PERFORMANCE OF ENZYME–LINKED IMMUNOSORBENT ASSAY VERSUS LATEX AGGLUTINATION TEST IN THE DIAGNOSIS OF ACUTE GASTROENTERITIS BY ROTA VIRUS

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Abstract

In this study we use two types of methods for detecting Rota virus in 91 stool specimens from children with acute gastroenteritis.

The aim of this study to determine the performance (sensitivity & specificity) of latex agglutination (LAT) and enzyme–linked immunos-orbentassay (ELISA) for evaluation children acute gastroenteritis by Rotavirus.

Fecal samples were collected from ninety one children suffering from acute gastroentritis. their age ranged between(1–132) months. The highest sensitivity was(92.5%) obtained with LAT followed by ELISA (84.09%). while the highest specificity was(93.6%) obtained with ELISA followed by LAT(86.3%). the highest predictive positivity value was obtained with ELISA(92.5%) followed by LAT (84.09%).

LAT is easy to performance and gave high sensitivity with accepted specificity therefore, could be applied successfully for routine diagnosis and Epidemiological study. But ELISA techniques allow quantitative estimation of Rotavirus antigens.

Keywords : Rotavirus, LAT, ELISA, sensitivity, specificity.

Introduction

Gastroenteritis is a leading disease causing death among infants in developing countries [1,2].Rotavirus group A has represented the most ordinary cause of world wide childhood acute diarrhea. in developed countries, it was estimated that gastroenteritis associated to these etiological agents has been responsible for 600000 to 870000 death / year, which means 20 to 25 % of death due to diarrheic disease, as well as 6% of global mortality among children below five years old [1].

Several techniques have been developed for Rotavirus diagnosis. In the first Rotavirus surveys, the viral agent detection was performed by electronic microscopy; after wards, other techniques were developed, such as poliacrylamide gel electrophoresis(PAGE), immune- fluorescence, radio immune assay, reverse passive hem agglutination, enzyme – linked immunosorbent assay (ELISA), latex agglutination (LAT) and more recently, a reverse transcription polymerase chain reaction (RT-PCR) and immunoch-romatography [2,3,4,5,6]. Among these assays, LAT has reported as simple and fast assay, as required for rapid diagnosis and illness control on hospital level. however, the sensitivity and specificity of LAT and ELISA may according to the commercial kit used

Aim of the Study

This work aimed to evaluate the performance (sensitivity & specificity) of LAT and ELISA test for Rotavirus diagnosis.

Materials and Methods

Selection of Patients

Ninety one stool samples from children aged (1–132) month with acute gastroenteritis, 50 male with mean age (15.88 ± 12.99)months and 41 female with mean age (14.67 ± 14.96) months collected from January, 2007 to February,2008 from children care hospital in Baghdad city.

The stool sample were collected in sterile containers, then separated in to two parts, the first part was sent to laboratory for immediate testing by using LAT(biokit, Spain), the kit contain latex particles coated with anti-rotavirus Antibodies. a sample was considered positive for Rota virus when agglutination was observed within two minutes reaction, as recommended by the kit manufacturer.and the other part of stool specimens were stored at -20°c until examination by using ELISA

(biomanguinhos, RT, Brazil). The assay is a double sandwich method, in which the antigens are captured by antibodies that attached to a solid phase, the assay was carried out according to the manufacturer specifications.

Statistical analysis

Data were analyzed with by SPSS for window TM version 11 and Microsoft excel for windows 2007. Use Pearson's test (rho) for correlation between the variables and two test.

The level of significant was 0.05 (two tail), and the significant of correlations include also 0.01 (twotail).

Results

Ninety one of children aged from (1-132) month with acute gastroenteritis, were studied to instigated the Rotavirus in stool specimens Rotavirus was detected in 44 (48.4%) by LAT, 40 (43.97%) by ELISA Table (1).

The result revealed that significant a positive correlation between the age and LAT test ($p<0.05$), and there is a significant correlation between LAT and ELISA test ($p<0.01$) Table (2).

Table (1)
Rotavirus antigen detection by LAT and ELISA in children with gastroenteritis ELISA.

LAT	Positive	Negative	Total
positive	37	7	44
negative	3	44	47
total	40	51	91

LAT: latex agglutination ; ELISA : enzyme–linked immunosorbent assay.

Table (2)
Correlation among age, LAT test, and ELISA test.

	Pearson correlation	LAT	ELISA
Age	PN	0.260	-0.030
	P*	0.020	0.795
LAT	PN		0.583
	P**		0.000

****correlation is significant at the 0.05 level (2- tail).***
***** correlation is significant at the0.0 1 level (2- tail).***

The result observed in this work demonstrated good specificity (86.3%) and high sensitivity (92.5%) to LAT test, but demonstrated good sensitivity (84.09%) and high specificity (93.6%) to ELISA test. Table (3). The correlation degree in regard of ELISA was a good, with a high predictive value (92.5%). This study also verified that the percentage of false positive reaction was 7.7% (7 /91) in LAT compare with 3.3% (3/91) in ELISA.

Other wise, Rotavirus antigen was not detected by the ELISA on seven LAT positive samples, showing a false negative reaction index of was 7.7%(7/91) as shown in Table(3).

Table (3)
Parameters of LAT test and ELISA test.

