Comparison of multiplex real time PCR with ELISA for diagnosis of viral Gastroenteritis in children of Al-Muthana province

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Abstract

Simultaneous detection of Gastroenteritis viruses in children is important to prevent outbreak and control infection. In this study, evaluated the multiplex real time PCR technique with ELISA test fordetecting three enteric viruses (Rotavirus, Astrovirus, and Norovirus) from 335 stool specimens collected from children of Al-Muthana province. The overall rates of incidences were 79(23.6%), 39(11.6%) and 8(2.4%) respectively by using multiplex PCR technique and 20.3, 10.7, and 2.4% overall rate detection with ELISA, the sensitivity and specificity diagnostic values for PCR test were higher than of ELIAS test.

Introduction

Acute Gastroenteritis is a common infection in children. The this infection mostly associated with dehydration and a leading cause of admission to children hospital in industrialized countries as well as the major source of prevalence rate and mortality in developing countries (Parkin, *et al.*,2009). Most enteric viruses have been recognized as the most important etiological agents of disease, and the Gastroenteritis is frequently caused by Rotavirus and Norovirus, therefore, there are increasing number of reports of other viruses (Ferreiraa, *et al*., 2012).

The three categories of enteric viruses are being considered clinically relevant: group A, Norovirus (family/Caliciviridae), Rotavirus(family/Reoviridae)and Astrovirus (Levidiotou, *et al.,* 2009).

Simultaneous detection of pathogenic viruses in a given sample, even when the pathogens have similar symptoms and signs, is essential to giving the true pathogen spectrum (Briese, *et al.*, 2005). In present study, RT-PCR assay used multiplex PCR with two channels (FAM, Cy5) to solve the main difficulties in simultaneous detection of Rotavirus, Astrovirus, and Norovirus (Van Maarseveen, *et al*., 2010).

The aim of this study is thedetermination of the efficiency of simultaneous detection for RT-PCR technique in comparison withELISA assay in the detection of enteric viruses (Rotavirus, Astrovirus and Norovirus) among the children in Al-Muthana province/ Iraq.

Materials and Methods

Patients and samples collection

From January to November 2012, fecal specimens collected from children (n = 335; mean age= 2.1 ± SD 1.81 year) who were admitted to maternal and children hospital of Al-Samawa city/ Al- Muthana province for signs of Gastroenteritis using routine clinical laboratory procedure (Belliot, *et al.*, 2008) for each of stool sample taken from these children, Rotavirus group A, Astrovirus, and Norovirus group I and II were detected by multiplex RT-PCR technique and ELISA assay in the same time.

ELISA assay

ELISA technique was performed according to manufacture recommendations (Qiagene, germany).

RT-PCR Technique

The stool suspension was made by 0.5 g of stool (0.5 ml for fluid) to 5 ml of phosphate-buffered saline (PBS) with 10% chloroform (Abebe, *et al*., 1992). The

second step, the suspension was centrifuged at 6000 rpm/15 min, and then the supernatant was collected for the following test. RNA was extracted from 100 ml of each sample using RNA extraction kit (Sacace biotechnologies.Inc.). RT-PCR was performed by one step in same tube, two channels (FAM, Cy5 dyes) for primers of Rotavirus, Astrovirus and same the channels which used for Norovirus, and internal control (internal control used as inhibition indicator. The positive and negative results were detected by measurement of threshold cycle Ct values.

Statistical analysis

Statistical analysis was carried out with Sigma plot software (version 11.0) that used to plot the standard curve of ELISA test, means ± SD (standard deviations). The Unpaired *t*-test was used to compare between each two groups of positive results (Sack, *et al.*, 2006).The diagnostics sensitivity and specificity were calculated according to Zhou, *et al.*, (2002).

Results and Discussion

From 335 children suffering from Gastroenteritis,the overall rates of incidences of Rotavirus, Astrovirus, and Norovirus were 79(23.6%), 39(11.6%) and 8(2.4%) respectively by using multiplex PCR technique. Single infections cases were diagnosed in 102(30.4%) of the 335 study children, the Rotavirus, Norovirus, and Astrovirus single infection cases were 67(65.7%), 31(30.4%), and 4(3.9%) respectively as figure 3(A),

whereas mixed viruses infection cases were detected in 12(3.6%) of the same children. Rotavirus was associated in 12 mixed infection cases, figure 1 and 2. The most dual gastroenteritis infections were Rotavirus and Norevirus8(66.6%)out of 12, whereas Rotavirus and Astrovirus mixed infections in 4(33.3%) out of 12, figure 3(B).

