risk of rotavirus diarrhea. [5] The death of approximately 11 percent of children under the age of five in Eritrea is mainly due to diarrhea. [6]

Rotavirus is a ubiquitous organism that causes human disease independent of socioeconomic status. The incidence of rotavirus infection among young children is not affected by improvements in public health measures, and rotavirus infection is associated with equal morbidity in high and low-income countries. It also causes approximately 39% of hospitalizations due to diarrheal disease in developing countries. [2] Rotavirus is transmitted by the fecal–oral route. Infection occurs worldwide and is characterized by nausea, vomiting and watery diarrhea. Seasonality of rotavirus infections varied across Africa reflecting the difference in the climatic conditions. The children under 5 years of age will have dehydration and electrolyte imbalance. As this virus is shed in the feces in large numbers it can be detected in the stool by using Enzyme Linked Immuno Sorbent Assay. [7]

Rotavirus infection is not routinely diagnosed in most Eritrean hospitals and its clinical spectrum of signs and symptoms are similar to other gastroenteritis infection. Understanding the burden and magnitude of rotavirus associated infections among less than five years old children will reveal important information on incidence of rotavirus. Moreover the study will reveal an important comparison data to assess the impact of rotavirus vaccination program. The aim of the study was to assess the incidence of rotavirus infection among children visiting Mendefera and Orotta pediatric referral hospitals in Eritrea and to assess the associated the possible risk factors among children. The research will generate a baseline data that could be used for future studies at a higher scale.

MATERIALS AND METHODS

Study area and population

This cross sectional study was conducted in the pediatric outpatient and in-patient departments of Mendefera Referral Hospital and Orotta Pediatric National Referral Hospital in Eritrea, North East Africa. The study population includes children under five years of age with the symptoms of watery diarrhea who were attending the hospitals for the treatment. Out of 114 children who attended the hospital in our study period, 82 children who had the symptoms of gastroenteritis of less or equal to 7 days that is with watery diarrhea and were admitted for treatment of

gastroenteritis as a primary illness were included in the study and the remaining 32 children who acquired gastroenteritis during their hospitalization for treatment of other diseases and showed bloody diarrhea were excluded from our study. Oral and written consent was taken from the participants.

A pre designed and coded questionnaire was used to collect data on study subjects pertinent to demographic characteristics, clinical conditions and possible risk factors for rotavirus infection among children and mothers. The ethical clearance for this research was granted by Asmara College of Health Sciences (ACHS) and Ministry of Health (MoH) ethical committee.

Sample collection and processing

The stool samples from the children were collected using a sterile waterproof container with screw-cap lid containers and these containers were labeled accordingly. 4-5 ml of diarrheic stool samples was collected from each child. The labeled stool samples were transported from Mendefera and Orotta Referral Hospitals on ice bag to Immunoserology Department of National Health Laboratory (NHL), where it was stored at -20°C until assayed for the detection of rotavirus specific antigen. Convenient sampling technique was used for collection of stool samples according to inclusion and exclusion criteria.

All the samples were analyzed for the detection of antigen of rotavirus using Enzyme immunoassay (EIA). [8] Prospect rotavirus micro plate assay was used to process samples (Oxoid Ltd, UK) which detects Group A rotavirus with VP6 structural protein because these group of rotavirus are highly prevalent globally. A common assay format for antigen detection is the double antibody sandwich-technique. In this assay, a capture antibody specific for the viral antigen being sought was bound to a reaction surface; for example, the wells of a plastic micro-titer tray or the surface of a plastic bead. The addition of an enzyme substrate produced a color change or light emission if the enzyme is present. Thus, color change or light emission indicated that viral antigen was present in the specimen being tested.

Statistical analysis

All the data derived from the questionnaires and processed data were entered to SPSS software package version 20 and statistically analyzed. Frequency tables and cross tabulations were drawn. Medians and standard deviation were calculated. Chi square test was also applied to see the difference between categorical and numerical variables.

