Rotavirus, Enteropathogenic Escherichia *coli* (EPEC) and Parasitic Etiology of Pediatric Diarrhea in Kirkuk city

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Abstract:

The present study was done to determine the etiology of gastrointestinal pathogens of acute diarrhea in children under 5 years of age in Kirkuk city.Various common enteropathogens (viral, bacterial and parasites) associated with diarrhea were investigated in children less than 5 years of age admitted to Kirkuk Pediatric Hospital. The identified enteropathogens was as follows: *Rotavirus* 31 (14.9%),Enteropathogenic *Escherichia coli* (EPEC)26(12.5 %),*Entamoeba histolytica* 30(14.4%), and *Giardia lamblia* 2 (0.96%) .Mixed infection between these enteropathogens also observed .*Rotavirus* investigated by latex and the positive results confermed by using ELISA.

Introduction

Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries [1] . Acute gastroenteritis is one of the leading causes of illness and death in infants and children throughout the world, especially in developing countries. An estimated 2.5 million gastroenteritis deaths occur each year in children less than 5 years of age [2]. Diarrheal infections may be caused by bacterial, viral, or parasitic pathogens. Some cases have a single defined cause,others do not have any defined cause, and a substantial number (one third) are caused by multiple pathogens , because each known diarrheal pathogen fulfills Koch's postulates and is capable of being the sole etiologic agent causing disease. Multiple pathogens are not essential for causing disease.How additional pathogens cause and contribute to the disease process is unknown. The source of the multiple pathogens in a patient could simply result from multiple pathogens in an urban environment of crowded impoverished conditions. If the various pathogens occurred independently in cases of disease, then each pathogen in a polymicrobial infection would be expected to occur in proportion to its presence in all patients with severe diarrhea[3] . 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%.[4].Rotavirus was coined from the Latin word (rota - meaning wheel), and is given because the viruses have a distinct wheel like shape.[5].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.[6].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.[4]. 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) estimates that 527,000 children under the age of five years die of rotavirus disease each year . Children in the poorest countries account for 82% of rotavirus deaths [7] .There is increasing evidence that polymicrobial infections in which microorganisms present specific pathologies may act in a synergistic or inhibitory fashion, impacting on either tissue, host cell destruction or the maintenance of health, among these, bacterial–bacterial, viral–viral, parasitic–parasitic- or viral–bacterial interactions have been well documented. For example, enteroaggregative *Escherichia coli* (EAEC) or enteropathogenic *E. coli* (EPEC) may provide a second pathogen with better conditions to invade the intestine and cause diarrhoe[8] .Enzyme immunoassays (EIAs) have replaced electron micoscropy (EM) as the standard method for the detection of rotaviruses in stool samples in the 1980s [9].

Materials and Methods

From Jan. to May. 2012, a total of 208 stool samples were collected from children < 5 years age admitted to the Kirkuk pediatric hospital, all of them had history of acute diarrhea and few of them presented with fever and vomiting . Macroscopic examination of the stool samples were done such as consistency, color, presence of mucus and blood. All samples were examined

microscopically for the presence of pus cells, (R B Cs) and ova/cysts

Detection of Rotavirus

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 or 0.2g of faeces to 2.0 ml of dilution buffer in centrifuge tubes. After thorough mixing by vortex, tubes were incubated at room temperature for 5-10 minutes. Samples then centrifuged at 800 g for 10 minutes and supernatant was separated. 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 according to latex agglutination were also tested by Enzyme-linked Immuno-sorbent Assay (ELISA) using rotavirus kit (Bio-pharm: Germany) following manufacturer's instructions.

Detection of EPEC

Stool samples collecteded in sterile containers were processed for bacterial enteropathogens using recommended standard procedure. All the enteropathogenic *Escherichia coli* strains isolated from diarrheal stool samples were confirmed by agglutination with specific antisera (*Escherichia coli* antisera Plasmatic laboratory product: UK). Liquid stable antisera for the determination of O antigens for the serological identification of pathogenic *Escherichia coli*.

Procedure of slide agglutination of heat treated bacteria:

1- Dense suspension of organim to be tested was prepared by taking 3 to 5 colonies of organisms from a fresh culture on nutrient agar and placed in 3 ml of 0. 85% saline .The suspension was heated to 100 C◦ for 60 min and centrifuged at 900 g for 20 min. the supernatant then removed and 0.5 ml of 0. 85% saline added to resuspend the precipitate. The suspension was mixed until be homogeneous and used as antigenic suspension for – O – antigen groping.

