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RAPID DIAGNOSIS OF VIRAL DIARRHEA IN CHILDREN UNDER 5 YEARS, TO STRENGTHEN THE EXISTING ADD PROGRAM

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ABSTRACT **Background:** Gastroenteritis in childhood is one of the most prevailing cause of morbidity and mortality across the globe. Rotavirus is the most common etiological agent among viruses causing gastroenteritis in children below five years. Adenovirus has been evidenced as the second most common cause of childhood gastroenteritis in certain parts of the world. The present study was conducted to estimate the incidence of Rotavirus and Adenovirus among diarrhoeal cases in children under 5 years of age. **Materials and methods:** A total of 40 children younger than 5 years of age suffering from acute diarrhoea were included in the present study. A total of 40 samples were collected and analyzed for Rotavirus and Adenovirus using commercially available Immunochromatography kit. **Results:** The prevalence of Rotavirus and Adenovirus was found to be 7.5% and 2.5% respectively among children under five years of age with acute diarrhoeal disease. **Conclusion:** The disease burden of Rota viral illness has decreased due to increased coverage of Rotavirus vaccination through inclusion in National immunization schedule and thus effective and efficient vaccination coverage is fundamental rationale in the process of strengthening of Acute Diarrhoeal Disease(ADD) program.

KEYWORDS : Acute diarrhoea, Rotavirus, Adenovirus, Immunochromatography.

INTRODUCTION

Gastroenteritis in the childhood is one of the most common cause of morbidity and mortality across the globe.[1] World Health Organization (WHO) reports that there are 2 billion cases of acute gastroenteritis every year worldwide.[2] The global burden of acute gastroenteritis is immense in the developing countries and especially India accounts for 3.86 lakhs childhood deaths every year.[3] Acute gastroenteritis is caused by bacteria, viruses, parasites, and rarely fungi. The viruses which bring about gastroenteritis in humans include Rotaviruses, Adenoviruses, Caliciviruses, Norwalk viruses and Astroviruses.[4]

The accurate diagnosis of viral gastroenteritis is essential, though it will not influence any initiation of specific antiviral agents, it will definitely reduce the irrational use of antimicrobials.[5] In developing countries like India, there are meager number of studies done on viral gastroenteritis when compared to bacterial gastroenteritis. This could be mainly credited for failure in viral identification & scarcity of viral diagnostic facilities in the developing countries.[6]

Rotavirus is the most common causative agent among viruses causing childhood gastroenteritis. Rotavirus is responsible for around 2 million healthcare admissions and 450,000 deaths among childhood gastroenteritis annually.[7,8] Adenovirus has been evidenced as the second most common cause of childhood gastroenteritis after Rotavirus in certain parts of the world.[9]. There are several studies done on Rotavirus, but role of enteric adenoviruses are less explored and analyzed. This warrants the need of local, regional and national epidemiological data to go over on the most common agents causing childhood gastro-enteritis to aid the clinicians following appropriate treatment protocol, Public health officials to implement control measures and for Researchers to develop suitable vaccines.

The present study was conducted to estimate the incidence of Rotavirus and adenovirus among childhood gastroenteritis and its epidemiological importance in a tertiary care teaching hospital. Enzyme Immunoassays (EI) and polymerase chain reaction (PCR) are commonly used for the diagnosis of Rotavirus and Adenovirus diarrhea from stool samples[10,11]. This study uses a simple and rapid immunochromatography method to detect Rotavirus and Adenovirus antigens from stool samples. The impact of factor, Rotavirus immunization status that can contribute to the prevalence of the disease will also be evaluated and recommendations will be formulated for disease prevention.

Objectives:

- To estimate the incidence of Rotavirus diarrhoea among children under 5 years.
- To estimate the incidence of Adenovirus diarrhoea among children under 5 years.

MATERIALS AND METHODS:

Children below 5 years of age attending Tirunelveli Medical College Hospital, with history of diarrhea, vomiting and fever of less than 3 days were included in this present study. The sample size was 40 and the study period was 3 months. Both out-patients and in-patients were included in the study. Socio demographic details such as age, gender, duration of illness, fever, vomiting, nutritional status, history of antibiotic intake, Rota virus vaccination status were obtained using a detailed structured proforma. Ethical committee approval and informed consent were obtained for the present study.

Specimen collection and processing

About 15-20 ml of stool specimen was collected in a sterile wide mouth container. The stool sample was transported to the laboratory immediately in cold chain maintenance without any delay and stored at -70°C. The specimens were processed in Microbiology laboratory of Tirunelveli Medical College Hospital.

A commercial Rotavirus and Adenovirus antigen detection kit (SD Bioline Rota/Adeno Rapid Test kit, Standard Diagnostics, Korea) was used to detect the presence of Rotavirus and Adenovirus antigen from the stool sample. The sensitivity and specificity of the kit were 97.4% and 100% respectively. This kit was based on immunochromatography principle and had an advantage of detecting Rotavirus and Adenovirus simultaneously with a single kit. The specimen was processed as per kit manufacturer's recommendations.