RESULTS

Demographic characteristics of study subject

All children less than 60 months of age presenting to Orotta referral pediatric hospital and Mendefera referral hospital with acute gastroenteritis were included in the study. Out of 114 children participated in the study, 32 children were not selected as they did not satisfy the inclusion criteria; so only the remaining 82 children who were finally qualified participated in the study. Out of those 45(54.9%) were males and remaining 37(45.1%) were females giving a male to female ratio of 1.2:1.The mean age (SD) of the study subjects was 14.2 months (± 8.73 months). With regard to place of residence by zoba, most of the participants were from Zoba Maekel 63(76.8%) followed by Zoba Debub 12(14.6%), Anseba 5(6.1%), and Northern red sea and Gash barka 1(1.2%) respectively. Sixty three (76.8%) of the study subjects reside in urban areas and the rest 19(23.2%) of the study participants reside in rural areas (Table 1).

Incidence of Rotavirus

The incidence of rotavirus in children was 9.8%. Out of the 82 stool samples examined for rotavirus antigen by ELISA only 8(9.8%) were found positive whereas the remaining 74(90.2%) were negative. Six (13.3%) out of 45 males were found to be positive for rotavirus antigen and the remaining 2(5.4%) out of 37 female cases showed positive result. Among

rotavirus positive cases 75% were males and 25% were females (Figure 1).

Table 1: General description of the study subject

Parameter	Frequency	Percentage (%)
Age in months		
<6	7	8.5
≥6	75	91.5
Sex		
Male	45	54.9
Female	37	45.1
Zone		
Maekel	63	76.8
Debub	12	14.6
Anseba	5	6.1
Norhthern red sea	1	1.2
Gashbarka	1	1.2
Southern red sea	0	0
Residence		
Urban	63	76.8
Rural	19	23.2

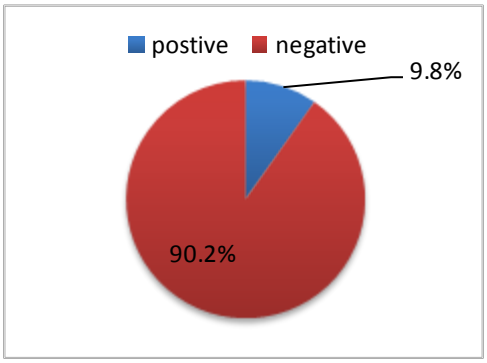

Figure-1 Incidence of rotavirus among children under 5 years of age

Clinical information on children with and without Rotavirus

Parents/caretakers were interviewed and medical records were reviewed by the site staff to complete the study questionnaire and/or case report form. Pertinent clinical data on children enrolled in the research was collected including date of onset of gastroenteritis, vomiting, diarrhea, duration of diarrhea or vomiting, treatment and degree of dehydration were also recorded. A comparison of clinical variables between children with or without rotavirus infection is shown in Table 2. Out of 8 rotavirus positive children, 3 (37.5%) showed vomiting only while remaining 5(62.5%) had vomiting along with fever. Vomiting only was observed among 22(29.7%) rotavirus negative cases while 34 (46%) had fever and vomiting. None of the rotavirus positive cases showed upper respiratory infection.

Table 2: Clinical information on the study subject with and without rotavirus infection

Variables	**Rotavirus positive n (%) N=8**	**Rotavirus negative n (%) N=74**
Vomiting only	3(37.5)	22(29.7)
Fever only	0(0.0)	6(8.1)
Fever and vomiting	5(62.5)	34(46)
Without fever and vomiting	0(0.0)	12(16.2)
Upper Respiratory Infection		
Yes	0(0.0)	19(25.7)
No	8(100)	55(74.3)
Dehydration		
Severe	5(62.5)	48(64.9)
Moderate	3(37.5)	23(31)
None	0(0.0)	3(4)

Association of demographic characteristics of children and mother with the incidence of Rotavirus

Assessment of demographic characteristic of children with laboratory finding was done by using cross tab statistical analysis concerning sex, age and residence as shown in Table 3. The association of demographic characteristic of children with results indicated that there is no statistically significant association with a p-value of greater than 0.05.

