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Comparison of Three Different Rotavirus Antigen Tests for Rotavirus Detection in Fecal Samples: A Retrospective Analysis

Dışkı Örneklerinde Rotavirüs Tespitinde Üç Farklı Antijen Testinin Karşılaştırılması: Retrospektif Bir Analiz

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Abstract

Objective: Direct antigen tests are the most commonly used methods in most laboratories to detect rotavirus rapidly in stool samples. This study aimed to evaluate the performance of three commercially available test methods for detecting rotaviruses in fecal specimens and compare the results with those of the reverse transcription-polymerase chain reaction (RT-PCR), which is considered a gold standard test.

Materials and Methods: The presence of rotavirus antigens in stool samples was investigated by an enzyme-linked immunosorbent assay (ELISA), an immunochromatographic test (ICT), and a latex agglutination test (LAT), which were commercially available. The results of these tests were compared with those of a multiplex RT-PCR as a reference test. Sensitivity, specificity, and positive and negative predictive values were calculated, and agreement with RT-PCR was evaluated by Cohen's kappa test.

Results: A total of 85 patients (51.8% male and 48.2% female, aged 0-32 years) were included in this study. The sensitivities of the ICT, LAT, and ELISA tests were 78.6%, 78.6%, and 96.4%, respectively; the specificities of the tests were 69.0%, 72.4%, and 69.0%, respectively. According to kappa tests, moderate agreement was found between RT-PCR and ICT (κ=0.464, p<0.001); moderate agreement was found between RT-PCR and LAT (κ=0.493, p<0.001); substantial agreement was found between RT-PCR and ELISA (κ=0.694, p<0.001). The ELISA test showed the highest sensitivity and a high level of agreement with RT-PCR.

Conclusion: ICT and LAT are quick and practical tests for rotavirus detection. However, in this study, it was seen that they were not superior to the ELISA test in terms of accuracy of diagnosis.

Öz

Amaç: Rotavirüsün hızlı tespitinde direkt antijen testleri çoğu laboratuvarda en yaygın olarak kullanılan yöntemlerden biridir. Bu çalışmanın amacı, dışkı örneklerinde rotavirüs antijenlerinin tespiti için ticari olarak piyasada bulunan üç farklı tanı yönteminin performanslarını değerlendirmek ve sonuçlarını altın standart test olarak kabul edilen ters transkripsiyon-polimeraz zincir reaksiyonu (RT-PCR) testinin sonuçlarıyla karşılaştırmaktır.

Gereç ve Yöntemler: Dışkı örneklerinde rotavirus antijenlerinin varlığı ticari enzyme-linked immunosorbent assay (ELISA), immünokromatografik test (ICT), ve lateks aglütinasyon testi (LAT) ile araştırıldı. Testlerin sonuçları referans test olarak bir multipleks RT-PCR'ınkilerle karşılaştırıldı. Duyarlılık, özgüllük, pozitif ve negatif prediktif değer hesaplandı ve RT-PCR ile uyumluluk Cohen'in kappa testi ile değerlendirildi.

Bulgular: Çalışmaya toplam 85 hasta (%51,8 erkek ve %48,2 kadın, 0-32 yaş) dahil edildi. ICT, LAT ve ELISA testlerinin duyarlılıkları sırasıyla %78,6, %78,6 ve %96,4; testlerin özgüllükleri sırasıyla %69,0, %72,4 ve %69,0 idi. Kappa testlerine göre, RT-PCR ve ICT arasında orta düzeyde (κ=0,464, p<0,001), RT-PCR ve LAT arasında orta düzeyde (κ=0,493, p<0,001), RT-PCR ve ELISA arasında ise iyi derecede bir uyum belirlendi (κ=0,694, p<0,001). ELISA testi, RT-PCR ile en yüksek duyarlılık ve yüksek düzeyde uyum gösterdi.

Sonuç: ICT ve LAT, rotavirüs tespiti için hızlı ve pratik testlerdir. Ancak bu çalışmada tanı doğruluğu açısından ELISA testinden daha üstün olmadıkları görülmüştür.