Antigen extraction from stool was done by first allowing the test device and stool sample to settle to room temperature prior to testing. An assay diluent was transferred into the sample collection tube (provided in the kit) and filled up to the marked line. A small portion of faeces (about 50 mg) was collected with the sample collection swab (provided in the kit)

and it was inserted into the sample collection tube containing diluent and rolled at least 10-times properly to release the stool specimen. The swab was later discarded after squeezing it against the wall of tube.

The test device was removed from the foil pouch and placed on a flat, dry surface. A dropping cap (provided in the kit) was assembled on the sample collection tube. About 4- 5 drops (about 100-125 μl) of the mixture from sample collection tube was added into the sample well of the test device. The results were interpreted within 20 minutes.

There are three different dark red coloured bands that can be seen in the test device depending on the test result. Starting from the side of the sample well, the first band was T1 (Adenovirus), followed by T2 (Rotavirus) and C (Control). The presence of only control band (C) within the result window indicated negative result.

The presence of two colour bands (T1 and C band) within the result window indicated Adenovirus positive. The presence of two colour bands (T2 and C) within the result window indicated Rotavirus positive. The presence of three colour bands (T1, T2 and C) within the result window indicated both Adenovirus and Rotavirus positive. The approximate duration for entire procedure was 30 minutes.

RESULTS:

This study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli for three months period from June 2021 to August 2021. Stool samples were collected from a total of 40 children who had diarrhoea.

All the samples were subjected to Rotavirus and Adenovirus antigen detection by rapid immunochromatography – RICT (Rota/Adeno Rapid).

Table 1: Demographic characteristics of children under 5 years of age who presented with acute diarrhea

Age in months	Male		Female	
	Total No (Out of 23)	%	Total No (Out of 17)	%
0-6	1	4.3	1	5.8
7-12	12	52.2	10	58.9
13-24	6	26.1	4	23.5
25-60	4	17.4	2	11.8
Total	23	100	17	100

Out of 40 samples collected from childhood diarrhoeal cases, 2 cases (5%) were in the age group of 0-6months, 22 cases (55%) belonged to the age group of 7-12months, followed by 10 cases (25%) in the age group of 13-24 months, 6 cases (15%) in the age group of 25- 60 months. Majority of the diarrhoea case belonged to the age group of 7-24months.**(Table-1)**

Out of the 40 cases, 23(57.5) were male children and 17(42.5) were female. There is a slight male predominance.**(Table-2)**

Table 2: Distribution & Percentage of Rotavirus & Adenovirus Positivity

Test Results	No	%
Rotavirus positive	3	7.5
Adenovirus positive	1	2.5
Negative for either Rotavirus & Adenovirus	36	90
Total	40	100

Among the Rotavirus positive cases, 2(66.7%) belonged to 7-12 months age group, 1(33.3%) to 13-24 months, showing an increased frequency of rotavirus in the age group of 7-12months.

Adenovirus positivity was seen in 7-12 months age group.**(Figure 1)**
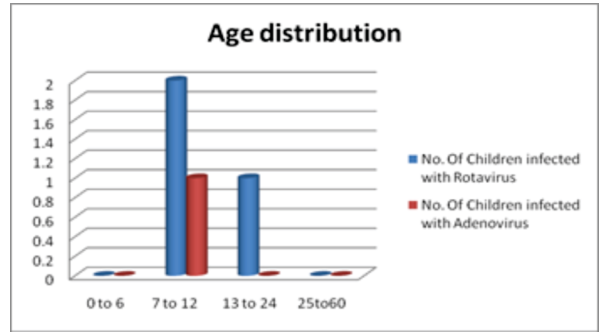

Figure 1 Age-wise distribution of children infected with Rotavirus and or Adenovirus

Out of the 3 Rotavirus infected children, 2(66.7%) were male children and 1(33.3%) were female children. There is a slight male predominance. The only Adenovirus infected child was female.**(Table-3)**

Table 3: Gender distribution of children infected with Rotavirus and or Adenovirus

Gender	No. Of Children infected with Rotavirus	No. Of Children infected with Adenovirus
Male	2(66.7%)	0
Female	1(33.3%)	1(100%)
Total	3(100 %)	1(100%)

All the Rotavirus positive children 3 (100%) had associated fever and vomiting along with diarrhoea. Adenovirus positive children 1(100%) had associated fever along with diarrhoea. Regarding the hydration status of the Rotavirus infected children, 2(66.7%) presented with severe dehydration and 1(33.3%) had signs of moderate dehydration . Adenovirus infected child 1 (100%) had signs of moderate dehydration. **(Table-4)**

Table 4: Dehydration status of children infected with Rotavirus and or Adenovirus

Dehydration	No. Of Rotavirus positive children	No. Of Adenovirus positive children
Nil	0	0
Mild	0	0
Moderate	01	01
Severe	02	0
Total	03	01

