Two different diagnostic methods for detection of rotavirus in Iraqi young Childreen

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Abstract

ELISA (Premier Rotaclone) was compared with latex agglutination test (LT) (Bio Kit) for detection of Rota virus in fecal samples from clinically suspected cases of viral gastroenteritis in children. Out of 40 samples 12(30%) were positive for Rota virus antigen by ELISA kit. While 30 samples (75%) samples were positive for Rota virus by latex agglutination test (LT). All controls were negative for viral antigen by ELISA and LT test. ELISA and Latex kits were found to be economically sensitive for screening and rapid diagnosis of rota virus diarrhea. In conclusion, our study showed that the latex agglutination is clearly a reliable and rapid method for detection rotavirus but Elisa is more sensitive than the latex agglutination.

طريقتان تشخيصية مختلفة للكشف عن فايروس روتا في الأطفال العراقيين

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الخلاصة

قورنت عدة فحص التفاعل المناعي الأنزيمي (الأليزا) (برايمر روتا كلون) مع عدة فحص تلازن اللاتكس على الشريحة (بايوكت) في الكشف عن فايروس الروتا في نماذج الخروج من حالات التهاب أمعاء مشكوك من أنها التهاب أمعاء فايروسي. من مجموع أربعين نموذج فحصت كانت 12 (30%) موجبة لفحص التفاعل المناعي الأنزيمي و28 كانت سالبة للفحص أعلاه. كذلك وجد عند فحص النماذج أعلاه ان 30 (75%) كانت موجبة لفحص تلازن اللاتكس على الشريحة و10 كانت سالبة للفحص أعلاه. جميع نماذج السيطرة كانت سالبة لفايروس الروتا عند فحصها بكلا الطريقتين. وجد ان طريقة التفاعل المناعي الأنزيمي وكذلك طريقة تلازن اللاتكس على الشريحة كانت اقتصادية وذات حساسية عالية للمسح السريع في تشخيص فايروس الروتا المسبب للإسهال في الأطفال.

Introduction

Rota virus are non enveloped viruses belonging to genus Rota virus in the family reoviridae. they are the major cause of dehydration and diarrhea in young children causing death among infants in developing countries (1).

In 1973, Bishop identified for the first time the rota virus by electron microscopy(2). The size of the virus is of 70nm. The virus was denominated rota virus because it has the shape of a wheel (wheel=rota in latin). It is round and double shelled. it includes a genome of 11 segments of double stranded RNA, at least 7 distinct serotype have been identified but the main human pathogen are the rota virus in the group A subtype (4).

Several tests are used routinely in diagnostic laboratories for the detection of rotavirus in faecal samples. Include Enzyme Linked Immunosorbent Assay (ELISA)(5), Electron microscopy(EM), Virus Isolation (VI), Passive Hemagglutination(PHA) , and Latex agglutination assay(LT) (3).

In this study we compared a latex agglutination test with ELISA kit for sensitivity in detection rota virus in fecal samples.

Materials and Methods

- **Fecal collection and preparation:** Forty fecal specimens obtained from young children(from 1 month to 33 month) with acute gastroenteritis were submitted to central public health laboratory (CPHL) in Baghdad, between April 2008 and October 2008. The negative and positive control samples were from healthy and unhealthy young children. Fecal samples were prepared as either 10% (wt/v) suspension of solid or semisolid feces in 0.01M PBS (PH7) or as 20% (vol/vol) suspension of liquid feces in 0.01M PBS (PH7). All specimens were centrifuged at 2000rpm, and the supernatants were tested and then stored in sterile vials at (-20ºC) for further study.
- **Latex agglutination test:** The latex test (Rotagene from biokit, Spain): rapid qualitative test for the direct detection of rotavirus antigen in feces by agglutination of latex particles on slide.
- **Specimens collection:** Specimens collected in a clean, dry container, free of detergent. At least 2-3 ml of representitve specimens were collected. A swab may also be used for collection as long as minimum of 0.2 ml of sample obtained.
- **Procedure:** 0.2 ml of fecal specimen were added to 1ml of diluents, vortexed to homogenize suspension and then centrifuged for 10 min at 1500 rpm, the supernatants were tested with rotagene reagent. This is a rapid slide test in which latex particles are coated with antibodies specific for group A rota virus antigen present in fecal supernatant. This test was read with naked eyes in 5 minutes.
- **ELISA Test:** The ELISA kit (Premier rotaclone, Germany) an assay appropriate for human diagnostic testing, was used in this study. Fecal samples were testing following the manufacturers instructions for human fecal specimens.
- **Specimens preparation:** One ml of specimens diluents was added to properly marked tube, using a transfer pipette and then 0.2grams of stool spcimens, were added, thereafter it was centrifuged and supernatant was collected.
- **Procedure:** 100ul of fecal specimens, positive control and negative control were added to each well, then 100ul of enzyme conjugated were added to each well, mixed by gently swirling on tabletop and then incubated at room temperature for 60 minute. Then poured out of the wells into discard vessel, washed with deionize water for 6 times, and then 100ul of substrate solution were added to each well and incubated for 10 minute at room temperature. Visual determination can be made after 10 minute incubations, samples with blue color grater than the negative control were positive,and the samples showing colorless were negative.