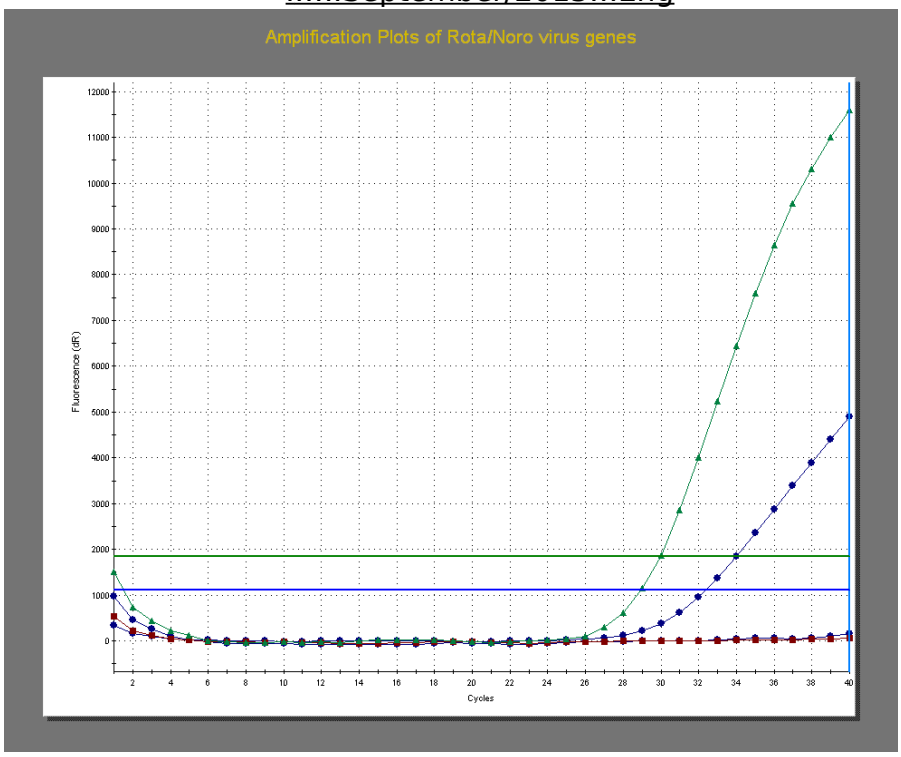

Figure1. Positive RT-PCR tests of mixed Rota/Astrovirus infections. Green and red curves indicate the expression of Rotavirus (22.4 C_t) and Astrovirus (26 C_t) respectively. The other curves indicate the negative cases.