Introduction

Rotavirus is a leading cause of acute viral gastroenteritis throughout the world, and most children are infected by 5 years of age (1). Many studies conducted in both developing and developed countries report that group A rotaviruses are responsible for 13-50% of all cases of viral gastroenteritis in children under 5 years of age (2). Despite the availability of a rotavirus vaccine, more than 200,000 deaths occur per year under the age of five worldwide (1). In studies conducted in Turkey, rates of rotavirus positivity in children with gastroenteritis ranged from 18.7% to 53% (3-10).

The rapid and accurate diagnostic tests for the detection of the virus in patients with acute gastroenteritis are important not only for the diagnosis of viral gastroenteritis but also to prevent the spread of the disease (11). The specific methods available for detecting the rotavirus in stool specimens include electron microscopy (EM), immuno-EM, cultivation techniques, rapid antigen tests, polyacrylamide gel electrophoresis (PAGE), and reverse transcriptase-polymerase chain reaction (RT-PCR). EM, cultivation, and PAGE are not recommended because they are expensive, time-consuming, and technically difficult (4). Several rapid antigen tests, such as the latex agglutination test (LAT), the immunochromatographic test (ICT), and the enzyme-linked immunosorbent assay (ELISA), are inexpensive, easy-to-perform, and commercially available.

In routine diagnostics, it is important to know the sensitivity and specificity of these rapid tests. Rapid and accurate diagnosis could prevent unnecessary and potentially harmful antibiotic treatment and improve knowledge of the epidemiology of rotavirus infections (12). These rapid tests have good performance for determining rotavirus, and they are frequently used by physicians as an aid to diagnosis. The RT-PCR is the most sensitive molecular method to detect and confirm rotavirus (13,14).

The objective of the presented study is to evaluate the performance, sensitivity, and specificity of three commercially available rotavirus antigen tests: ELISA, ICT, and LAT, compared with a reference test, a multiplex reverse transcription-PCR (mRT-PCR), for detecting rotavirus in fecal specimens from patients with acute gastroenteritis over medical records retrospectively.

Materials and Methods

In this study, the results of three different commercial rotavirus antigen tests, ELISA, ICT, and LAT, which were studied to detect rotavirus in stocked stool samples sent to our laboratory from patients with acute gastroenteritis between January and December 2014, were compared with the results of the RT-PCR, which was used as a reference test. The study was approved by the Aydın Adnan Menderes University Ethics Committee (protocol no: 2022/176, date: 10/11/2022).

Detection of rotavirus by ELISA: The ProSpecTTM® Rotavirus Microplate Assay (Oxoid, Ltd., Basingstoke, Hampshire, UK) is a qualitative sandwich ELISA and was used to detect rotavirus group A antigen in stool samples.

Detection of rotavirus by ICT: The CerTest® Rota-Adeno Card Test (CerTest, Biotec, Spain) was used to detect rotavirus antigen in stool samples. This test is a one-step lateral flow ICT that simultaneously detects group A rotavirus and adenovirus in stool samples.

Detection of rotavirus by LAT: The Virotect Rota (Omega Diagnostics, Scotland, UK) wasbasically a rapid

LAT for the detection of rotavirus in fecal samples.

Multiplex RT-PCR: The commercially available Seeplex® Diarrhea ACE Detection multiplex PCR (Seegene, Seoul, Korea) was used to simultaneously detect group A rotaviruses, AdV 40 and 41 (species F), noroviruses GI and GII, and astroviruses. Nucleic acids were extracted from fecal suspensions by using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and the QIAcube platform (Qiagen). The nucleic acid was amplified using a PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA), and the PCR products were visualized after electrophoresis on a 1.5% agarose gel. Under ultraviolet light, the DNA products with 650 base pairs (bp) for Astrovirus, 411 bp for enteric adenovirus, 541 bp for group A rotavirus, 304 bp for norovirus group I, and 214 bp for norovirus group II showed positive results. All test kits were performed according to the manufacturer's instructions.

Statistical Analysis

Descriptive statistics were presented as percentages, the mean with standard deviation, or the median with minimum and maximum values. Based on the RT-PCR results (15), sensitivity, specificity, and positive and negative predictive values were calculated for the ICT, LAT, and ELISA. Test agreement with the RT-PCR results accepted as the gold standard was assessed using Cohen's kappa with 95% confidence intervals (CI). The strength of agreement was based on Cohen's kappa value: <0 poor, 0.0-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial, and 0.81-1.00 almost perfect (16). All data entries were made in SPSS 26.0, and the tables were prepared with it. Calculations were performed and evaluated according to the references mentioned before.

