An evaluation of rapid antigen tests diagnostic performance in the detection of SARS-CoV-2 infection

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Abstract

Aim: The study aimed simultaneously compare the diagnostic performance of the rapid antigen test (CoVard COVID-19 Rapid Antigen Test Kit, Turkey) and the RT-PCR test used in the detection of SARS-CoV-2 infection.

Materials and Methods: In this study, the results of rapid antigen tests and RT-PCR tests applied simultaneously to the diagnosis of SARS-CoV-2 infection on nasopharyngeal swab specimens taken from patients applying to the Mersin University Hospital Emergency Unit with various symptoms were retrospectively analyzed. The study included 308 patients with or without respiratory tract infection symptoms who underwent COVID-19 rapid antigen and RT-PCR testing.

Results: It was observed that 157 (51.0%) of the patients had symptoms related to COVID-19. A total of 50 (16.2%; 95%CI: 12.0-20.5%) of the patients' rapid antigen test and 97 (31.5%; 95%CI: 26.3-37.0) of the patients' RT-PCR test were positive. The rapid antigen test for 41 (42.3%; 95%CI: 32.0-52.9) out of 97 specimens with a positive RT-PCR test was also positive. The rapid antigen test was positive in nine (18%; 95%CI: 8.6-28.6) specimens while the RT-PCR test was negative. The concordance between the rapid antigen test and the RT-PCR was intermediate (k=0.437, p<0.0005).

Conclusion: Compared to nucleic acid-based tests, rapid antigen tests are practical and fast, as well as not requiring experienced personnel and special laboratory infrastructure. It was concluded that the use of rapid antigen tests will help provide rapid triage in emergency services, especially during the times when cases with COVID-19 are on the rise.

Introduction

Due to the pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, more than 489 million cases and more than six million deaths have been reported in different age groups worldwide [1]. Despite the mass vaccination applications carried out in the later stages of the pandemic, rapid detection, treatment, and follow-up of individuals infected with SARS-CoV-2 continued to be an important strategy in the control of the epidemic. Computed tomography (CT), molecular, and immunological techniques have been used in the laboratory diagnosis of SARS-CoV-2 infection in our country and around the world since the beginning of the epidemic [2]. In the guide published by the World Health Organization on March 2, 2020, the real-time polymerase chain reaction (RT-PCR) technique was recommended as the primary diagnostic test in the diagnosis of asymptomatic and symptomatic patients suspected of having COVID-19 [3].

During the pandemic, the RT-PCR technique has become a widely used test for the diagnosis of SARS-CoV-2 infection. The RT-PCR test detects the viral load in patients with suspected COVID-19 with the cycle threshold (Ct) and this value is determined inversely with the viral load [4]. The Ct value obtained as a result of RT-PCR has been reported as an important parameter for the evaluation of the risk of viral spread and the control of infection [5]. When the Ct value is ≥ 35 and above, it is determined that the presence of infectious virus particles in the cell culture is 6.9% and the risk of viral spread continues [4,6]. Patients with a Ct value <24 are classified in the high-risk group, those with Ct ≤ 24 and <31 in the intermediate risk group, and those between 31 and 38 in the low-risk group [7]. Although the performance of the RT-PCR technique in the diagnosis of SARS-CoV-2 infection is high, it is an

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expensive, time-consuming method that requires appropriate laboratory infrastructure, equipment, and well-trained staff. The first of the difficulties that arose in the implementation of the RT-PCR test was the difficulty of controlling infection in health institutions in countries with limited resources that did not have the appropriate laboratory infrastructure for RT-PCR, and the second was that the laboratories worked at over their capacity due to the rapid increase in the number of suspected COVID-19 cases. At this point, rapid antigen tests offered a practical, low-cost, fast, and economical alternative that did not require expertise in detecting suspected cases of COVID-19 [8]. Rapid antigen tests applied to nasopharyngeal and oropharyngeal specimens directly detect SARS-CoV-2 virus antigens. Rapid antigen tests are divided into two groups: antigen (Ag) tests directly detecting the SARS-CoV-2 virus antigen (nucleoprotein/nucleocapsid protein) and antibody (Ab) tests detecting one or more types of antibodies [9]. More than 150 rapid antigen test kits have been approved after the United States (US) Food and Drug Administration approved the first SARS-CoV-2 rapid antigen test in May 2020 [10]. In studies on the clinical use of rapid antigen tests, it was determined that the sensitivity of these tests depends on various factors such as the time from the onset of infection, the virus concentration in the specimen, and the formulation of the reagents in the test kits [11,12].

The main objective of the study was to simultaneously compare the diagnostic performances of the rapid antigen test (CoVard COVID-19 Rapid Antigen Test Kit, Turkey) and the RT-PCR test used in the detection of SARS-CoV-2 infection.

