ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Rapid Antigen Test in the Diagnosis of COVID-19

COVID-19 Tanısında Hızlı Antijen Testi

Feray Ferda Senol[1], Altay Babacan[2], Mete Ozcan[3], Esra Timurkaan[4], Yuksel Akaya[5], Ozlem Aytac[1], Pinar Oner[1], Zulal Asci Toraman[6]

[1]Elazig Fethi Sekin City Hospital Microbiology, Elazig; 2Ufuk University Faculty of Medicine, Department of Pediatric Hematology Oncology, Ankara; [3]Firat University Faculty of Medicine, Department of Biophysics; [4]Elazig Fethi Sekin Hospital Internal Medicine, Elazig; [5]Health Sciences University Hamidiye Faculty of Medicine, Department of Microbiology, Istanbul; [6]Firat University Faculty of Medicine, Department of Microbiology, Elazig, Türkiye

ABSTRACT

Aim: *The COVID-19 pandemic has shown the importance of laboratory-based diagnosis in the control of infectious diseases. Early and accurate diagnosis of COVID-19 is crucial for limiting the spread of infection and determining the treatment to be applied to patients. Our study compared the results of real-time polymerase chain reaction (RT-PCR) and rapid antigen tests to diagnose COVID-19.*

Material and Method: *Nasal and throat swab samples sent from 500 patients to our laboratory were studied by RT-PCR method with Multiplex RT-qPCR Diagnostic Kit 1000 rxn (CORONEX, Türkiye) and by Abbot COVID-19 Ag Rapid Test Device (Germany) immunochromotographic method by the recommendations of the manufacturer companies. The patient's Demographic information in the study was taken from the hospital information automation system.*

Results: *Of the 500 patients participating in the study, 202 (40.4%) were women, and 298 (59.6%) were men. While 57 patients (11.4%) were detected positively by the RT-PCR method, 54(10.4%) were detected positively by a rapid antigen test method. Of the 57 patients found positive by the RT-PCR method, 8 (14%) were negative by a rapid antigen test method. Of the 54 patients found positive by the rapid antigen test method, 5 (9.25%) were negative by the RT-PCR method. According to the RT-PCR method, the sensitivity of the rapid antigen test test was 90.74%, and the specificity was 98.21%.*

Conclusion: *Although the RT-PCR test is the gold standard method for COVID-19 detection, rapid antigen tests may be useful as a screening test or as a supportive test in diagnosis during periods of intense virus activity or epidemic situations.*

Key words: *COVID-19 diagnosis; RT-PCR; rapid antigen test*

ÖZET

Amaç: *COVID-19 pandemisi, bulaşıcı hastalıkların kontrolünde laboratuvara dayalı tanının ne kadar önemli olduğunu göstermiştir. COVID-19'un erken ve doğru tanısı hastalara uygulanacak tedavinin yanı sıra enfeksiyonun yayılımını sınırlanması için de çok büyük önem arz eder. Çalışmamızda, COVID-19 tanısı için Gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) ve Hızlı antijen test sonuçlarının karşılaştırılması amaçlandı.*

Materyal ve Metot: *Laboratuvarımıza, 500 hastadan gönderilen nazal ve boğaz sürüntü örnekleri Multipleks RT-qPCR Tanı Kiti 1000 rxn (CORONEX, Türkiye) ile RT-PCR yöntemiyle ve Abbot COVID-19 Ag Rapid Test Device (Germany) immünokromotografik yöntemiyle üretici firmaların önerileri doğrultusunda çalışıldı. Çalışmaya katılan hastalara ait demografik bilgiler hastane bilgi otomasyon sisteminden alındı.*

Bulgular: *Çalışmaya katılan 500 hastanın 202'si (%40,4) kadın 298'i (%59,6) erkekti. Hastaların 57'si (%11,4) RT-PCR yöntemiyle pozitif tespit edilirken, Hızlı antijen test yöntemiyle hastaların 54'ü (%10,4) pozitif olarak tespit edildi. RT-PCR yöntemiyle pozitif tespit edilen 57 hastanın 8'i (%14) Hızlı antijen test yöntemiyle negatif olarak tespit edildi. Hızlı antijen test yöntemiyle pozitif tespit edilen 54 hastanın 5'i (%9,25) RT-PCR yöntemiyle negatif olarak tespit edildi. RT-PCR yöntemine göre Hızlı antijen test testinin duyarlılığı (sensitivity) %90,74, özgüllüğü (specificity) %98,21 olarak tespit edildi.*