Results

In the study, which included 85 people in total, 25 (29.4%) of them were under 1-year-old and 43 (50.6%) were under 5-years-old. The median age was two years (0-32), and 41 were female and 44 were male.

Out of the 85 samples, 56 (65.9 %) tested positive for rotavirus by RT-PCR. Out of all the samples, 63 (74.1%), 53 (62.4%), and 52 (61.2%) were found to be positive in the ELISA, ICT, and LAT kits, respectively.

In Table 1, it was shown that the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the RT-PCR as a gold standard. These values for the ICT test were found to be 78.6%, 69.0%, 83.0%, and 62.5%, respectively. These values were 78.6%, 72.4%, 84.6%, and 63.6% for the LAT and 96.4%, 69.0%, 85.7%, and 90.9% for the ELISA, respectively.

Considering Cohen's kappa, moderate agreement was found between the RT-PCR and the ICT (κ=0.464 (95% CI, 0.268-0.660), p<0.001); moderate agreement was found between the RT-PCR and the LAT (κ=0.493 (95% CI, 0.303-0.683), p<0.001); and substantial agreement was found between the RT-PCR and the ELISA (κ=0.694 (95% CI, 0.529-0.859), p<0.001) (Table 1).

Discussion

Rapid and accurate rotavirus detection is required to ensure the administration of appropriate treatment plans and infection control. Several rapid test kits, including latex agglutination, ICTs, and enzyme immunoassays for detection of rotavirus infection, are used in routine diagnosis. In our study, we evaluated the performance, sensitivity, and specificity of three commercial rotavirus antigen tests compared with a mRT-PCR assay for detecting rotavirus in fecal specimens from patients with acute diarrhea.

In the presented study, RT-PCR was accepted as the reference method, the ELISA was the antigen test with the highest sensitivity (96.4%), and the LAT was the test with the highest specificity (72.4%) among the three tests compared. When PPV and NPV were examined, it was observed that the ELISA had the highest values (85.7% and 90.9%, respectively). The test with the highest agreement with the RT-PCR was found to be ELISA (κ=0.694). Considering our findings, it can be thought that the performances of all three tests are not at the desired level; nevertheless, ELISA is the best among these tests.

The ICT is rapid and easy to perform (17). We found that out of 85 samples, 53 (62.4%) were positive in the ICT. The sensitivity and specificity of ICT were 78% and 69%, respectively. Studies comparing the ICT test with the reference method, the PCR test, showed that the sensitivity of the ICT test was 80-100% and the specificity was 89-100%. In the presented study, the sensitivity and specificity of the ICT test were lower than in previous studies. The latex agglutination assay is faster and simpler but less sensitive than the ELISA

Table 1. The sensitivity, specificity, positive and negative predictive value, and agreement for rotavirus group A detection in fecal samples by three tests compared with the RT-PCR

	ICT	LAT	ELISA
Sensitivity (%)	78.6	78.6	96.4
Specificity (%)	69.0	72.4	69.0
Positive predictive value (%)	83.0	84.6	85.7
Negative predictive value (%)	62.5	63.6	90.9
Cohen's kappa with 95% CI	0.464 (0.268-0.660)	0.493 (0.303-0.683)	0.694 (0.529-0.859)

LAT: Latex agglutination test, ICT: Immunochromatographic test, ELISA: Enzyme-linked immunosorbent assay, RT-PCR: Reverse transcription-polymerase chain reaction, CI: Confidence interval

(17). In our study, while the LAT test was similar to the ICT, it was less sensitive and specific than the ELISA test. In a previous study, it was reported that the latex agglutination assay had a sensitivity of 63.6% and a specificity of 86.8% when compared with the RT-PCR (18). Xiang et al. (19) found 81.03% and 97.44% for the LAT sensitivity and specificity, respectively. Unlike previous studies, we found 78.6% sensitivity and 72.4% specificity for the LAT.

