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STUDY OF THE ASSOCIATION OF ROTAVIRUS AND ASTROVIRUS IN CHILDREN INFECTED WITH ACUTE GASTROENTERITIS IN BAGHDAD CITY

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Abstract

Acute gastroenteritis is one of the most prevalent human diseases and is linked globally with elevated mortality and morbidity, particularly among babies and young kids. To avoid outbreak and control infection, it is essential to simultaneously detect gastroenteritis viruses in children. In this research, 49 stool samples gathered from kids with acute gastroenteritis and 49 stool samples gathered from the control group in Baghdad city to evaluate the multiplex real-time PCR method with chromatography immunoassay test to detect two enteric viruses (Rotavirus and astrovirus). The overall rates of incidences in children with acute gastroenteritis were 17(17.3%), 5(5.2%) respectively by using multiplex PCR technique and overall rate detection with chromatography immunoassay 14(14.2%) for rotavirus, the sensitivity of diagnostic values for PCR test was higher than of chromatography immunoassay test. Rotavirus more associated with clinical symptoms then astrovirus, but no statistical differences in CRP level, WBCs count among both viruses. With sluggishly elevate of lymphocytes with rotavirus infection.

Key words: Rotavirus, Astrovirus, Gastroenteritis.

Introduction

Acute gastroenteritis (AGE) is one of the most prevalent human diseases and is linked globally with elevated mortality and morbidity, particularly among children. Furthermore, elderly and immunocompromised people are also a higher danger of diarrheal disease (Pratte Santos *et al*., 2019). AGE implies intestinal mucosal inflammation. The onset of diarrhea with or without vomiting, fever or abdominal pain characterizes it. Most enteric viruses have been identified as the most significant etiological agents of the disease and gastroenteritis is often caused by rotavirus and norovirus, hence the amount of records of other viruses is growing (Walker *et al*., 2013). Rotaviruses, astroviruses, noroviruses, adenoviruses and sapoviruses are common viruses that cause acute gastroenteritis (Thongprachum *et al*., 2016).

The main symptoms of virus-associated gastroenteritis are non-bloody watery diarrhea, fever and vomiting which usually persist for more than three days; other symptoms include headache and abdominal cramps (Chi *et al.*, 2018). Diarrhea symptoms can last between one and nine days (Elawad *et al*., 2015). Rotavirus is the leading cause of severe diarrhea in children worldwide. It is estimated to cause 36% of diarrhea hospitalizations among children aged less than 5 years (Burnett *et al*., 2018). Also, Astroviruses (AstV) are common causes of AGE in children, the elderly and immunocompromised individuals, with a peak of astrovirus detections at 6 to 11 months of age (Olortegui *et al*., 2018).

Simultaneous detection of pathogenic viruses in a specified sample, even if the pathogen has comparable symptoms and signs, is crucial in order to provide the real pathogen spectrum (Mitab, 2013). In the present study, used multiplex PCR to solve the main difficulties in simultaneous detection of Rotavirus, Astrovirus. And used internal control as a monitor for the protocol as well as positive and negative control.

The aim of this study is a determination of the efficiency of simultaneous detection for RT-PCR

technique in comparison with chromatography immunoassay in the detection of enteric viruses among the children under five years and using of C-reactive protein titter, WBCs as a marker for acute gastroenteritis in children.

Materials and Methods

Patients and samples collection

From the beginning of October, 2018 to the end of January, 2019, in two hospitals which are Children's Protection Teaching Hospital and Al-Alawiya Children's Hospital in Baghdad City. During the period of study, 98 stool specimens and 98 blood samples were collected from the patient and control group, their ages under five years old. The information from patients including age, sex, type of feeding, residence, clinical features (diarrhea, fever, vomiting, abdominal pain, dehydration and frequency bowel motion) and rotavirus vaccination was taken.

Chromatography immunoassay method

Rotavirus A was detected in stool samples by chromatographic immunoassay method using rotavirus combo rapid test cassette kit/ Biozek medical.