2- Two loopfuls or drops (5-10ul) of antigenic suspension was placed onto a carefully cleaned microscope slide. The slide then partitioned using a chinagraph pencil.

3- A drop of polyvalent antiserum was placed onto one of the drops of emulsified isolate and on the other a drop of saline was placed as a control.

Note: antiserum dropper bottle do not allowed to be contaminated with organism.

4- The reagents was mixed by tilting the slide back and forth for 60 seconds while viewing it under light against a dark background.

5- Clumping or agglutination was distncted within this period, without clumping in the saline control (autoagglutination), regarded as a positive result. Weak agglutination regarded as negative.

Parasite detection

A smear of feces in 0.9% saline was examined microscopically for the presence of leukocytes, (R B Cs), *E. histolytica* and *Giardia lamblia* cysts and trophozoites. This was carried out by wet mount method by mixing a flecks of stool from the tip of wooden applicator with one drop of physiological saline and another with drop of lugols iodine solution on a microscopically slide .The slide was first examined under low power (100X) then under high power (400X)to identify protozoal cyct and trophozoites of *E. histolytica* and *Giardia lamblia.*

RESULTS

During the study period a total of 208 children < 5 years of age were investigated for detection of the etiological agents of diarrhea including viral ,bacterial , protozoal and yeasts. From the total children, 31(14.9 %) were infected by *rotavirus*. among these cases 12(38.7%) were femals while, 19(61.2%) were males (table1). Cases which were positive with latex agglutination were also tested using ELISA. Only 24/31(77.4%) cases were positive with ELISA technique (table2). Incidence of *rotavirus* diarrhea in relation to residence also observed since 113(54.3%) of patients were living in urban area, while 95(45.6%) patients were living in rural areas (table 3). In addition to *rotavirus* the other enteropathogens also isolated these include 26(12.5%) cases were infected with EPEC .Protozoal infection appeared in 32(15.3%)cases ,these were *E. histolytica, 30 (14.4%),Giardia lamblia* 2(0.96%). Also monillia observed in 67(32.2%) cases (table 4) .In this study mixed infections were observed :Rotavirus and EPEC in 3 (1.4%), rotavirus and *E. histolytica* in 5(2.4%), rotavirus and monillia in 11(5.2%) , (table 5).

Table1: Detection of rotavirus by latex agglutination according to sex

Sex	Tested samples No. (%)	RV positive No. (%)	RV Negative No. (%)
Male	122(58.6)	19(61.2)	103(58.1)
Female	86(41.3)	12(38.7)	74(41.8)
Total	208 (100)	31(100)	177(100)

Table2 :Detection of Rotavirus by latex and the positive result compared by ELISA techniqu

Results	No.Of cases by ELISA(%)	No.Of cases by Latex agglutination (%)
Posive results(%)	24(77,4)	31(14.9)
Negative results(%)	7(22.5)	177(85.0)
Total	31(100)	208(100%)

Table(3) :Distribution of rotavirus diarrhea according to residency.

Residence	No.of cases	Positive cases No. (%)	Negative cases No. (%)
Urban	113	18(15.9)	95(84.0)
Rural	95	13(13.6)	82(86.3)
Total	208	31(14.9)	177(85.0)

Table:(4).The etiological agents identified in stool specimens .

Etiology	Positive cases No. (%)	Negative cases No. (%)	Total No.(%)
Rotavirus	31(14.9)	177(85.0)	208(100)
EPEC	26(12.5)	168(87.5)	208(100)
Giardia lamblia	2(0.96)	206(99.0)	208(100)
E. histolytica	30(14.4)	178(85.5)	208(100)
C.albicans	22(10.5)	186(89.4)	208(100)

Table (5): Mixed infection detected in the studied cases .

