

Prevalence of Rotavirus, Adenovirus, and Rotavirus denovirus Coinfection among children less than 5 years in Kirkuk city

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Abstract

Infectious gastroenteritis is one of most important causes of morbidity and mortality in children < 5 years age. Rotavirus and enteric adenoviruses are the most important agents of infectious gastroenteritis. Little is known about the epidemiology of rotavirus and adenovirus gastroenteritis in our region. This study was aimed to determine the frequency of rotavirus and enteric adenovirus gastroenteritis in pediatric patients admitted to Kirkuk pediatric hospital. Fresh stool specimens of 177 children < 5 years age patients admitted to Kirkuk pediatric hospital during Jan-May 2012 due to acute gastroenteritis were studied. Rotavirus and enteric adenovirus antigens were investigated in the fresh stool specimens with the qualitative Latex for Rotavirus and **Rota-Adeno immunochromatographic test** for both Rotavirus and Adenovirus. Using latex agglutination viral antigens were determined In 30 (16.9%) of the fresh stool specimens. Of these 30 specimens, rotavirus was established in 10 (33.3%), enteric adenovirus in 2 (6.6%), both rotavirus and enteric adenovirus in 10 (33.3%) ,and 8 (26.6%) were negative for both Rota-Adenovirus using immunochromatographic test.. Rotavirus antigen was detected in 36.6 % of patients infants between 7 and 12 months of age were most frequently infected

Introduction :

The World Health Organization defined diarrhea as the voiding of more than two unformed watery stools in any 24 hour period, or any voiding of watery stools if accompanied by fever, abdominal pain and/or vomiting. [1]. Diarrheal disease is one of the leading causes of illness in young children in developing countries. [2,3,4]. Diarrheal disease is responsible for 25-30% of deaths among children younger than five years of age.[2].The public health significance of Diarrheal disease cannot be overemphasised. Diarrhea diseases are the cause of almost three million deaths annually mainly among children younger than five years of age. Although extensive investigation of Diarrhea have not been reported, the Diarrhea – specific mortality in children younger than five years of age in Africa has been estimated at about 106 per 1000.[4].After pneumonia, diarrhea is the second commonest cause of infection-related death in under five years children of Bangladesh. [5].Acute gastroenteritis continues to be a significant cause of morbidity and mortality worldwide. An estimated of more than 700 million cases of acute diarrhea occurred in children under five years of age every year. The mortality rate has been estimated to be 3-5 million cases per year, the majority of these which occur in developing countries. [6] .Viral gastroenteritis is a common infection in all age groups worldwide. Usually four types of viruses are the main causes of this infection in all over the world: Rotaviruses, caliciviruses, astroviruses and adenoviruses types 40 and 41. [7].Rotavirus remains the commonest cause of severe dehydrating diarrhea among children worldwide. Children in developing countries die more because of several factors including poorer access to hydration therapy. The reported

prevalence of rotavirus diarrhea from global surveillance networks and hospital based studies ranges from 6% to 56%.[8].Rotavirus was coined from the Latin word (rota - meaning wheel), and is given because the viruses have a distinct wheel like shape.[9].Rotaviruses are non-enveloped, double-stranded RNA viruses (dsRNA) comprising a genus within the family *Reoviridae*. The 11 segments of dsRNA genome code for 6 structural and 6 non-structural proteins. Seven groups of rotavirus (A–G) have been described, with group A rotaviruses being the leading cause of severe dehydrating gastroenteritis in children < 5 years of age worldwide.[10].It is not possible to diagnose rotavirus infection by clinical presentation because the clinical features of rotavirus gastroenteritis do not differ from those of gastroenteritis caused by other pathogens. Confirmation of rotavirus infection by laboratory testing is necessary for reliable rotavirus surveillance and can be useful in clinical settings to avoid inappropriate use of antimicrobial therapy. Rotavirus is shed in high concentration in the stool of children with gastroenteritis and a fecal specimen is the preferred specimen for diagnosis.[8].The main symptoms of rotavirus gastroenteritis (RVGE) are fever, abdominal pain, lethargy, diarrhea and vomiting that may lead to hypovolemic shock and dehydration . Severe cases may lead to death . The World Health Organization (WHO) estimated that 527,000 children under the age of five years were died of rotavirus disease each year. Children in the poorest countries account for 82% of rotavirus deaths.[3]. Rotaviruses and enteric adenovirus types 40 and 41 are the most prevalent viruses in the aetiology of childhood gastroenteritis in developed countries.[11]. After