Result were read by ELISA reader by adding 100ul of stop solution (sulfuric acid) to each well, at 450nm filter and specimens with absorbance units (A450), greater than

0.150 were considered positive and specimens with absorbance equal to or less than 0.150 were considered negative.

Result

Forty fecal samples from diarrheic young children were collected from April 2008 to October 2008 and screened by latex test and ELISA test. Out of 40 specimens screened by latex test 30(90%) positive for rota virus antigen compared with ELISA which showed that out of 40 tested specimens 12(30%) were positive for rota virus antigen as shown in (Table 1).

Table (1) Comparison of results from two testing methods for Rotavirus in 40 fecal samples

Total	Latex (+ve)	Latex(-ve)	ELISA (+ve)	ELISA (-ve)
40	**30 (90%)**	**10**	**12 (30%)**	**28**

The concordance of results between the two tests is shown in (Table 1). thirty specimens (90%) were positive by latex test as it shown in (Table 1) while twelve (30%) were positive by ELISA as it shown in (Table 2). There was statistical difference ($p<0.05$) in detection of rotavairus incidence betwen two different methods. When antigen positivity in relation with age groups, the positivity ratio between two year and two years with few months old group was meaningful with all two tests as it shown in (Table 3).

Table (2) Rotavirus antigen detection by ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	**0.056**	**0.056**	**0.054**	**1.19**	**0.57**	**1.86**	**0.09**	**0.06**	**0.06**	**0.05**	**0.06**	**0.027**
B	**0.068**	**1.01**	**0.05**	**0.06**	**0.07**	**1.14**	**0.06**	**0.05**	**0.06**	**0.12**	**0.05**	**0.07**
C	**0.05**	**1.08**	**0.06**	**0.05**	**0.06**	**0.18**	**0.23**	**1.12**	**0.09**	**0.30**	**0.04**	**1.21**
D	**0.05**	**0.81**	**0.07**	**0.23**	**0.05**	**0.04**	**0.04**	**0.04**	**0.48**	**0.11**	**0.05**	**0.04**
E												
F												
G												
H												

A1 = Blank
A2 = Negative control 1
A3 = Negative control 2
A4 = positive control
A5-D12 =patient samples
Validation of the test
Positive control must be > 0.3
Sample considered positive if OD > 0.150
Sample considered negative if OD < 0.150

Table (3) Rotavirus Antigen Detection by Two Different methods in children with Diarrhea with different ages

	Total		Latex		ELISA	
Age (month)	N	%	N	%	N	%
0-6	10	25%	10	100%	6	60%
7-12	18	45%	13	72.2%	5	27%
13-20	8	20%	5	62.2%	1	12.5%
21-26	3	7.5%	1	33.3%	0	0%
27-33	1	2.5%	1	33.3%	0	0%
TOTAL	40	100%	30		12	