Materials and Methods

In this study, the results of rapid antigen tests and RT-PCR tests applied simultaneously to the diagnosis of SARS-CoV-2 infection on nasopharyngeal swab specimens taken from patients applying to the Mersin University Hospital Emergency Unit with various symptoms between February 25, 2022 and May 20, 2022 were retrospectively analyzed. This study was reviewed and approved by the Mersin University Clinical Research Ethics Committee (Date: 20/07/2022 and Decision No: 2022/498). The study included 308 patients with or without respiratory tract infection symptoms who underwent COVID-19 rapid antigen and RT-PCR testing. Demographic characteristics and symptom information for patients with positive rapid antigen test or SARS-CoV-2 RT-PCR test were obtained from the hospital information system.

Rapid antigen test

The CoVard COVID-19 (Turkey) rapid antigen test kit is a lateral flow sandwich test. This test uses a double-antibody sandwich method to detect SARS-CoV-2 infection from nasopharyngeal and oropharyngeal swab specimens. The monoclonal antibody in the test kit binds to the SARS-CoV-2 antigen in the patient specimen and forms a complex. The reaction complex forms a red reaction line in the control area (C) in the test cassette. If the patient specimen contains the SARS-CoV-2 antigen, a red reaction line will emerge in the T region. While nasopharyngeal and oropharyngeal swab specimens taken from the patients were dipped into the lysis solution in the test tube, the specimen was completely eluted into the buffer solution. The specimens in the test tube were transferred to the specimen well in the test cassette with the help of a dropper. After a waiting period of 15 minutes, the reaction line formation in the C and T regions of the test cassette was evaluated.

RT-PCR test

Nasopharyngeal and oropharyngeal swab specimens were utilized for evaluation of SARS-CoV-2 by RT-PCR. After isolation of viral RNA with the vNAT solution, a RT-PCR test was performed. The DS CORONEX COVID-19 (Ver.2.0) (DS Bio ve Nano Teknoloji, Turkey) kit used in the RT-PCR test targets the N and Orf1ab gene region specific to SARS-CoV-2. The RT-PCR test was performed by Qiagen Rotor-Gene as 35 cycles at 95°C for 5 seconds, at 55°C for 1 second, and after 5 minutes of pre-denaturation at 45°C and 1 minute at 95°C in accordance with the protocol recommended by the manufacturer. It was performed on a Q5plex real time PCR device.

Statistical analysis

Statistical analyzes were performed using the statistical software IBM SPSS Statistics (version 20, Armonk, NY: IBM Corp). Continuous data were presented as mean, median, min-max, and confidence interval (CI), and categorical data were presented in numbers and percentage. The Kolmogorov Smirnov test was analyzed for the normality test. An independent variable t-test was applied to those with normal distribution. The SARS-CoV-2 RT-PCR was considered the gold standard method, Cohen's kappa coefficient (κ) was used to evaluate the concordance between tests, and a p-value less than or equal to 0.05 was considered significant.

Results

The mean age of the 308 patients included in the study was 47.7±29.7 years. Of these, 142 (46.1%) were female and 166 (53.9%) were male. It was observed that 157 (51.0%) of the patients had symptoms related to COVID-19. A total of 50 (16.2%; 95% CI: 12.0-20.5) of the patients' rapid antigen test and 97 (31.5%; 95% CI: 26.3-37.0) of the patients' RT-PCR test were positive. The rapid antigen test for 41 (42.3%; 95% CI: 32.0-52.9) out of 97 specimens with a positive RT-PCR test was also positive (Table 1). The rapid antigen test was positive in nine (18%; 95% CI: 8.6-28.6) specimens while the RT-PCR test was negative. Rapid antigen test false negativity was found to be significantly lower in specimens with positive RT-PCR test and a Ct value <15 compared to specimens with a Ct value ≥15 (36.7%-67.2%; p=0.005, Pearson Chi-square test). Accuracy, specificity, sensitivity, negative predictive value, and positive predictive values of rapid antigen test were 78.9%, 95.7%, 42.3%, 78.3%, and 82.0% respectively. Rapid antigen test positivity was detected in 21.7% (n=34) of the patients with COVID-19 related symptoms (p=0.009), and RT-PCR test positivity was detected in

Table 1. Comparison of RT-PCR test and rapid antigen test.