Among the Rotavirus as well as Adenovirus infected children, none of the children were adequately breast fed, showing that there is higher occurrence of diarrhoea in children who were not adequately breast fed. Out of the 40 cases, 35(87.5%) were fully immunized, 4(10%) were partially immunized and 1(2.5%) was non-immunized for Rotavirus. Rotavirus infection was not seen among fully immunized children. .**(Table-5)**

Table 5: Vaccination status of children infected with acute diarrhoea

Vaccination status	Total No	Percentage
Fully immunized for Rotavirus	35	87.5
Partially immunized for Rotavirus	4	10
Non-immunized for Rotavirus	1	2.5
Total	40	100

DISCUSSION:

Acute infectious diarrhoea is one of the major causes of mortality among children worldwide, particularly in developing countries like India leading to economic losses which is a major public issue. It is necessary to determine the main causative organisms for the purpose of diagnosis, treatment and follow up and containment of outbreak of these infections. Rotavirus and adenovirus are recognized as the

commonest etiological agents causing acute diarrhoea in children. This study was conducted to determine Rotavirus and Adenovirus incidence in children under 5 years of age with acute diarrhoeal disease.

This study reveals majority of the children with acute diarrhoea (55%) belonged to the age group of 7-12months of age followed by 13-24 months.(Table 1). This correlates with the study by Mohammad Youssef et al12. Only 4.3% belonged to the age group of 0- 6 months. This could be due to protection of infants from pathogens by maternal antibodies. And there was a slight male predominance (57.5 %) but statistically not significant. This could be due to more susceptibility of male children to infection or higher likelihood of their being brought for medical care as stated by WHO scientific working group.[13]

In the present study, incidence of Rotavirus was found to be 7.5% (Table 2) which is lower when compared to other studies in India where they have reported Rotavirus detection rate of 23.5% to 49.4%[14]Decrease in incidence of Rotavirus in our study could be due to inclusion of Rotavirus vaccine in our routine immunization schedule since 2016. The decrease in incidence could also be due to season of the period the study was carried out whereas increased incidence was seen in winter months as stated by other studies.[9]

Furthermore we have used rapid immunochromatography method for detection of these viruses which would have influenced the rate of infection but we have not compared this method with other methods like Enzyme Linked Immunosorbent Assay (ELISA) or Polymerase Chain Reaction(PCR). Further rapid immunochromatography kit used was economical and practical compared to other methods.[16]

In our study, incidence of Adenovirus was found to be 2.5% which is comparable to the Adenovirus positivity of 2-31% reported in developing countries as stated by Wilhelmi et al.[17]Co infection of more than one viral agents are not uncommon in gastroenteritis and certain studies have reported co-infection of 1.3% to 8%[18]In the present study, there was no co-infection of Rotavirus and Adenovirus.

In this present study, we observed an increased frequency (66.7%) of Rotavirus in the age group of 7-12 months and Adenovirus positivity in 7-12 months age group but it was statistically not significant. WHO scientific group has too observed a peak incidence of Rotavirus infection at 9-12 months age group.[13]There was slight male predominance among Rotavirus infected children but statistically not significant which correlates with the study done by Motamedifar et al.[15]

In the present study, all the Rotavirus positive children 3(100%) had associated fever and vomiting along with diarrhoea which was statistically significant.(P<0.05). This correlates with the study done by Arvind et al where they have observed diarrhoea, fever and vomiting as common clinical presentation for Rotavirus infection.[18]

This study reveals that Rotavirus infected children presented 2(66.7%) with severe dehydration which correlates with several studies in which more than 80% children had presented with severe dehydration thus indicating the importance of early diagnosis of infection as well as immunization.[16]

In our study, children infected with Rotavirus as well as Adenovirus were inadequately breast fed when compared with other children who presented with acute diarrhoea. Still the contribution of breast feeding could not be determined since most of the children in the study were above six months of age and on complimentary feeding,

In the present study, Rotavirus infection was not recognized among fully immunized children which was statistically significant (P<0.05) and thus proves effectiveness of Rotavirus vaccine included in routine immunization schedule in prevention of Rotavirus infection.[19]

CONCLUSION:

Incidence of Rotavirus and Adenovirus was found to be 7.5% and 2.5% respectively among children with acute diarrhoeal disease. Majority of children infected with Rotavirus were in the age group of 7 to 12 months, among which most of them presented with severe dehydration. Immunochromatography is found to be the rapid and cost effective method, can be used to detect viral infections in stool samples even in peripheral care settings thus helping in early diagnosis and management, even at the point of care and containment of diarrhoeal outbreaks at field level.The disease burden of Rota viral illness has decreased due to increased coverage of Rotavirus vaccination through inclusion in National immunization schedule and thus vaccination coverage is the main key in the process of strengthening of Acute Diarrhoeal Disease(ADD) program.

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