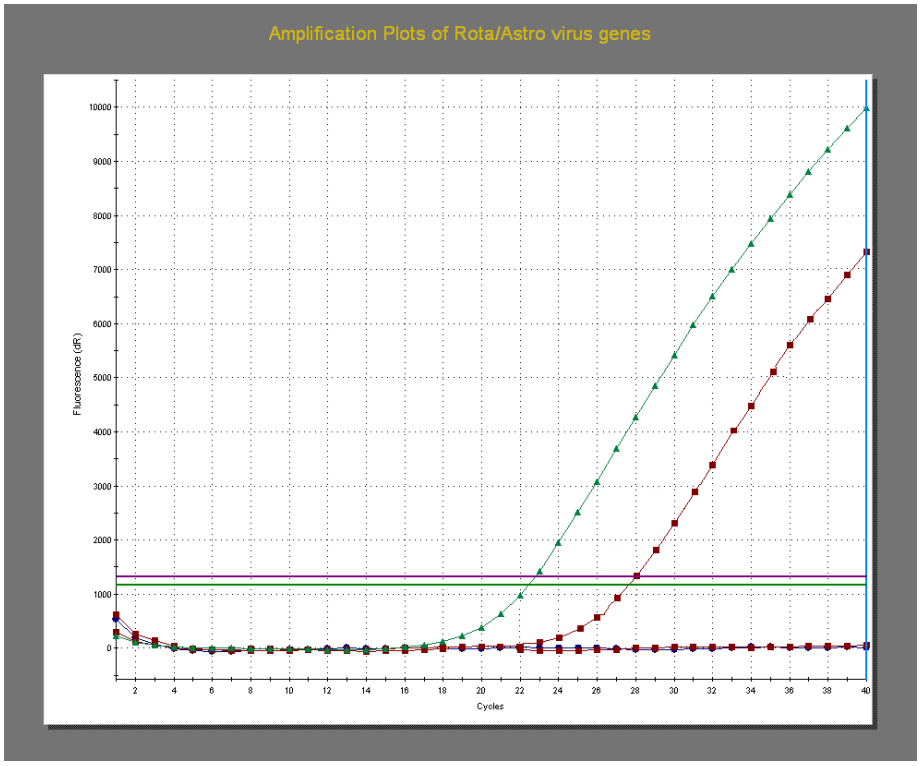

Figure2. Positive RT-PCR tests of mixed Rota/Norovirus infections. Green and blue curves indicate the expression of Rotavirus (29.9 C_t) and Norovirus (32.3 C_t) respectively. The other curves indicate the negative cases.

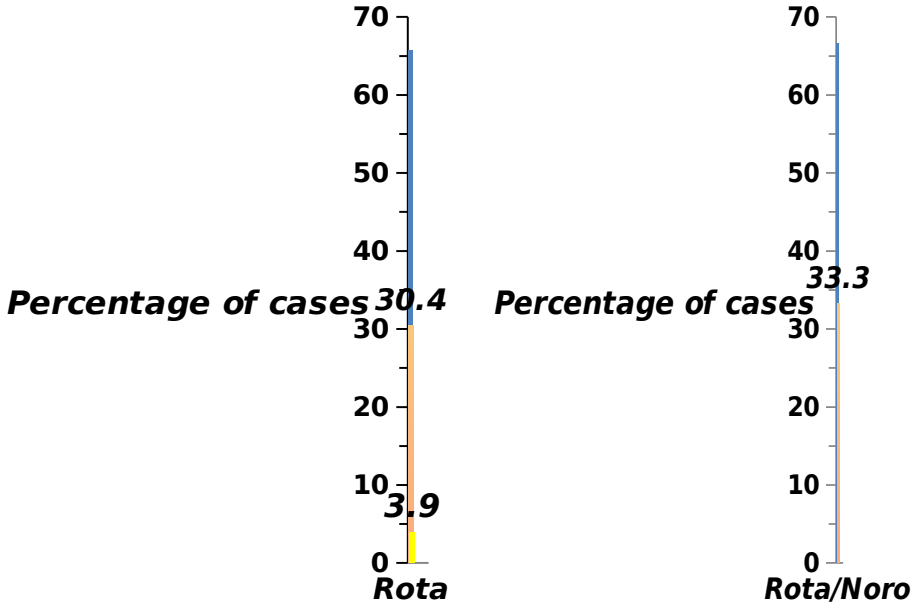

Figure 3. (A) Percentage of single Rota, Noro and Astro viruses infections of 102 total single infection cases, (B) percentage of mixed Rota/Norovirus infections of 12 mixed infection cases that detected by RT- PCR.

Infection with single virus rarely associated with severe clinical syndrome but the infection with multi viruses mostly results in severe cases with rapid progressive cardiopulmonary and neurological complications, therefore, the infections with other enteric viruses can be overcome by the major virus (Cardosa, *et al.,* 1999; Han, *et al*, 2011).

ELISA technique results shown 68 (20.3%), 36 (10.7%) and 8 (2.4%) as overall rates of incidences for Rotavirus, Norovirus and Astrovirus respectively; their single infection cases were 92(27.5%) that included 58(63%), 30(32.6%) and 4(4.3%) for single infection cases of Rotavirus, Norovirus and Astrovirus respectively (figure 4 A) whereas the mixed virus infections were detected in 10 (3%) and the Rotavirus was mixed with Norovirus in 6(60%) and with Asterovirus in 4(40%) cases, (figure 4 B).