Microorganisms involved in mixed infections	No. of cases	percentage (%)
E. histolytica + *Rotavirus*	5	*22.7*
E. histolytica + EPEC	3	*13.6*
Rotavirus + EPEC (II)	3	*13.6*
Rotavirus + Monellia	11	*50.0*
Total	22	*100*

Discussion

The routine diagnosis of rotavirus is based on rapid detection of group A antigen in faeces, generally by latex agglutination or enzyme immunoassay[10]. The findings of this study showed that rotavirus is most common among children .This is consistent with a number of studies carried out in Saudi Arabia and other developing countries, Similarly rotavirus was also found to be an important causative agent of viral gastroenteritis among children in some physician-based studies carried out in the community of France and Netherlands. A study by Mohammad Yousif *et al*. [1] in Jordan mentioned multiple pathogens were identified in 41 (15.5%) stools, and none were identified in 89 (33.6%). Among the 36 patients who had received antibiotic therapy before hospitalization, bacterial enteropathogens were isolated from 11 (30.6%). Rotavirus was the most common enteropathogen, detected in 86 (32.5%) patients, followed by EPEC in 34 (12.8%) patients.This result similar to our result regarding rotavirus and EPEC.Other study by Brianna Lindsay *et al*. [3] in india mentioned that Polymicrobial infections frequently contained *Vibrio cholerae* O1 and rotavirus. Study by Lipson and Zelinesky–Papez. [11] mentioned that Eighty-two stool specimens obtained from children with gastrointestinal disease were tested for the presence of antigen to rotavirus by latex agglutination (LA) (Virogen (VR), Meritec (MER), Wellcome (WEL), Slidex Rotatest (SRT), and enzyme-linked immunosorbent assays (Rotaclone [TRC], Rotazyme II [RTZ], Pathfinder [PTH]). Confirmatory testing was performed by isolation of rotavirus from stool specimens with the use of a shell vial centrifugation, antigen-detection tissue culture amplification method. The sensitivities and negative predictive values of VR, MER, WEL, SRT, TRC, RTZ, and PTH tests were 85, 89, 95, 91, 98, and 100%, respectively. Each test demonstrated 100% specificity and positive predictive values except the SRT, which showed a specificity of 95%. The WEL LA test may be used as a preliminary rapid screening assay following a stat request. The Kallestad PTH ELISA, however, was determined to be the rotavirus antigen detection kit of choice for routine laboratory diagnostic testing[11] . In our study ELISA technique was more sensitive 77.4% than Latex agglutination. It is concluded that strongly positive reactions found by Latex agglutination may be regarded as true positive reactions, whereas samples producing weakly positive and/or negative reactions should be retested in a more specific and sensitive assay, such as enzyme linked immunosorbent-assay (ELISA).It is a proven fact that ELISA is more sensitive than latex agglutination test. However, in our study samples were latex positive but ELISA negative, this may be due to false positive latex tests as ELISA is

more sensitive, or that because of the subjectivity of the reader since latex is read by naked eye. It is very important when reading latex tests to compare with positive and negative controls.The other reason could be that the testing samples with latex after high-speed centrifugation affects results and may be a possible cause of false positive results. Therefore all the new generation latex tests now on market use a filtration process of the stool samples. *Candida albicans* was isolated as the only causative agent from 22 children (10.5%) .In Iraq, researchers found a higher prevalence of *C. albicans* in infectious diarrhea, for example Al-Marzoqi (9.5%) and Al-Jebouri (24%) as sited by Alrifai [12]. This is because they considered the presence of *C.albicans* in stool as an opportunistic infection beside other pathogens, as well as the abuse of antibiotics as the reason for the overgrowth of this opportunistic pathogen.Lastly many specimens which shown positive results for rotavirus were containing fatty drops15(48.3%). This coexisting may be one of the factors which leading to viral infection and this point need additional researchs.

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مسببات الاسهال (الفايروسية والبكتيرية والطفيلية) لدى الاطفال في محافظة كركوك

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الملخص

اجريت الدراسة الحالية لتحديد مسببات اخماج المعدة – الامعاء المسببة للاسهال لدى الاطفال الاقل من 5 سنوات عمرا في مدينة كركوك بالعديد من المسببات الفيروسية (الفايروسية والبكتيرية والطفيليةوتم تحديدها وقد اظهرت نتائج هذه الدراسة ايجابية الفحص لروتافايرس في 31(14.9 %) عينة اما البكتريا المعوية الممرضة في 26(12.5 %) عينة والاميبا النسيجية في 30(14.4%) عينة والجيارديا في 2(0,96 %) عينة وكما اظهرت النتائج وجود الاصابات المشتركة بين بعض هذه العوامل الممرضة كما تم مقارنه النتائج لفايروس الروتا بفحصي *اللاتكس و الاليز ا.*