rotavirus infection, adenoviruses are the second cause of severe and acute gastroenteritis in children under five years. Adenoviruses are non-enveloped, icosahedral with linear dsDNA genome. Based on hemagglutination (HI) and neutralization tests, six groups (A-F) and 51 serotypes of adenoviruses have been determined. Group F of adenovirus including types 40 and 41 are the main etiologies of 1-20% of acute gastroenteritis and 50% of all adenoviruses found in stool specimens are types 40 and 41 [12]. The ability of adenoviruses to cause acute infection in both the gastrointestinal tract and the respiratory system, makes them quite important. It has been reported that they cause about 5% of all infectious diseases in infants and nearly 3% in children from 2-4 yr old. Other studies revealed that adenovirus types 40 and 41 are the sole adenoviruses that have been found associated with gastroenteritis in infants and young children, and may be the second major causative agent of gastroenteritis after rotaviruses. [7]. Prevention can be achieved by vaccination; however, not in the first 2 months. Breastfeeding is considered to protect against infections. However, epidemiological findings on the protective effect of breastfeeding are inconsistent. Some studies did not show the effect of breastfeeding against rotavirus infection and others found that breastfeeding may alleviate the severity of symptoms. However, a majority of investigations reported a protective effect against RV infections [13].

Material and Methods:

From Jan to May of 2012, fecal specimens were collected from children with acute gastroenteritis less than 5 years old. An informed consent was obtained from the parent (either mother or father) of the child before inclusion in the study. The parents were informed about the procedure, making certain that he/she was fully competent of understanding the procedure carried out to obtain the specimen from the child. From each case at least 5ml or 5g of faeces was collected in a clean, sterile container, and labelled accordingly. All faecal samples were tested with commercially available Rotavirus latex agglutination kit (Rotavirus latex test kit; Plasmatec laboratory products, UK). The tests were performed following manufacturer's instructions. Briefly, faecal samples were prepared by adding 0.2ml/0.2g of faeces to 2.0 ml of dilution buffer in centrifuge tubes. After mixing by vortex, tubes were incubated at room temperature for 5-10 minutes. Samples were then centrifuged at 800 g for 10 minutes and supernatant was taken. One drop of supernatant from each sample was mixed with one drop of rotavirus latex reagent and observed for agglutination after few seconds. A clear

agglutination was considered positive for rotavirus. Positive and negative controls were performed with each batch of the tests. All positive samples and ten negative samples for rotavirus by latex agglutination were also tested by (CERTEST ROTAVIRUS-ADENOVIRUS: SPAIN) following manufacturer's instructions. Briefly, fresh aliquot processing as indicated above was used to perform the Rota - Adeno immunochromatographic test. 100 µl of processed stool samples was dropped into immunochromatographic well, after 10 min incubation at room temperature, the results of test were read by observation of colored indicator:

-Negative: Only one GREEN band appears across the central well in the site marked with the letter C (control line).

-Rotavirus positive: In addition to the GREEN control band, a RED band (Rotavirus test line) also appears in the site marked with the letter T (Result line).

-Adenovirus positive: In addition to the GREEN control band, a Blue band (Adenovirus test line) also appears in the site marked with the letter T (Result line).

.Rotavirus –Adenovirus Positive: All the lines described above (a GREEN control band in the control region, a RED and BLUE band in the result region) could appear at the same time during the test performance due to a simultaneous infection of Rotavirus and Adenovirus.

Invalid: A total absence of the control coloured band GREEN regardless the appearance or not of the result lines (RED/BLUE).

Results:

Stool samples were examined respectively, these specimens from children, less than 5 years of age, referred for fever, abdominal pain, vomiting and/or acute diarrhea. Rotavirus antigens were detected from fecal samples by latex agglutination (LA). Among the children with Diarrhea, Rotavirus was identified in 30 (16.9%) of the total cases studied. Out of these 30 patients infected with rotavirus, 19 (63.3%) were males and 11 (36.3%) females, also, Rotavirus antigen was detected in 36.6 % of Infants between 7 and 12 months of age who were most frequently affected. Age distribution of children with less than 5 years and those excreting rotavirus antigen RVA detected by latex agglutination are shown in table 1.

In this study rotavirus was detected using latex agglutination technique. Thirty positive and 10 negative samples by latex agglutination were also tested using Rota-Adeno immunochromatographic test. Results of 10 negative samples by both methods were similar

while, Out of 30 stool specimens tested by using Rota-Adeno immunochromatographic test, 10 (33.3%) were positive for rotaviruses Moreover, adenovirus infections accounted for 2 (6.6%).

Mixed infections were observed in 10(33.3%), negative for both Rota-Adenovirus observed in 8 (26.6 %) cases (Table 2).

Table 1: Age distribution of children those excreting Rotavirus antigen RVA detected by latex agglutination.