Molecular detection of enteric viruses by real-time PCR

The enteric viruses include rotavirus and astrovirus was submitted for detection by real-time PCR using acute intestinal infections (A.I.I) screen real-TM kit. The assay is based on three major processes: isolation of RNA for rotavirus and astrovirus using Ribo-Sorb extraction kit (Sacace Biotechnologies/ Italy) from stool samples that collected from children under 5 years, then, reverse transcription of the RNA to cDNA and Real-Time amplification of cDNA. The test contains an internal control (IC) which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. The RT-PCR program that was adopted for the detection of rotavirus, astrovirus according to the manufacture manual by Real-Time PCR device 7500 Applied biosystem/ Singapore. The results were interpreted by the device software through the presence of crossing of fluorescence curve with the threshold line (CT), were rotavirus A was detected on the FAM (blue) channel and astrovirus on the JOE (red) channel detected with PCR-mix-1 rotavirus/ astrovirus and Internal control (IC) was detected on the FAM (blue) channel with PCR-mix- IC.

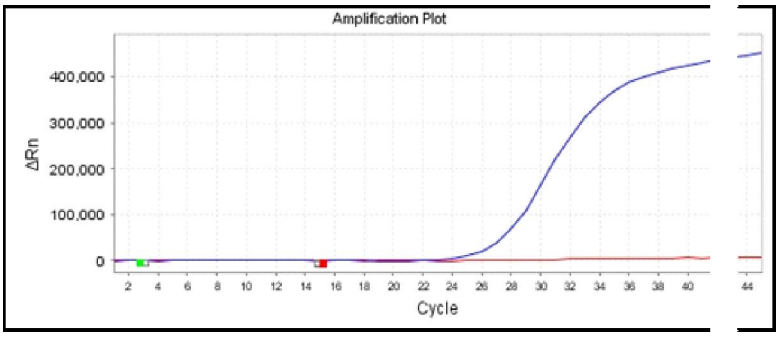

Fig. 1: The positive RT-PCR multiplex amplification of rotavirus A. Blue curve indicates the expression of rotavirus A (24.4 Ct) and red curves indicate the no expression of astrovirus.

Statistical analysis

The data in this study were analyzed using SPSS by licensed materials version 25 computer software. The data were presented as mean ± standard deviation (Mean ± SD) using the chi-square test and independent-samples T-test to compare mean and obtainment the correlation among the studied parameters with infection. The range test at (p <0.05) was considered to be statistically significant, a highly significant when (p <0.001) and non-significant when (p<0.05). In addition, Kappa Coefficient test was used to compare the two diagnostic methods (RT-PCR and chromatography immunoassay).

Results and Discussion

During the period of study, from the beginning of October 2018 to the end of January 2019, ninety eight fecal samples from children under five years (range from 2 month to 5 years) was compiled at multiplex RT-PCR and included 21 (21.4%) of samples positive for one of the two viruses (Rotavirus A and astrovirus). For the patient group, 49 children's infected with acute gastroenteritis as a disease group, with a mean age of 1.9 ±1.2 years was included 17 samples positive for at lees one enteric-virus infected. And for controls, 49 children's apparently healthy as a case-control for diseases, with a mean age of 2.1 ±1.2 years, was included 4(8.1%) samples positive for rotavirus infection. Stratifying the age into classes revealed that the age group 12-24 month were the most affected group which represented 12.2% among positive for enteric viral infection, while the most common age groups among Acute gastroenteritis were <1 year and 12-24 months which represented 33.6% and 27.5% respectively. Statistical analysis revealed no significant differences in age distribution between the different group's studies (Singh *et al*., 2017 and Mutlak *et al*., 2018).

Overall, the rotavirus A detection by chromatographic immunoassay method was in 14(14.2%) out of 98(100%) stool samples, among them, rotavirus A positive in 11 (22.4%) of out of 49 (100%) stool samples that collect from children with acute gastroenteritis and

Table 1: The correlation of RT-PCR test results with a chromatographic immunoassay method for detection of Rotavirus infections.

Cassette rotavirus	Real time PCR rotavirus		P. value	Sensitivity	Specificity
	Negative	Positive			
Negative	81 (82.7%)	3 (3.1%)	0.000	82.4%	100.0%
Positive	0 (0.0%)	14 (14.3%)			
Total	81 (82.7%)	17 (17.3%)			

three (6.1%) stool samples were in asymptomatic children. The rotavirus detection results by chromatographic immunoassay method in stool samples that collected from children with acute gastroenteritis were close related to result by Al-Shuwaikh, (2016) in Baghdad city.