Sonuç: *COVID-19 tespitinde RT-PCR testi altın standart yöntem olmakla birlikte Hızlı antijen testleri, virüs aktivitesinin yoğun olduğu dönem veya salgın durumlarında tanıda tarama testi olarak ya da destekleyici test olarak kullanımının faydalı olabileceği sonucuna varıldı.*

Anahtar kelimeler: *COVID-19 tanı; RT-PCR; hızlı antijen test*

İletişim/Contact: *Feray Ferda Senol, Elazig Fethi Sekin City Hospital Microbiology, Elazig, Türkiye* • ***Tel:*** *0505 776 84 76* •
E-mail: *drferdasenol@yahoo.com* • ***Geliş/Received:*** *06.11.2023* • ***Kabul/Accepted:*** *15.08.2024*

ORCID: *Feray Ferda Şenol: 0000-0003-4705-5757 • Altay Babacan: 0000-0002-1508-1056 • Mete Özcan: 0000-0002-5551-4880 • Esra Timurkaan: 0000-0001-5654-7070 • Yüksel Akkaya: 0000-0002-3167-8055 • Özlem Aytaç: 0000-0002-3305-6284 • Pınar Öner: 0000-0001-9592-5986 • Zülal Aşçı Toraman: 0000-0001-5202-8564*

Introduction

On Sunday, December 31, 2019, the World Health Organization (WHO) reported that deaths due to acute respiratory failure and pneumonia, the cause of which could not be determined, were observed in the fish and live animal market in Wuhan, Hubei province of China, and that a new virus[1] may have caused this situation. On 30 January 2020, WHO declared COVID-19 an "International public health emergency." With COVID-19 patients being seen intensively in 113 countries, COVID-19 was declared a pandemic on March 11, 2020.

SARS-CoV-2 is an enveloped virus containing a single-stranded positive-stranded RNA genome within the family Coronaviridae. As a result of genome studies, they have reported that it is a member of the betacoronavirus family, including MERS-CoV and SARS-COV, which have caused outbreaks in different countries in the past two decades. As a genomic structure, SARS-CoV-2 was 79% similar to SARS-CoV and 50% similar to MERS-CoV[2]. There are four structural proteins belonging to the virus, M (membrane protein), E (envelope protein), S (spike protein), N (nucleocapsid protein). Among these proteins, the S protein plays a very important role, especially in the attachment of the virus to the host and its entry into the cell[3].

Transmission routes for SARS-CoV-2, as in other coronaviruses, are indicated to develop after inhalation of virus-containing particles and direct or indirect contact with the oral mucosa, conjunctiva, and nasal tract. The primary target receptor points for the host are Angiotensin-converting enzyme 2 (ACE2) receptors located in the oropharynx and upper respiratory tract, which are human respiratory epithelial cells. It may also be involved in SARS-CoV-2 transmission in our gastrointestinal tract[4,5].

Accurate and timely diagnosis of COVID-19 is necessary for new patients to be identified and for the pandemic process to be followed[6]. Microbiologically, three basic methods that are routinely used for the diagnosis of COVID-19 are at the forefront. Of these methods, molecular tests are based on replicating the nucleic acid belonging to the virus and are very important in diagnosing acute infections. Secondly, antigen tests help diagnose early infection periods when it is impossible to perform molecular tests and virus antigens are present in high quantities[7]. Thirdly, antibody tests are methods that can contribute to the diagnosis of encountering the infection at a later stage of the infection or determining whether an immune response has developed[8].