(month)	No.	(%)
≤ 6	6	20
7-12	11	36.6
13-18	4	13.3
19-24	3	10
25-30	1	3.3
31-36	0	0
37-60	5	16.6

Table 2 : rotavirus and adenovirus in stool samples of 30 children (those excreting 2:Occurance of Rotavirus antigen RVA detected by latex agglutination.) using(CERTEST ROTAVIRUS-ADENOVIRUS immunochromatographic:SPAIN)

Virus	No.	(%)
Rotavirus only	10	(33.3)
Adenovirus only	2	(6.6)
Rota-Adenovirus	10	(33.3)
Negative	8	(26.8)
Total	30	(100)

Discussion:

Rotavirus is endemic worldwide; the infection is associated with high rates of morbidity throughout the world and high rates of mortality in developing countries. In the United states, rotavirus infection are responsible for approximately 3 million cases of diarrhea and an estimated 55,000 hospitalization for diarrhea and dehydration in children under 5 years old each year, although these infections cause relatively few deaths in united States. However, in developing countries, rotavirus gastroenteritis account for more than 800,000 childhood deaths per year due to poor nutrition and health care. [9].Rotavirus infection is also known as infantile diarrhea (since almost all children are infected in thier first few years of life when they are especially at risk for the disease) or winter diarrhea (because in the United states, the disease occurs more often during winter and spring with the most activity occurring from November to May). It is a highly contagious and unpredictable disease and may lead to severe dehydration (loss of body fluid) and even death. [3].The routine diagnosis of rotavirus is based on rapid detection of group A antigen in faeces, generally by latex agglutination or enzyme immunoassay.[14] In this study, viral antigens were determined by latex agglutination in 30 (16.9%) of 177 fresh stool specimens. Of these 30 specimens, rotavirus was established in 10

(33.3%) , adenovirus in 2 (6.6%) , both rotavirus and enteric adenovirus in 10 (33.3%) by using (CERTEST ROTAVIRUS-ADENOVIRUS immunochromatographic: SPAIN). Study by Alicem Tekin in Turkey In 170 (18.1%) of fresh stool specimens, viral antigens were determined. Of these 170 specimens, rotavirus was established in 157 (16.7%), enteric adenovirus in 9 (1.0%), both rotavirus and enteric adenovirus were in 4 (0.4%). The viral antigen positive cases were most frequently seen during autumn and winter months in children between 5-24 months of age[15]. Other study by Zamani, H. *et al.* mentioned that Rotavirus antigen was detected in(15.3%) of patients. Infants between 6 and 12 months of age were most frequently affected. Rotavirus infection was significantly less frequent in breast-fed than among bottle-fed babies. Watery diarrhea was present in 68.5% of children. Detection rate was highest in the spring and lowest in summer. Rotavirus can be regarded as a major etiologic agent of acute diarrhea in infants and children up to 5-years-old in Iran, immunization at birth may protect the children before their first symptomatic infection.[16] Other study in Makkah included cases of acute Diarrhea with rotavirus infection in children below 5 years of age; 62% of the cases were under 2 years of age.[14]. In our study 81% of the cases were under 2 years of age .Similar studies have been conducted both in developing

and developed world. The results of this study suggest that rotavirus infection is an important pathogen of acute Diarrhea in infants and children, as the patients were from major government hospitals referred from the entire community of Makkah[14]. Rotavirus had multiple strong positive associations odds ratios (ORs); (ORs >2) with the other gastrointestinal pathogens detected in the fecal samples, even after considering age and season. The positive association of rotavirus, an RNA virus that affects cells covered in microvilli, and adenovirus, a DNA virus that affects cells that

are dividing to generate new cells with microvilli, may represent an interaction between pathogens to cause more severe diarrhea. In a previous study, found that among children in Korea with virus-caused diarrhea, adenoviruses occurred preferentially in the presence of rotavirus and that rotavirus and norovirus, although most common, occurred in polymicrobial infections in proportion to their numbers in the samples.[17]. Rotavirus is most important agent in acute gastroenteritis of childhood in our region too. It should be investigated routinely in fresh stool specimens.

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انتشار الإصابة المشتركة لفايروس الروتا مع الالدينو فايروس لدى الاطفال اقل من 5 سنوات عمرا في كركوك

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الملخص

ان النزلات المعوية تعد من المسببات المهمة للوفيات لدى الاطفال اقل من 5 سنوات عمرا ويعد فايروس الروتا والالدينوفايروس من العوامل المهمة في هذا المجال وهناك القليل من الدراسات الوبائية لكلا الفيروسين اجريت هذه الدراسة لغرض التعرف على مدى انتشار الفيروسين عند الاطفال الاقل من خمس سنوات المراجعين لمستشفى الاطفال في كركوك للفترة من كانون الثاني لغاية شهر ايار لسنة 2012 حيث تم اجراء الفحص على 177 عينة براز المستحصلة من الاطفال دون 5 سنوات للبحث عن فايروس الروتا بطريقة فحص اللاتكس وكانت النتيجة ان 30(16.9%) عينة اظهرت النتيجة الموجبة لهذا الفحص وتم اعادة فحص العينات الموجبة بفحص الكروماتوكرافي المناعي وكانت نتيجة الروتافايروس لوحده 10 (33.3 %) حالات والالدينوفايروس لوحده 2(6.6%) حالة اما الإصابة المشتركة بكلا الفيروسين فكانت 10(33.3%) وكما اظهرت الدراسة ان 8 (26.6%) كانت سلبية النتيجة لكلا الفيروسين. اظهرت هذه الدراسة ان اغلب الاصابات بهذه الفايروسات تقع ضمن الفئة العمرية من 7 – 12 شهر .