Although the ELISA is the standard test for detecting rotaviruses, it is time-consuming and not cost-effective. In this study, 63 (74.1%) samples were positive for rotavirus by the ELISA and the sensitivity and specificity of the ELISA were 96.4% and 69%, respectively, when compared with the RT-PCR. Gautam et al. (20) conducted a comparative analysis study of three commercial EIA kits. Using the RT-PCR as the gold standard, the sensitivities were between 75 and 82.1% and all the specificities were found to be 100%. Ibrahim et al. (13) observed that the ELISA had a good performance with 88% sensitivity and 100% specificity when compared with the RT-PCR results. Considering our study, the ELISA test was more sensitive than the the other ELISA tests mentioned but less specific.

In a study in which 95 stool samples were studied for LAT, three ICTs, and ELISA tests and compared with the RT-PCR, it was reported that the sensitivity and specificity were 85.7% and 100% for LAT, 100% and 95% for two ICTs, 86.7% and 87.5% for another ICT, and 98.1% and 97.3% for the ELISA, respectively (21). We found the sensitivity of the ELISA to be higher than LAT and ICT, and the specificity to be lower. In another study comparing rapid tests, three different commercial immunologic tests for rapid detection of group A rotavirus (the ICT method, LAT, and ELISA) were used to evaluate 228 stool specimens obtained from children with acute gastroenteritis. The sensitivity and specificity values of the ELISA, ICT, and LAT methods were 96% and 68%, 99% and 99%, 99%, and 96%, respectively. In the study conducted by Wilhelmi et al. (22), the sensitivity and specificity for the ELISA and ICT were found to be higher than LAT.

Agreement with the RT-PCR for the ELISA was the highest compared with the ICT and LAT. In a previous study (23), the analytical and clinical performance of ICT was investigated, and the test results were compared to the ELISA and RT-PCR, found a high level of agreement (κ=0.857). Although there was lower agreement, we also found the highest agreement with the RT-PCR in our study with the ELISA.

The LAT and ICT were rapid and easy to perform but showed lower sensitivity than the ELISA. The ELISA was the best test in terms of sensitivity and specificity but had limitations, such as generating results that were difficult to interpret and being time-consuming. Because of the nature of convenience sampling, the results of this study are not generalizable. However, similar results have been obtained in many studies. In this study, the samples were not fresh; frozen samples were thawed and studied.

Conclusion

In summary, the ELISA test for rotavirus detection showed the highest sensitivity and a high level of agreement with the RT-PCR. Even though it is a less sensitive test, the ICT and LAT may be used alternatively for the rapid screening of group A rotavirus in stool samples, especially during the acute gastroenteritis outbreak seasons. In clinical practice, the possibility of false positive and false negative results with rotavirus should be kept in mind. A false positive antigen test could be caused by cross reactivity with other microorganisms or interference.

When testing with rapid kits yields a negative result in suspected patients, testing with RT-PCR should not be delayed.

Ethics

Ethics Committee Approval: The study was approved by the Aydın Adnan Menderes University Ethics Committee (protocol no: 2022/176, date: 10/11/2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.K., N.E., Design: S.K., N.E., Data Collection or Processing: S.K., N.E., F.K., V.Y., M.A., Analysis or Interpretation: S.K., H.Ö., Literature Search: S.K., N.E., H.Ö., M.A., Writing: S.K., N.E., H.Ö.