The RT-PCR method is the most preferred test for diagnosing COVID-19 and is considered the gold standard. The RNA extraction process can be performed from oropharyngeal and nasopharyngeal upper respiratory tract swab samples and lower respiratory tract samples such as bronchoalveolar lavage and sputum. It has been stated that the results of the samples taken from the nasopharynx are two times better than those from the oropharynx. The SARS-CoV-2 RNA has been isolated in blood, urine and fecal samples[9]. However, it was found that the reliability of these samples was less than that of respiratory tract samples. In collecting upper respiratory tract samples, taking them within a few days from the onset of complaints is recommended. The RT-PCR test is known not to detect other viruses by cross-reaction other than SARS-CoV-2, so its originality is quite high. Although a clear rate cannot be given for sensitivity, it is stated that it is between 63–78%[9]. The SARS-CoV-2 rapid antigen test test sensitivity is between 0% and 94%. The sensitivity of the SARS-CoV-2 rapid antigen test tests is Dec. To diagnose SARS-CoV-2, WHO recommends a minimum sensitivity of 80% and specificity of 97% for rapid diagnostic tests that can be used in patients with symptoms compatible with COVID-19[10].

The study aims to compare RT-PCR and rapid antigen tests used in diagnosing COVID-19 and to determine their diagnostic performance.

Materials and Methods

Five hundred samples of COVID-19 suspects, 230 women and 270 men, were included in this study between March 25, 2022, and March 30, 2022. The lowest age of the people was 20, the highest was 53, and the average was 36.2±3.29. The study was done with a nose and throat sample on a single swab. Samples For the RT-PCR test, a Multiplex RT-qPCR Diagnostic Kit [1000 rxn] was used with 15 µl extract and 5 µl patient sample for a total of 20 µl by the manufacturer's recommendations. Human RNaseP (Ribonuclease P) genes were targeted with SARS-CoV-2-specific 'ORFLAB'and' N'genes. For amplification, it has been worked with CFX96 (Biorad, USA) in the test analysis. Ct significance ≤35 values have been accepted as positive. For the rapid antigen test, the COVID-19 Ag Rapid Test (Abbot, Germany) was performed using the immunochromatographic method per the manufacturer' s recommendations. The operations were carried out in the biosafety cabinet. In the case of the color change of the test band, the result was evaluated as positive.

Statistical Analysis

Receiver operating characteristic (ROC) was used to identify the optimal rapid antigen test cut-off level and determine its sensitivity and specificity for COVID-19. In addition, the area under the ROC curve (AUC) was used to assess the diagnostic performance of rapid antigen testing in COVID-19. Finally, the Hosmer-Lemeshow fit test was conducted to determine the agreement between observed and model-predicted proportions of COVID-19. All statistical calculations were performed using the IBM Statistical Package for Social Sciences (SPSS) program version 21.0 commercial software (IBM Inc, Chicago, II, USA).

Results

Of the 500 patients in this study, 202 (47.6%) were women, 298 (52.4%) were men, and the average age was 48. Fifty seven patients (11%) tested positive, and 443 (88.6%) tested negative with the RT-PCR method. Fifty four out of 500 patients have tested negative, and 446 out of them have tested positive with the rapid antigen test method. 8 patients who have tested positive with RT-PCR have been negative with the rapid antigen test method. Meanwhile, 5 patients who tested negative with RT-PCR tested positive with rapid antigen test method (Table 1).

Table 1. *The sensitivity and specificity of the RAT method according to the RT-PCR method*

Gender	Number of patients	RT PCR(+)	RAT(+)	Sensitivity	Specificity
Male	298	35	33	%87.88	%97.74
Female	202	22	21	%95.24	%98.90
TOTAL	500	57	54	%90.74	%98.21

The obtained results indicated that the rapid antigen test successfully discriminates COVID-19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 1. The obtained results indicated that the rapid antigen test successfully discriminated -19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 1.

The obtained results in male patients indicated that the rapid antigen test successfully discriminated COVID-19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 2.