| RT-PCR Result | Total n | Rapid antigen test result | | | |
| | | Positive | | Negative | |
		n (%)	Ct value Median (Min-Max)	n (%)	Ct value Median (Min-Max)
Positive					
Ct<15	30	19 (63.3)	13.1 (7.6-14.8)	11 (36.7)	13.67 (6.2-14.8)
15≤Ct<20	38	17 (44.7)	17.0 (15.0-19.4)	21 (55.3)	18.1 (15.6-19.8)
20≤Ct<25	21	3 (14.3)	23.8 (20.5-24.2)	18 (85.7)	22.7 (20.5-24.6)
25≤	8	2 (25.0)	25.6 (25.1-26.2)	6 (75.0)	26.2 (25.8-27.1)
Total	97	41 (42.3)	15.2 (7.6-26.2)	56 (57.7)	18.9 (6.2-27.1)
Negative	211	9 (4.3)	-	202 (95.7)	-
Total	308	50 (16.2)	-	258 (83.8)	-

Table 2. RT-PCR and rapid antigen test results according to patient characteristics.

| Characteristics | Total n (%) | RT-PCR Positive | | | | Rapid antigen positive | |
		n (%)	p	Ct(Mean) (95% CI)	p	n (%)	p
Age							
<65	182 (59.1)	56 (30.8)	0.742	19.2 (18.0-20.3)	0.001	29 (15.9)	0.864
≥65	126 (40.9)	41 (32.5)		16.0 (14.6-17.4)		21 (16.7)	
Sex							
Female	142 (46.1)	41 (28.9)	0.360	17.6 (16.2-19.1)	0.699	17 (12.0)	0.061
Male	166 (53.9)	56 (33.7)		18.0 (16.8-19.1)		33 (19.9)	
COVID-19 related symptoms							
Yes	157 (51.0)	68 (43.3)	<0.0005	17.4 (16.3-18.6)	0.141	34 (21.7)	0.009
No	151 (49.0)	29 (19.2)		18.9 (17.5-20.2)		16 (10.6)	
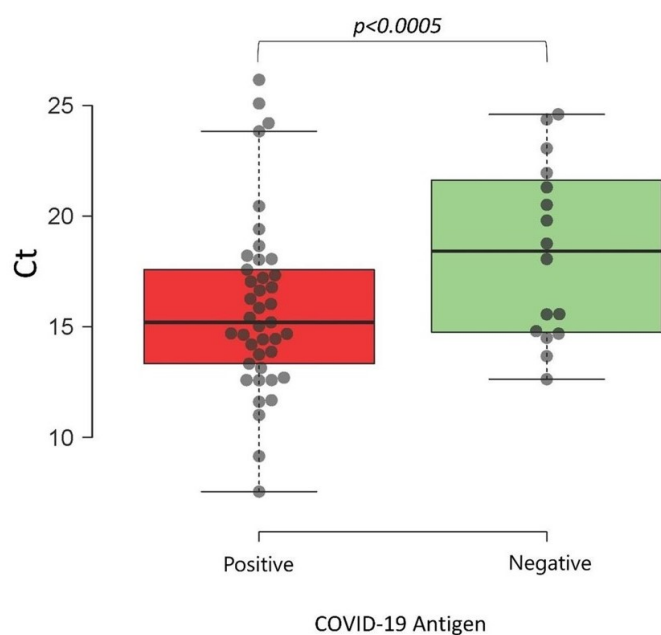
Figure 1. Distribution of Ct values according to the COVID-19 rapid antigen test results.

43.3% (n=68) (p<0.0005) (Table 2). The concordance between the COVID-19 rapid antigen test and the RT-PCR was intermediate (k=0.437, p<0.0005). The mean Ct value of the patients with rapid antigen test positivity was found to be significantly lower than the patients with a negative COVID-19 antigen test (rapid antigen test positive mean Ct: 15.88±4.00, rapid antigen test negative mean Ct: 19.25±4.59; p<0.0005, independent variable t-test) (Figure 1). The mean Ct value in the <65 age group was 19.2 (95% CI: 18.0-20.3) while the mean Ct value in the ≥65 group was 16 (95% CI: 14.6-17.4) (p=0.001) (Table 2).

The RT-PCR test was positive in 43.3% and the rapid antigen test was positive in 21.7% (p<0.05) of patients with COVID-19 related symptoms. While the RT-PCR test was positive in 19.2% of the patients who did not have COVID-19 symptoms or who had come to the emergency unit for other reasons, the rapid antigen test was positive in 10.6% of them. There was no significant difference in mean Ct values between those with and without COVID-19 symptoms (95% CI:17.5-20.2; p=0.141). There was no statistically significant difference in RT-PCR and rapid antigen test results in male and female patients.