Table 3: Association of demographic characteristics of children with the incidence of rotavirus

Parameter		**Rotavirus positive n (%) N=8**	**Rotavirus negative n (%) N=74**	**Total cases n (%) N=82**	***p-value***
Sex	Male	6(75)	39(52.7)	45(54.9)	
	Female	2(25)	35(47.3)	37(45.1)	0.205
Age(months)	<6	0(0.0)	7(9.5)	7(8.6)	
	≥6	8(100)	67(90.5)	74(90.2)	0.473
Residence	Urban	6(75)	57(77)	63(76.9)	
	Rural	2(25)	17(23)	19(23.1)	0.596

Analysis on demographic characteristic of mother with ELISA result was done regarding the education background, occupation and livestock as shown in Table 4. Among parents who were literate, seven (87.5%) were found to be

positive and 1(12.5%) from illiterate parents was found to be positive. With regard to occupation, one was found to be positive from employed parents and seven unemployed out of eight were positive for parents having livestock. The p value for all the results was found to be ($p > 0.05$).

Table 4: Association on demographic characteristics of mother with ELISA result

Variables/Parents		Positive (%)	Negative (%)	Total (%)	*p-value*
Education Background	Literate	7(87.5)	68(91.9)	75(91.4)	
	Illiterate	1(12.5)	6(8.1)	7(8.6)	0.527
Occupation	Employed	1(12.5)	8(10.8)	9(11)	
	Unemployed	7(87.5)	66(89.2)	73(89)	0.623
Livestock	Yes	2(25)	4(5.4)	6(7.3)	
	No	6(75)	70(94.6)	76(92.7)	0.103

General association of risk factors with ELISA result

The research also incorporated assessment of potential risk factors which expose children to rotavirus infection. Generally the possible risk factors for rotavirus infection are source water drinking, washing child hand after defecation, often play with other children ,comorbidity, parental feeding habit, washing hand(after going to toilet, after helping child defecation, before feeding child, before preparing food), defecation place at home and dispose of household garbage. Based on the data collected from the questionnaire, the association of risk factors for rotavirus infection is performed using SPSS computer software. However, the association of risk factors with results indicated that there is no statistically significant association with a p-value of greater than 0.05 (Table 5).

Table 5: Incidence of rotavirus among children in relation to possible risk factors

Variables		Positive (%)	Negative (%)	Total (%)	*P value*
Co morbidity	Yes	3(37.5)	19(25.7)	22(26.9)	
	No	5(62.5)	55(74.3)	60(73.1)	0.365
Drinking Water source	Chlorinated	7(87.5)	55(74.3)	62(75.7)	
	Non chlorinated	1(12.5)	19(25.7)	20(24.3)	0.371
Washing hand after toilet	Sometimes	6(75)	45(60.8)	51(62.1)	
	Usually	2(25)	29(39.2)	31(37.9)	0.354
Washing hand after helping child defecation	Sometimes	6(75)	44(59.4)	50(61)	
	Usually	2(25)	30(40.5)	32(39)	0.326
Washing hand before feeding child	Sometimes	6(75)	43(58.1)	49(59.8)	
	Usually	2(25)	31(41.9)	33(40.2)	0.299
Washing hand before preparing food for child	Sometimes	6(75)	44(59.4)	50(61)	
	Usually	2(25)	30(40.6)	32(39)	0.326
Defecation at home	Toilet	2(25)	44(59.4)	46(56)	
	Open	6(75)	30(40.6)	36(44)	0.068
Disposal of household garbage	Rubbish pit	2(25)	31(41.9)	33(40.2)	
	Open	6(75)	43(58.1)	49(59.8)	0.299

DISCUSSION

Rota virus is believed to be found worldwide and accounts for about 40 to 60% of cases of acute gastroenteritis occurring during the cooler months in infants and children. These viruses have been detected in intestinal contents and in tissues from the upper gastrointestinal tract. Children who are under 5 years of age are mainly infected with rotavirus. Cross-sectional studies of hospitalized infants and young children with diarrheal illnesses have yielded the most compelling evidence for the importance of rotaviruses as etiologic agents of severe diarrhea of early life. Due to genetic diversity in different parts of the world, knowledge of the molecular epidemiology of rotavirus in circulation is important, and appropriate rotavirus-surveillance in a community before, during, and after the introduction of a vaccine campaign is essential to detect uncommon and novel types which might help to explain vaccine failure. For diarrheal disease control to be successful through vaccination,

continuous monitoring of the rotavirus types is needed. [3]