While rotavirus A was detected in 13(26.5%) samples out of 49 stool samples in the children with acute gastroenteritis by RT-PCR. Rotavirus À was detected on the FAM (blue) channel and Astrovirus on the JOE (red) channel with tube contain PCR-mix-1 Rotavirus/ Astrovirus (Fig. 1). These results corresponding with results of a study to determine the incidence of rotavirus in Baghdad, was 21%, in patients, while 7% in case-control (Mahmood *et al*., 2015). Also, this result matched to Rahajamanana *et al*., (2018) they demonstrate that the median proportion of annual hospitalizations due to diarrhea was 26% by rotavirus. In Iraq studies for rotavirus prevalence, one study showed all samples tested by using PCR and revealed result was 35% positive specimens (Abdulazeez *et al*., 2018). Another state the overall rates of incidences were 23.6% of rotavirus infection (Mitab, 2013). Individually detected of rotavirus was in 13 stool samples, when one co-detection of rotavirus with astrovirus in the same sample on the same plat wall (Fig. 2). The present study demonstrates that the rotavirus was the major detection enteric virus in-patient, especially affected children less two years. This matched with study in Saudi children Meqdam and Thwiny, (2007).

Astrovirus was detected in five samples (10.2%) out of 49 stool samples from the patient. Among them, one co-detection of rotavirus with astrovirus in only one patient (2%) of all patients with AGE and that represent 20% of the astrovirus-positive sample (Fig. 3).

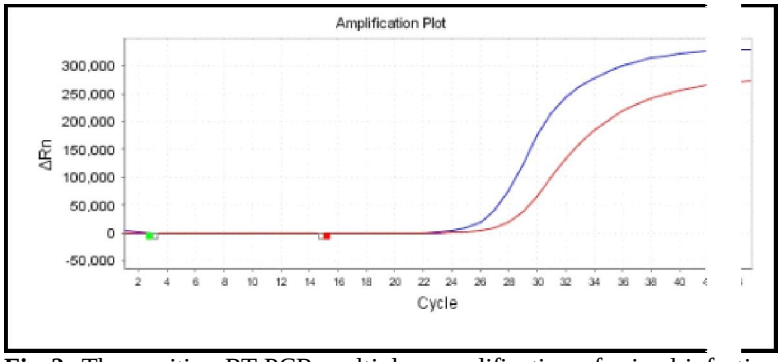

Fig. 2: The positive RT-PCR multiplex amplification of mixed-infection rotavirus/ astrovirus. Blue and red curves indicate the expression of rotavirus A (24.0 Ct) and astrovirus (25.6 Ct) respectively.

Recent epidemiological studies have used diagnostic tests to highlight the effect of gastroenteritis associated with astrovirus. It causes acute gastroenteritis in humans with overall prevalence rates of up to 10% (Khamrin, 2016). Many infections may be sub-clinical, but in many studies, 2 to 9% of infantile acute non-bacterial diarrhea is attributable to astroviruses (Wohlgemuth *et al*., 2019).

Another study state the overall positivity observed of astrovirus was 6% of the patients (Quintero-Ochoa *et al*., 2019). Higher frequency of astrovirus was detected in children less than two years of age (60%) of astrovirus-positive samples. That agrees with (Khamrin, 2016).

Compare chromatography immunoassay with RT-PCR in rotavirus detection

Rotavirus diagnostic by two methods was compared to sensitivity and specificity. As shown in table 1, RT-PCR method more Sensitive (100%) than chromatography immunoassay in (82.4%) in the diagnosis of rotavirus, although the specificity of both was 100% in the detection of rotavirus in stool samples agreement between RT-PCR and chromatography immunoassay in the diagnosis of rotavirus (kappa=0.885), Negative predictive value= 96.4% and Positive predictive value=100% (Table 1).

In close related diagnostic to another study, the sensitivity was assessed as 84.2% for EIA and 82.5% for rapid diagnostic immunochromatographic test. The specificity of those tests was calculated as 96.4% for a rapid diagnostic immuno-chromatographic test (Moutelikova *et al*., 2018). In contrast, another study results revealed that 47.2% and 35.2% were rotavirus positive using chromatographic immunoassay and PCR techniques, respectively (Abdulazeez *et al*., 2018).