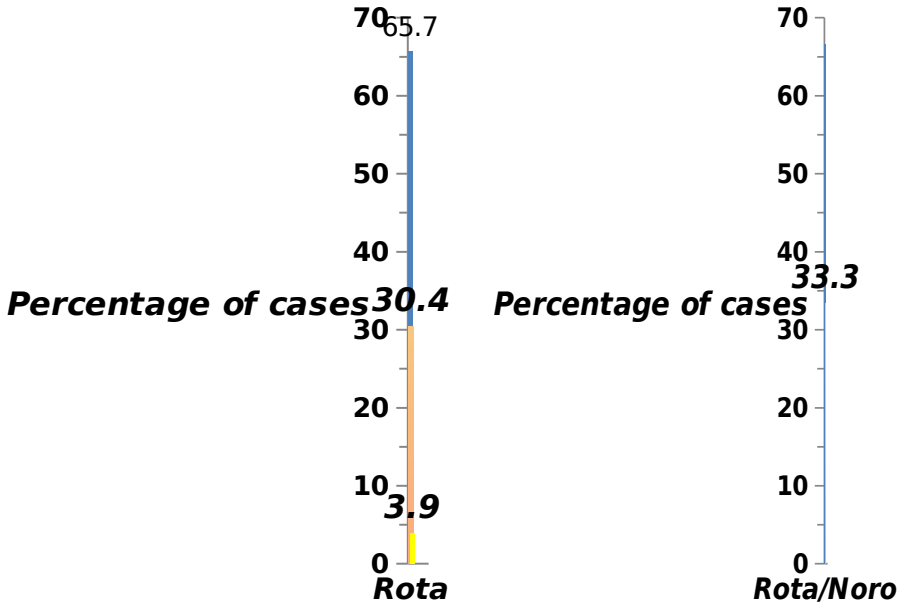

Figure 4. (A) Percentage of single Rota, Noro and Astro viruses infections of 92 total single infection cases. (B) percentage of mixed Rota/Norovirus infections of 10 mixed infection cases that detected by ELISA.

To evaluate PCR assay in detection of study Rotaviruses, The PCR test results were compared to ELISA assay. ELISA and PCR tests were detected 68/79 (%) and 256/267 (%) as true positive and negative cases respectively, whereas the false negative cases were 11:267 without any false positive for PCR test to produce sensitivity and specificity diagnostic values with 86.1% and 100% respectively(Table 1). The sensitivity and specificity values for detection Norovirus and Astrovirus by using PCR assay were 100%,92.3% and 100%, 100% respectively, (Table 2 and 3).

Table (1): Correlation of PCR test results with ELISA test for detection of Rotavirus infections.

Parametersfor PCR assay		Total	ELISA test		PCR assay
Specificity	Sensitivity		Negative	Positive	
% 100	% 86.1	79	11	68	Positive
		256	256	0	Negative
		335	267	68	Total

Table (2): Correlation of PCR test results with ELISA test for detection of Astrovirus infections.

Parametersfor PCR assay		Total	ELISA test		PCR assay
Specificity	Sensitivity		Negative	Positive	
% 100	% 100	8	0	8	Positive
		327	327	0	Negative
		335	327	8	Total

Table (3): Correlation of PCR test results with ELISA test for detection of Norovirus infections.

Parametersfor PCR assay		Total	ELISA test		PCR assay
Specificity	Sensitivity		Negative	Positive	
% 100	% 92.3	39	3	36	Positive
		296	296	0	Negative
		335	299	36	Total

Used RT-PCR assay provided main advantages, such as sensitivity and specificity, can also be used to diagnosis viruses in mixed infected samples. During our present study, we diagnosed 12 samplesmixed infected with two viruses. Multiplex assays, such as PCR, which are capable of good and simultaneous detection of multiple pathogenic viruses in a single tube as well as can prevent delays in treatment and misdiagnosis without adding other steps to the process or increasing costs (Yan, *et al.*, 2003).

So future development will include the development of full automation, the reduction of the possibility of technics errors and decreasing the assay time.Based on our findings, we believe that this test (RT-PCR) may serve as an alternative and good method for the improvement of clinical detection of mixed infections in diagnostic applications and surveillance.

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مقارنة تقنية real time PCR مع ELISA لتشخيص الالتهاب المعوي الفيروسي في أطفال محافظة المثنى

الخلاصة

الكشف الفيروسي الآني لحالات التهاب المعدة والأمعاء عند الأطفال مهم لمنع تفشي المرض والسيطرة على العدوى.في هذه الدراسة تم تقييم اختبار RT-PCR بمقارنته مع تقنية ELISA هي الكشف عن فيروسات Rotavirus , Norovirus و Astrovirus في عينات البراز لأطفال في محافظة المثنى ، حيث بلغت أجمالي نسب الإصابة لهذه الفيروسات من 335 عينة براز هي ، 79 (23.6٪)، و 39 (11.6٪) و 8 (2.4٪) على التوالي باستخدام تقنية real time PCR وكذلك 20.3%, 10.7 % و 2.4٪ باستخدام إل ELISA على التوالي.كما بلغت القيم التشخيصية لحساسية وخصوصية اختبار PCR نسب أعلى عند مقارنتها مع اختبار ELISA.