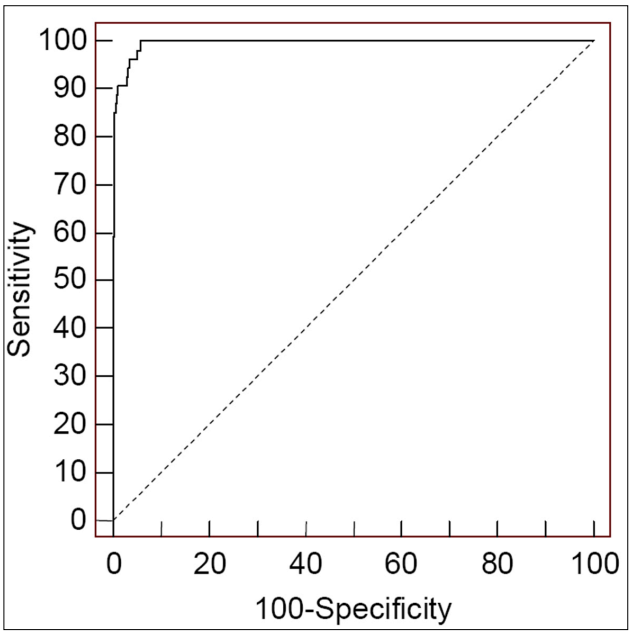

Figure 1. *ROC analysis of RAT tests in male and female patients.*

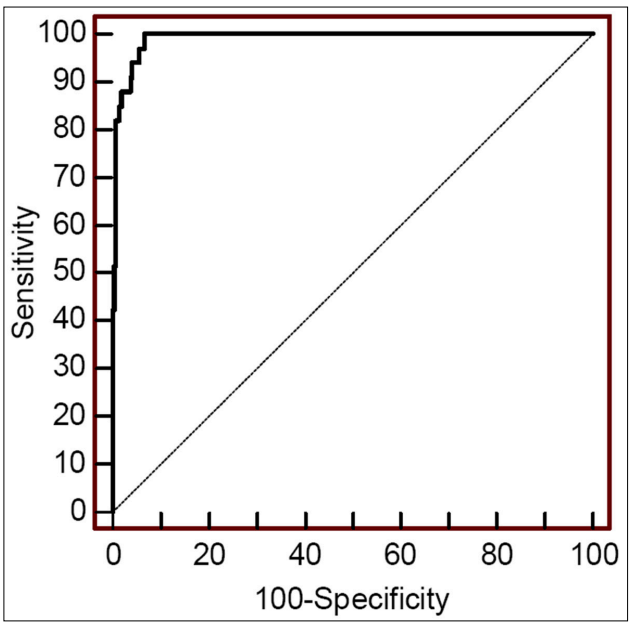

Figure 2. *ROC analysis of RAT tests in male patients.*

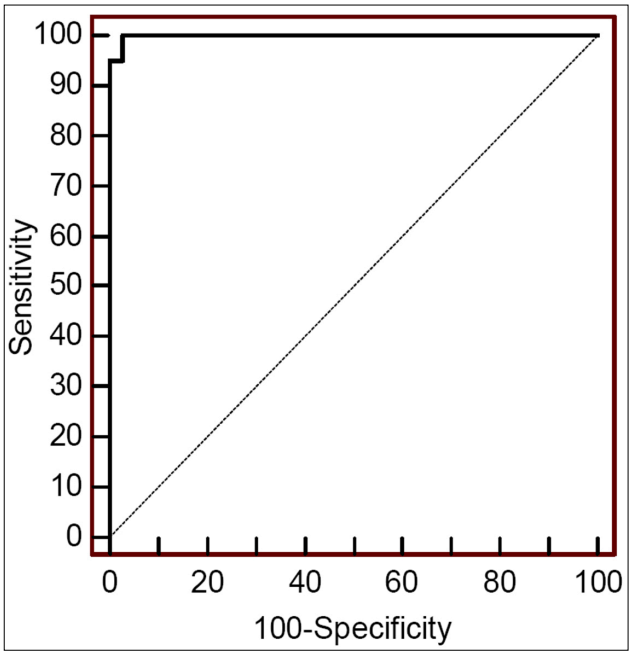

Figure 3. *ROC analysis of the RAT tests in female patients.*

The obtained results in female patients indicated that the rapid antigen test successfully discriminated -19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 3.

Discussion

Pneumonia caused by COVID-19 needs to be differentiated because it can be confused with viral pneumonias such as influenza, adenovirus, respiratory syncytial virus, and pneumonia caused by mycoplasma. Respiratory tract infections caused by COVID-19 can be seen simultaneously with other viral respiratory tract infections, and no specific clinical difference distinguishes them from each other[11].