The verified real-time PCR method is an easy, fast and accurate method for monitoring the diagnosis and treatment outcomes in a clinical setting more than other methods (Hwang *et al*., 2018). Chromatographic Immunoassay is the simple and good standard methods for detection of rotavirus. These methods, however, require low-cost equipment and simple experience, which is available in many laboratories. Some researchers for detecting rotavirus infection have used PCR as a reliable (Abdulazeez *et al*., 2018). In addition to its simplicity, PCR is robust, speedy, flexible and

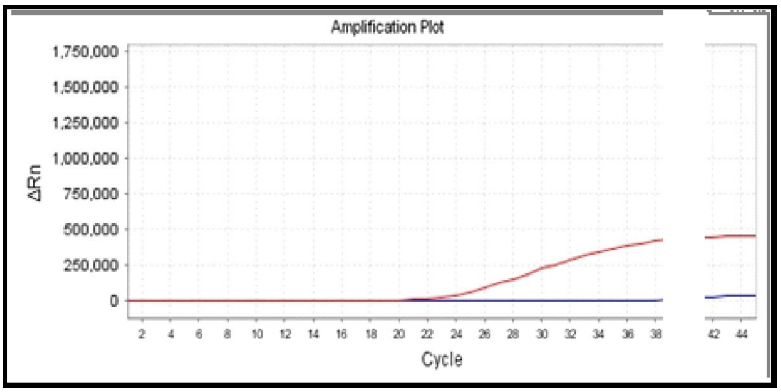

Fig. 3: The positive RT-PCR multiplex amplification of astrovirus. The blue curve indicates no expression of rotavirus A (negative) and red curves indicate the expression of astrovirus (20.9 CT).

sensitive (Green and Sambrook, 2019). RT-qPCR is more sensitive for surveillance of rotavirus gastroenteritis than routinely used EIA or rapid diagnostic methods. The specificity of both evaluated tests was very high. Comparisons of the RT-qPCR tests showed very good agreement of results (Moutelikova *et al*., 2018).

CRP and hematological test

The minimum CRP level in serum of patient was (6mg/l) while the maximum was (96 mg/l), with average mean 24.6± 21.8 mg/l in contrast, for control group the minimum CRP level was (6mg/l) while the maximum was (12 mg/l) and with average mean 7.3± 3.0 mg/l. That was significantly difference ($p<0.05$) in CRP in serum between patient and control group. Patients early in the onset of disease could present with low values of inflammatory markers despite the underlying cause, whereas the chance of an urgent condition in patients with low values of inflammatory markers after a longer duration of symptoms would decrease (Toorenvliet *et al*., 2010). There was no signiûcant difference between the four enteric viral infections in the level of CRP. Also, viral gastroenteritis caused lower serum level of CRP compared to non-viral gastroenteritis (Chung *et al*., 2017).

The hematological test was revealed leucocytes were 10±3.6 inpatient and 6.9±2.4 in control group, which was significant important ($p<0.05$). In general, leucocytes were elevated in 19.4% children and were low in 7.1%. The differentiation WBC showed the percentages of neutrophils, lymphocytes and monocytes were 47±12, 41±11 and 1.9 ±1.2, respectively, in children with a viral infection that compared with 51±11, 38±10 and 5.9±1.9, respectively were in non-viral infection group. Furthermore, the sluggish high lymphocytosis average was observed in the children with the virally infected group (Raboni *et al.,* 2014)

Rotavirus A infection in-patient was fever 10(76%), vomiting 8(61%), dehydration 6(46%) and abdominal pain 3(23%). Also, in children with astrovirus infection, the fever was 2(40%) vomiting 2(40%), dehydration 2(40%) and abdominal pain 1(20%). Rotavirus positive children with respect to clinical symptoms such as fever, vomiting and dehydration were high in this study compared astrovirus which reported a high percentage of clinical symptoms (Chung *et al*., 2017; Imade *et al*., 2015).

Conclusions

This study exhibits a significant association between rotavirus infection and acute gastroenteritis incidence in children less than 5 years, severe clinical symptoms. Poor discriminative value of CRP, WBCs count and deferential WBCs in enteric virus infection Real-time PCR applications for the detection of enteric viruses robust and sensitive compared with chromatography immunoassay, require low-cost equipment, raped and simple experience.

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