Discussion

The rapid and accurate diagnosis of infected cases still maintains its importance in controlling the spread of SARS-CoV-2 infection. The increased demand for RT-PCR tests in the periods when the cases with COVID-19 increased caused delays in the reporting of positive patients, making it difficult to follow up and check on contacted individuals. Besides that, the difficulty in accessing molecular tests and inadequate laboratory infrastructure in many health centers in developing or low-income countries increased the tendency to use rapid antigen tests as a preliminary screening test [13,14]. However, another reason for the widespread use of rapid antigen tests is the low cost compared to the RT-PCR test [8]. The Cochrane systematic review evaluating the rapid antigen tests available in the diagnosis of SARS-CoV-2 infection in the early stages of the pandemic reported that the sensitivity of these tests was 56.2% (95% CI: 29.5-79.8) and the specificity on average was 99.5% (95% CI: 98.1-99.9) [15].

In a study comparing five different rapid antigen tests, the sensitivity of these tests was found to be between 64.9% and 91.7% in the patient group with a Ct value of 30 [11]. In that study, it was determined that the sensitivity of rapid antigen tests was high in specimens with a Ct value of 30. Randriamahazo et al. determined the sensitivity and specificity of the rapid antigen test between 62.66% and 100% in specimens with a Ct value of 29 [8]. In another study comparing six rapid antigen tests, the overall sensitivity of rapid antigen tests was between 65% and 79%, and the specificity (for all) was 100% [16]. In the study, the sensitivity was found to be higher in specimens with an RT-PCR Ct below 25 and in specimens taken from patients presenting in the first week of symptoms. In a study conducted in Brazil, it was detected that the concordance between the rapid antigen test and the RT-PCR test was 97% at $\leq$25 Ct values while this rate decreased at $\geq$25 Ct values [14]. Shaw et al. reported that concordance between rapid antigen tests and RT-PCR tests was 54.5% [17]. Additionally, all specimens positive with a rapid antigen test were confirmed by RT-PCR, and specimens with Ct$\leq$28.8 could be detected by a rapid antigen test in that study. Bulilete et al. determined that the sensitivity of the rapid antigen test was 71.4% (95% CI: 63.1-78.7) [18]. Furthermore, researchers indicated that the sensitivity was 80.4% (95% CI: 70.5-88.1) for symptomatic patients and 83.1% (95% CI: 71.9-90.5) for patients reporting symptoms within five days [18]. Alghunaim M et al. reported that, as the viral load increased (Ct<25) in the symptomatic patient group, the diagnostic performance of rapid antigen tests increased [19]. The diagnostic performances of 12 rapid antigen test kits were evaluated in a study conducted in Turkey and the research indicated that the sensitivities of rapid antigen tests varied between 40.4-97.5% (Mean: 54.8%). That study reported that sensitivity of rapid antigen tests increased in specimens below Ct<25 [20]. Daloğlu et al. detected the sensitivity of rapid antigen tests as 92.6% in specimens with a Ct value of <17, 88.7% in specimens with a Ct value of <20, and 77.8% in specimens with a Ct of <22 [21]. Cirit et al. determined the sensitivity of the rapid antigen test as 82.7% in specimens with Ct<25 and 95.7% in specimens with 20$\leq$Ct

[22]. The rapid antigen test of 42.3% (95% CI: 32.0-52.9) of the RT-PCR positive specimens was also positive in this study. This ratio was found to be 63.3% in specimens with Ct <15. Similar to the studies in the literature, it was determined that the sensitivity of the rapid antigen test decreased as the Ct value increased.

The rapid antigen test was positive in only nine (2.9%) specimens while the RT-PCR test was negative. Even though specimens were taken simultaneously, possible differences during collection may be a reason for test inconsistencies between the two specimens.

In our study, it was observed that the mean Ct value (Ct:16.00) of the patients in the $\geq$65 group was significantly lower than the other age groups. It possible that the deficiencies in the immune system due to advanced age cause the virus to be found more in the respiratory tract and, as a result, the viral load in the specimens was high. However, it was expected that the mean Ct value (Ct: 17.4) of patients with COVID-19 symptoms was found to be lower than the others in our study. However, no significant decrease was observed in the values of these patients. This suggests that the viral load may also be high in the asymptomatic patient group.

Consequently, it has been determined that the sensitivity of rapid antigen tests increases in direct proportion to the increase in viral load when the Ct value is $\leq$20 based on recent studies. It was concluded that the analytical performance of rapid antigen tests varies with the viral load in the specimen. However, the tests of patients with suspected COVID-19 or symptoms of COVID-19 who are negative with rapid antigen tests should be confirmed by an RT-PCR test. Compared to nucleic acid-based tests, rapid antigen tests are practical and fast, as well as not requiring experienced personnel and special laboratory infrastructure. It was concluded that the use of rapid antigen tests will help provide rapid triage in emergency services, especially during the times when cases with COVID-19 are on the rise.

Ethical approval

This study was reviewed and approved by the Mersin University Clinical Research Ethics Committee (Date: 20/07/2022 and Decision No: 2022/498).

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