RT-PCR test is accepted as the gold standard in the diagnosis of COVID-19. However, regardless of the method used, the sensitivity and specificity of RT-PCR tests are less than 100%. Reasons such as sampling and transfer, virus dynamics in the person, extraction of RNA, and RT-PCR method may cause false negative results. The sensitivity of the RT-PCR method is estimated to be 70% and specificity 95%. However, there is no ideal test for comparison[12,13]. Chaimayo C et al. stated that 60 respiratory samples out of 454 were positive, and 394 were negative with RT-PCR. When the same samples worked with rapid SARS-CoV-2 antigen test, compared to RT-PCR, the sensitivity rate was 98.3%, and the specificity rate was 98.7%[14]. Torres I. et al. found the sensitivity with the CLINITEST rapid antigen test as 80.2%, specificity of 100% in symptomatic patients with suspected COVID-19 and 60.0% specificity, 100% in asymptomatic close contacts of COVID-19 patients[15]. Diao B et al., after detecting 80%.1 of the 251 patients tested positive for -19 with RT-PCR, these patients were studied with Fluorescent Immunochromatography (FIC) also compared to RT-PCR the sensitivity rate was 75.6%, the specific rate was 80.5%[16]. Nasopharyngeal swabs of 50 suspected patients for SARS-CoV-2 have been studied with Cori's coronavirus disease 2019 Ag Respi-Strip, RT-PCR Allplex 2019n-CoV tests and 11 out of these patients tested negative with both of these tests. Meanwhile, 27 of them tested negative with rapid antigen tests. In 39 patient samples that tested positive with RT-PCR, the average Ct rate was 22.78; in 12 patient samples that tested positive for both RT-PCR and rapid antigen test, the average Ct rate was 17.37. Compared to RT-PCR, the rapid antigen test's sensitivity rate was 30.77%, the specificity rate was 100%, and the antigen test had better performance with a high viral load. In contrast, with the lower viral load, it has been detected that it missed positivity[17]. In another study, 75 samples tested positive for SARS-CoV-2 RT-PCR, and 75 samples tested negative for SARS-CoV-2 RT-PCR have been researched for sensitivity and specificity for SARS-CoV-2 rapid antigen test. According to Ct rates of rapid antigen test sensitivity, It has been detected that in <25 is 100%, in 25–30 is 95%, in 30–35 is 44%.8 and in ≥35 is 22%.2; specificity of Ct rates was 96%. Eleven patients who have been detected positive with RT-PCR also detected negative with indicate rapid antigen test[18]. By Chiu RYT et al., these 11 patient's Ct rate was 32.56±4.59, and this rate increase of the Ct caused false negativity[19]. In the study by Jaaskelainen AE. et al.; Sofia (Quidel), Standard Q (SD Biosensor) and Panbio (Abbott) (three rapid tests) specificity rate was 100%, and the sensitivity rate for Pambio rapid antigen test was 87%, for Standard Q was 89% and for Sofia rapid antigen test was 92% compared to those which had a Ct rate below 25.)[20]. In our study, we found the sensitivity of the rapid antigen test kit to be 87.88%, the specificity as 97.74% in male patients, the sensitivity of the rapid antigen test kit as 95.24% and the specificity as 98.90% in female patients, and the sensitivity of the rapid antigen test kit as 90.74% and the specificity as 98.21% in all patients.

There was no significant difference between genders for rapid antigen tests. (p>0, 005)

As a result, it has been determined that the use of rapid antigen tests, which can be applied at the bedside, as an alternative to RT-PCR tests, can be beneficial in the diagnosis of COVID-19 due to reasons such as RT-PCR tests working in laboratory conditions, sample transfer, experienced personnel, high cost, working with the device, and evaluation of the result by the physician. However, as it is a qualitative test, it should be kept in mind that when false negative or false positive results of the test are considered, a negative result cannot exclude the possibility of COVID-19. For these reasons, the patient's clinical symptoms should be evaluated, and the diagnosis should be confirmed by RT-PCR test when necessary.

Statement of Ethics

Approval was obtained from the Firat University Faculty of Medicine/Türkiye Ethics Committee (Decision No: 2022/07–36 Date: 26.05.2022).

Conflict of Interest Statement

The authors declared no conflict of interest related to the article.

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