In our study out of the total 82 children who satisfied inclusion criteria, 12(14.6%) were from Mendefera referral hospital and remaining 70(85.3%) from Orotta pediatric referral hospital. Demographic information such as age, gender, and area of residence were recorded. The mean age of the study subjects were 14.72(SD=8.73). In a similar study conducted in republic of Ivory the mean age (SD) of the enrolled children was 13.6 months (±11.14 months). [9] Most of the study subject resided in urban with 63(76.8%) and remaining 19(23.2%) from rural. Mothers' education, working status, marital status and other socio demographic characteristics of guardians were assessed. The finding concerning working status is in agreement with that of other studies conducted in Ethiopia and Nigeria. [10,11] All of the mothers were married. 7(8.6%) were found literate which is similar to the findings of a study carried out by Dutta et al. [12] in Bahrain who showed 39.3% of rotavirus infection in children of mothers with university education. But in contrast to our study findings he reported high prevalence in higher income home.

In our study carried out for a short time 9.8% cases were found to be positive for rotavirus infection while the remaining 90.2% was negative. Since our study involved the assessing of the rotavirus infection after introduction of rotavirus vaccine in the national immunization program, an incidence of 9.8% was observed and the incidence rate is comparable with reports from Nigeria (13.8%) [11] and in Tanzania (9.7%). [1] High percentage more than these was observed in other African countries, 24% in Zambia, [13] 45.4% in Uganda [14] and 52% in Ghana. [15] Rotavirus incidence was higher in male than females, 6 out of 45(54.9%) male and 2 out of 37(45.1%) female pediatric patients were found to be rotavirus antigen positive which is in agreement with other studies. [3,11] But it is not clear whether this is due to a greater susceptibility to rotavirus exposure in boys or a greater likelihood of parents of affected boys seeking medical care. The main reason for the high rate may be due to the fact that most of the surveillance study was conducted during the peak of rotavirus activity as done in Ethiopia. [16]

Most of the infected children in the current study were between 9-22 month which is similar to the findings of other studies from African countries, Nigeria, [11] Ethiopia, [10] Republic of Ivary Coast [9] and from Asian countries like India. [17] Rotaviruses detected in this study from different age group were group A serotype. Elhag et al. [18] from Khartoum, Sudan, found that 95.2% type A which were further characterized by RT-PCR. Group A rotaviruses, as predominant and result in severe diarrheal diseases in infants and young children, was also reported in Colombia. [19] and in a relief camp in India [20] and in Tanzania. [21]

Surveillance period between March 2013 and March 2014 in Eritrea revealed a detection rate of 35.29% in Orotta hospital before the introduction of vaccine. High prevalence was also observed in April in Japan [22] and during cold season from November to January in China. [23] The difference between the studies can possibly be due to their respective durations, as our study was done only for four months while most of other studies covered all the seasons of the year and spanned for several months. We cannot estimate an annual prevalence based on short studies.

Comparison of the clinical characteristics and severity of the acute gastroenteritis among the rotavirus-positive patients indicated that vomiting, fever and dehydration were more frequently observed among diarrheal children with rotavirus, as reported in the other countries like Guinea-Bissau [24] and Iran. [25] Positive cases had watery diarrhea with greenish appearance of the stool which is predictive of rotavirus infection. In our study vomiting appeared to be more common lab finding as reported in other studies [10,18,26] in which children with

rotavirus vomiting (with mean 3), fever alone, was seen less frequent 6(7.3%) than those with rotavirus infection. Rotavirus was also significantly associated with vomiting, coryza, fever and severe dehydration which are similar to data from studies in Indian sub-continent. [17] Vomiting followed by fever appeared to be more (62.5%) in children with rotavirus diarrhea in this study; this is also correlated to study conducted in Jimma hospital south west Ethiopia by Al-Badani et al. [26] who found vomiting and fever in 51.2% rotavirus positive cases. The frequent association of fever and vomiting and the significant association of vomiting with rotavirus diarrhea are not conclusive in the clinical diagnosis of rotavirus infection, because such symptoms are constitutional and present with other causes of diarrhea. Nonetheless, the frequent vomiting with diarrhea leads to the risk of dehydration in rotavirus infection more than other diarrheal infections and therefore increases the need for hospitalization. [26]