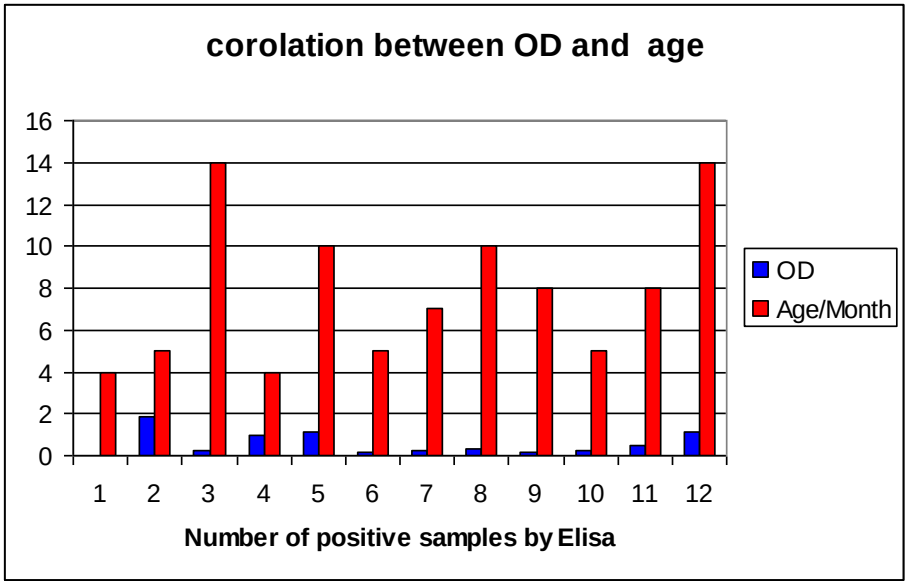

Fig. (1) Correlation between OD and Age

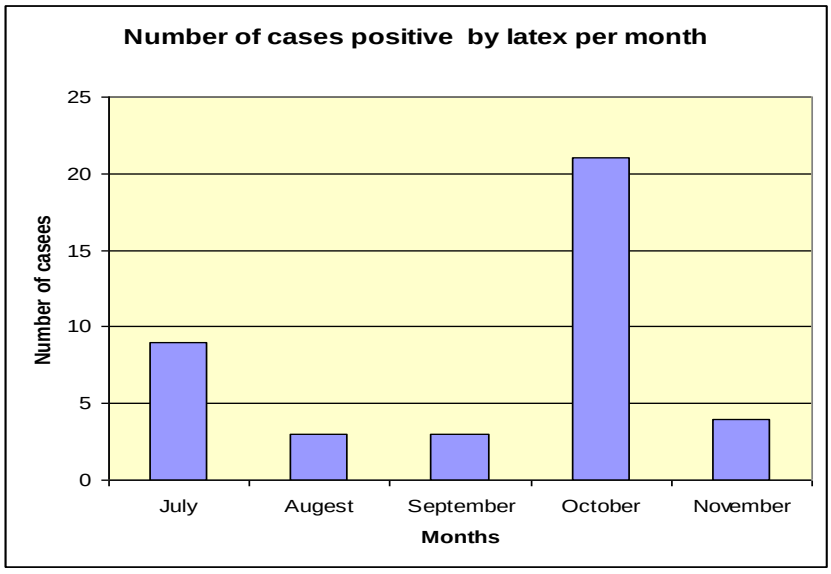

Fig. (2) Number of cases positive by latex per month

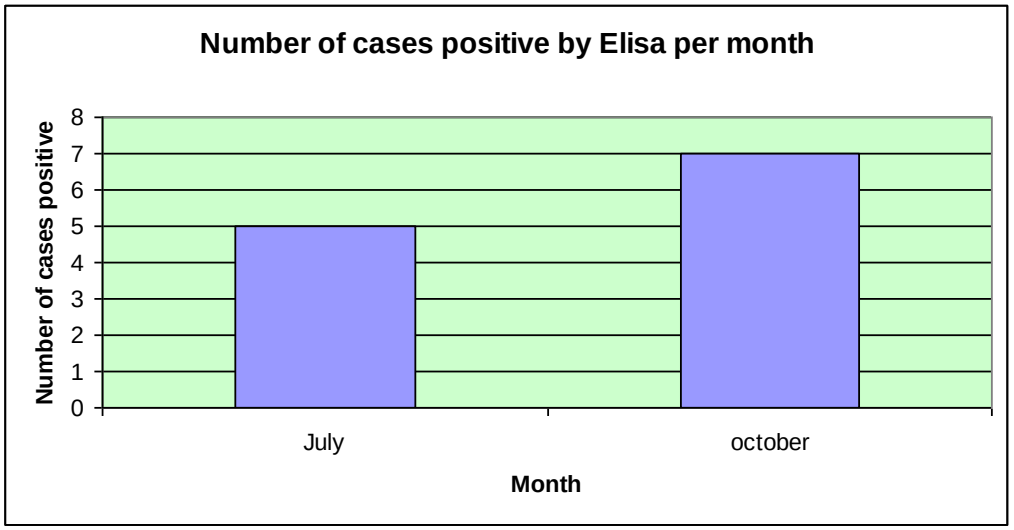

Fig. (3) Number of positive cases detected by ELISA per month

Discussion

Rotavirus is responsible for 20-30% patients with diarrhea younger than 5 years, and 35-50% hospitalized patients in Europe (6).

A rapid, simple, sensitive, and specific diagnostic technique for the detection of viral agents causing gastroenteritis is needed to facilitate timely treatment of the disease. Because rotavirus is a major agent associated with acute diarrhea in human and animal species (7). Many factors, including laboratory size, number of specimens per day, all influence the choice of protocols used for diagnostic testing (8). Various methods for the detection of rotavirus antigen in fecal samples have been developed. These include transmission electron microscopy(3), ELISA, immunoassay, and latex agglutination (9). ELISAs are used widely in diagnostic laboratories because they provide rapid detection of rotavirus antigen in a relatively short time in comparison to other tests.

Stool specimens as 12 of 40 by ELISA, 36 by 40 by latex agglutination give us ELISA most feasible for cohort screening test (10). Compared Elisa methods (premier Rota clone) with latex (biokit), these authors argued that Elisa test were considerably sensitive and coud be used for mass screening (14). Ibrahim, et al (11) report that latex was the most sensitive but least specific, those results were in accordance with our results.

30 specimens were positive by the latex and 12 specimens were positive by ELISA, this suggests the presence of non specific factors interfering with ELISA.

The results from this study show that the latex is a valuable tool in the diagnosis of rotavirus infection, the assay has the number of advantages including its simple format, rapidity and low cost, and it can be performed without the need for trained personnel or expensive equipment (12). In addition the latex has the advantage that it can be read with the naked eye, making it easy to perform in every laboratory, management of diarrheal disease demand, rapid, accurate diagnosis, therefore the use of the latex to detect rotavirus from diluted fecal samples is good alternative to ELISA (13).

In conclusion, our study showed that the latex agglutination is clearly a reliable and rapid method for detection rotavirus but ELISA is more sensitive than the latex agglutination assay, further study should be performed to develop Polymerase Chain Reaction (PCR) for detection RNA rotavirus.

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