Parameter	LAT	ELISA
Sensitivity	92.5%	84.09%
Specificity	86.3%	93.6%
Predictive positive value	84.09%	92.5%
Predictive negative value	93.6%	86.3%
False positive	7.7%	3.3%
False negative	3.3%	7.7%

Discussion

Specific diagnosis of infection with Rotavirus is made by identification of the virus in the patient's stool by enzyme immunoassay several licensed test kit are used which are sensitive, specific and detect all serotype of Rotavirus [7,8,9,10]. These kits are also used to diagnosis infections of animals [10]. Other methods, electron microscopy and polyacrylamide gel electrophoresis are used in research laboratories [11]. Reverse transcription polymerase chain reaction is used to detect and identify all species all serotypes of human Rotavirus [12]. In this study we evaluated the sensitivity and specificity of LAT was (92.5%, 86.3%) respectively compared ELISA was (84.09%, 93.6%) respectively with the high predictive positive value 84.09%, 92.5% for LAT and ELISA respectively. This study verified that the percentage of false negative reaction was 7.7% (7/91) to ELISA and 3.3% (3/91) to LAT. These false negatives reactions may be occurring due to many reasons: the monoclonal antibodies that sensitize the latex particle may not be detecting the serotype; the viral title could be lower than the technique sensitivity ; the feces may contain un specific inhibitors or IgA antibodies, resulting in weak agglutination reaction, not detected by the technician [3,4,5].These results were in a accordance with the documented in other papers, Altindi, etal(2004)[13]. Investigated Rotavirus in 135 children, 0 to 3 years old, and he found the sensitivity and specificity of LAT test and ELISA test was(93.75%, 94.96%), and (100%, 99.16%) respectively, and predictive positive value (71.43%, 94.12%) for LAT test and ELISA test respectively [13]. Ferreira, etal (2006) [14] demonstrated good sensitivity (82.9%) and high specificity (98.1%) of LAT test for evaluation children acute gastroenteritis by Rotavirus, predictive positive value (96.7%), predictive negative value (89.7%). In another project, the ELISA test showed the highest sensitivity to detect Rotavirus in feces [15].the LAT test for Rotavirus, was compared with direct electron microscopy (EM) and ELISA test for detection of Rotavirus in stools of children with diarrhea, the LAT test had a sensitivity of (81.7%) and specificity of (99.5%) compared with EM, predictive positive and negative value were (98% and 94.9%) respectively [16].

The result in this study indicate performed with great simplicity and speed. However, due to the occurrence of (3.3%and 7.7%) false negative reaction to LAT test and ELISA test, respectively representing (93.6% and 86.3%) predictive negative value to LAT test and ELISA test, respectively it is recommended that negative sample on one test be re evaluated by another assay higher sensitivity such as RT-PCR or PAGE. it is important to emphasize that the observed predicative negative value for LAT test and ELISA test was works, it reported (100% and 99.12%) to LAT and ELISA respectively Altindis, etal (2004) [13].and the predicative negative value for LAT was (94.9%) [16] the sensitivity of LAT and ELISA varied depending on the time of stool collection relative to the onset of symptom[15, 16]. in this study we found the significant positive correlation between age and LAT test that mean the excess of the shedding the virus (antigen) in the stool with to age because of Rotaviruses are transmitted by the fecal–oral route.person-to-person spread throw contaminated hand is probably the most important means by which Rotavirus transmitted in close communities and family homes [17]. The infective dose is 10 -100 infectious viral particles [18, 19, 20]. From all the previous causes the recurrent infections may be occur throughout life And the most recurrent infections are mild or asymptomatic [21].Symptomatic infection rates are highest in children under two years of age and lowest in adult [22,23,24,25,26]. Rotavirus infection of adults also occur, and frequent asymptomatic adult infection in the community [27]. there for the adult may be infected with Rotavirus and he do not know and the virus throw it replication, the viral RNA evades innate host immune responses [28,29]synthesis viral genome and the progeny viruses are release by the lysis of the cells [30,31,32,33].after that large numbers of viruses are in the feces (10^8-10^{10}) infectious particles / ml [17,21,34] therefore the reaction the stool specimens with LAT become clear and agglutination particles become large and more visible because of the large numbers of viral particles antigens that

equivalence the amount of antibodies in the reactants.

The result indicate that the LAT test used for Rotavirus diagnosis presented high sensitivity, good specificity, but ELISA test presented good sensitivity, high specificity the two test easy proceeding, providing fast diagnosis for Rotavirus infections, but LAT test suitable for Rotavirus diagnosis in hospital laboratories or even the physician's office for rapid diagnosis [3] but, ELISA test could be used for mass screening [8,35].we suggest that samples considered negative by LAT should be analyzed by a more sensitive second assay for assure an appropriate diagnosis for Rotavirus infection.

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الخلاصة

استخدم في هذه الدراسة نوعين من الطرق المستخدمة في الكشف عن فيروس الروتا في عينات الخروج لواحد وتسعون طفلا يعانون من التهاب المعوي المعدي الحاد.الهدف من هذه الدراسة هو تحديد كفاءة عمل وحساسية وخصوصية طريقة التلازن وطريقة الاليزا في الكشف عن فيروس الروتا المتسبب في الالتهاب المعوي المعدي الحاد.

جمعت عينات الخروج من(91) طفلا يعانون من التهاب المعوي المعدي الحاد، تتراوح اعمارهم بين (1-132) شهرا. وكانت اعلى حساسية فحص(92.5%) لفحص التلازن اما حساسية فحص الاليزا فكانت (84.09%). بينما كانت اعلى خصوصية فحص (93.6%) سجلت لفحص الاليزا اما خصوصية فحص التلازن كانت (86.3%).

وقد اثبتت النتائج سهولة عمل طريقة التلازن مع حساسية عالية وخصوصية جيدة لذلك يمكن استخدامها بنجاح للتشخيص اليومي وفي الدراسات الوبائية، اما طريقة الاليزا فيفضل استخدامها عندما يراد التقدير النوعي لمستضدات فيروس الروتا.