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References

1. LeClair C, McConnell K. Rotavirus. In: StatPearls. Treasure Island (Florida): StatPearls Publishing; 2022.
2. Kota M, Bino S, Delogu R, Simaku A, Neza B, Ruggeri FM, et al. Epidemiology of rotavirus diarrhoea in Albania. Arch Virol 2014; 159: 2491-5.
3. Gül M, Garipardıç M, Çıragil P, Aral M, Karabiber H, Güler İ. Investigation of Rotavirus and Adenovirus types 40/41 in Children with Gastroenteritis between 0-5 Years of Age. ANKEM Derg 2005; 19: 64-7.
4. Ozdemir S, Delialioğlu N, Emekdaş G. [Investigation of rotavirus, adenovirus and astrovirus frequencies in children with acute gastroenteritis and evaluation of epidemiological features]. Mikrobiyol Bul 2010; 44: 571-8.
5. Tapisiz A, Bedir Demirdag T, Cura Yayla BC, Gunes C, Ugraş Dikmen A, Tezer H, et al. Rotavirus infections in children in Turkey: A systematic review. Rev Med Virol 2019; 29: e2020.
6. Meral M, Bozdayı G, Ozkan S, Dalgıç B, Alp G, Ahmed K. [Rotavirus prevalence in children with acute gastroenteritis and the distribution of serotypes and electropherotypes]. Mikrobiyol Bul 2011; 45: 104-12.
7. Akan H, İzbırak G, Gürol Y, Sarıkaya S, Gündüz TS, Yılmaz G, et al. Rotavirus and adenovirus frequency among patients with acute gastroenteritis and their relationship to clinical parameters: a retrospective study in Turkey. Asia Pac Fam Med 2009; 8: 8.
8. Ceyhan M, Alhan E, Salman N, Kurugol Z, Yildirim I, Celik U, et al. Multicenter Prospective Study on the Burden of Rotavirus Gastroenteritis in Turkey, 2005-2006: A Hospital-Based Study. J Infect Dis 2009; 200: S234-8.
9. Bulut Y, Yenişehirli G, Durmaz R. Molecular Epidemiology of Rotavirus Strains in Under Five Children. Indian J Pediatr 2018; 85: 364-8.
10. Altindis M, Bányai K, Kalayci R, Gulamber C, Koken R, Apan T, et al. Molecular characterization of rotaviruses in mid-western Turkey, 2006-2007. Cent. Eur. J. Med. 2010; 5: 640-5.
11. Obi RK, OKE BO, Anjorin AA, Bidmos IK, Salu OB, Omilabu SA. Evaluation of techniques of rotavirus gastroenteritis in pediatric patients. Genes Rev 2015; 1: 6-27.
12. Roy S, Shamsuzzaman SM, Mamun KZ. Rapid detection of Rotavirus antigen in stool sample of acute diarrheic children. Bangladesh J Med Microbiol 2012; 6: 11-3.
13. Ibrahim SB, El-Bialy AA, Mohammed MS, El-Sheikh AO, Elhewala A, Bahgat S. Detection of Rotavirus in children with acute gastroenteritis in Zagazig University Hospitals in Egypt. Electron Physician 2015; 7: 1227-33.
14. Manjula G. Comparison of Immunochromatography with RT-PCR for Detection of Rotavirus in Fecal Samples. International Journal of Scientific Research 2012; 2: 479-81.
15. Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol 2008; 56: 45-50.
16. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-74.
17. Dhiman S, Devi B, Singh K, Devi P. Comparison of Enzyme-Linked Immunosorbent Assay and Immunochromatography for Rotavirus Detection in Children Below Five Years with Acute Gastroenteritis. J Clin Diagn Res 2015; 9: DC06-9.
18. Noppornpanth S, Theamboonlers A, Poovorawan Y. Predominant human rotavirus genotype G1P[8] infection in infants and children in Bangkok, Thailand. Asian Pac J Allergy Immunol 2001; 19: 49-53.
19. Xiang W, Peng Z, Xu J, Shen H, Li W. Evaluation of a commercial latex agglutination test for detecting rotavirus A and human adenovirus in children's stool specimens. J Clin Lab Anal 2020; 34: e23208.
20. Gautam R, Lyde F, Esona MD, Quaye O, Bowen MD. Comparison of PremierTM Rotaclone®, ProSpecTTM, and RIDASCREEN® rotavirus enzyme immunoassay kits for detection of rotavirus antigen in stool specimens. J Clin Virol 2013; 58: 292-4.
21. Lee SY, Hong JH, Lee SW, Lee M. Comparisons of Latex Agglutination, Immunochromatography and Enzyme Immunoassay Methods for the Detection of Rotavirus Antigen. Korean J Lab Med. 2007; 27: 437-41.
22. Wilhelmi I, Colomina J, Martín-Rodrigo D, Roman E, Sánchez-Fauquier A. New Immunochromatographic Method for Rapid Detection of Rotaviruses in Stool Samples Compared with Standard Enzyme Immunoassay and Latex Agglutination Techniques. Eur J Clin Microbiol Infect Dis 2001; 20: 741-3.
23. Kim J, Kim HS, Kim HS, Kim JS, Song W, Lee KM, et al. Evaluation of an immunochromatographic assay for the rapid and simultaneous detection of rotavirus and adenovirus in stool samples. Ann Lab Med 2014; 34: 216-22.