The association of risk factors such as drinking water source, co-morbidity, hand washing and nature of defecation when assessed with rotavirus infection there is no statistically significant association with a p-value of greater than 0.05. Similar results were reported in Yemen. [27] Also it was reported in England that no increased risk of rotavirus infection from contact with pets or other animals. [28] While there is substantial evidence that some rotavirus strains circulating in human populations may have originated from animal viruses, particularly in low income countries, it is likely that zoonotic transmission is rare, with subsequent spread of these viruses in humans occurring only after reassortment with a human rotavirus. A review of several studies in Bangladesh concluded that breastfeeding is a minor contributor to prevention of rotavirus diarrhea. [29] However, exclusive breastfeeding, particularly in infants, has been shown to offer protection against rotavirus. [30-32] Bottle feeding independently associated with rotavirus diarrhea was reported in children with watery diarrhea at a tertiary hospital in Indian air forces. [17] Due to the small numbers of exclusively breastfed children in our study we could not adequately examine the relationship between exclusive breastfeeding and rotavirus diarrhea.

Demographic characteristics of children and mother were cross tabulated and chi-square test was done. The association of risk factors with ELISA positive results in relation to sex indicated that there is no statistically significant association with a (*p-value*=0.205). This finding is in agreement with that of the study by Bizuneh et al. [10] from Ethiopia and in contrast to Juniad et al. [11] from Nigeria who found significant association in rotavirus excretion in males than females (*p-value*=0.015). Regarding area of residence, 23.1% of rotavirus cases were from rural area and 76.9% were from urban area, which is comparable to a study by Al-Badani et al. [26] from Yemen, who reported that 36.3% were urban dwellers and 56.7% were from rural area; only 7.0% of rotavirus infections were from the semi urban area, However, this difference was not statistically significant (*P* = 0.48). In this also the difference was not statistically significant (*p-value*= 0.059). Among the aforementioned socio-demographic characteristics of the mothers/caretakers no statistical differences were found pertaining to the presence of rotavirus infection in their children. This finding is similar to that of other studies from Ethiopia and Nigeria. [10,11] Nevertheless, there was no significant difference in rotavirus infection among all groups (*p-value* >0.05).

The latrine in home was not significantly associated with rotavirus infection in our study (*p-value*= 0.68). In contrast latrine usage in home was reported significantly associated with rotavirus infection in Yemen. [26] The role of exclusive breastfeeding needs to be explored further in study designed to establish relationship whether exclusive

breastfeeding protects against rotavirus diarrhea. This was in contrary with the study by Kurugol et al. [33] from Turkey who found significant association between rotavirus diarrhea and being not exclusively breast-fed in the first six months of life (*p-value*<0.05) compared with children who were exclusively breast fed for six months or longer. The association between breast-feeding and rotavirus infection may explain the poor personal hygiene practice. This link indicates their method of transmission, that is, the fecal-oral route. While breast-feeding protects against all cause diarrhea in infants, no evidence shows that breast feeding confers specific protection against viral gastrointestinal infection. [34] Several studies demonstrated that breast feeding did not prevent acquisition of rotavirus. [35,36]

CONCLUSION

Our study largely represents the burden of rotavirus diarrhea in hospitalized children. This hospital-based study results however may not be a true reflection of rotavirus burden in the community or outpatient clinics since the study was conducted from March through June during a peak rotavirus season in the study region.

To know the peak incidence of rotavirus infection nationwide study should be initiated. A long term assessment of rotavirus studies are important to have a good picture of the seasonal occurrence of rotavirus for health workers, and it will help to have necessary preparations and take necessary preparation to lower burden of the disease during the peak seasons. Within the limited time period an incidence of 9.8% is very high so vaccine should be implemented continuously in a wide range and in a periodic manner to lower and control the incidence of rotavirus.

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