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RESEARCH ARTICLE

Comparison of SARS-CoV-2 Rapid Antigen Test Performance Using RT-qPCR for Diagnosis of COVID-19

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ABSTRACT

Objective: The purpose of this study is to appraise the performance of the rapid antigen detection test (RADT) which is an immunochromatographic test to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen, compared to a real-time reverse transcriptase-polymerase chain reaction (RT-qPCR).

Materials and Methods: Nasopharyngeal samples were taken from 185 volunteers and SARS-CoV-2 RADT and RT-qPCR tests were performed simultaneously. The results were evaluated in the light of parameters such as age, gender, cycle threshold (Ct) values of RT-qPCR, and symptoms.

Results: RT-qPCR and SARS-CoV-2 RADT results of 148 participants from our study group were compatible, inconsistency was observed in the results of 37 participants. In general, the sensitivity and specificity for RADT were 63% and 100%, respectively. In the heterogeneous study group, the accuracy of the antigen test was found to be 80% (Cohen's K=0.690, 95%, p<0.001). When the Ct value was <20, the accuracy of the test was 85%>.

Conclusion: The results highlight that COVID-19 antigen detection with the RADT we used has the potential to present as an alternative diagnostic method in patients with high viral load, especially in the early and infective stages of disease.

Keywords: Rapid antigen detection test, RT-qPCR, SARS-CoV-2

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense RNA *virus* belonging to β-coronavirus genus. The World Health Organization (WHO) has been declared COVID-19 as a pandemic in March 2020.[1] The COVID-19 pandemic continues to spread rapidly and poses a challenge to health-care systems. The gold standard for the diagnosis of SARS-CoV-2 infection is to work with the real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) method.[2,3] In the RT-qPCR method, detection of nucleic acid takes hours. At the same time, special equipment, devices, and experts are required to perform the test. Therefore, there is a need for alternative tests that are easy to perform and give fast results. Rapid antigen detection tests (RADTs) that qualitatively detect SARS-CoV-2 antigen have been developed, RADTs are systems that give positive or negative results by recognizing the viral antigen with the SARS-CoV-2 antibody. There is no need for another device for the evaluation of the test results which can be obtained in a short time. The number of conformite europenne-approved rapid antigen products was more than 200 during this period. There is not enough scientific data about the accuracy and performance of RADTs.[4,5] In our study, we planned to compare the performance and accuracy of one of the commercially available SARS-CoV-2 RADT with the RT-qPCR.

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MATERIALS and METHODS

In this study, samples taken from 185 patients with respiratory symptoms or fever between July and September 2021 were examined. The sample was taken as a nasopharyngeal swab with the help of sterile swap. Swap was placed in the sample tube containing viral nucleic acid buffer, the cap of the tube was closed, and it was delivered to the diagnostic laboratory in appropriate transport standards. The samples were tested within 2 h. Nasopharyngeal samples were vortexed for 3–5 s before testing and studied in accordance with the operating instructions of the Diagnovital® SARS-CoV-2 Real-Time PCR Kit v2.0, (RTA Laboratories Inc, Istanbul, Turkey). Viral RNA extraction from samples was performed according to the company instructions. A negative sample (human specimen) was included in every RNA extraction procedure, and a non-template (water) sample was included in every RT-qPCR run as negative controls. An internal control amplification was performed to check RNA extraction and RT-qPCR quality. According to the applications of Roche SARS-CoV-2 rapid antigen test: 3 drops of the extracted sample were placed in the sample well of the test device at a 90° angle and after 15–30 min, the result has been read.

Informed consent was obtained from all volunteers. All procedures were approved by the Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee, (Date: July 08, 2021, No: 80929729-000-11896). It was studied in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

The "Statistical Package for the Social Sciences 27.0 program (IBM Corp., Armonk, NY, USA)" was used in statistics. While analyzing the data descriptive statistical methods (mean, percentage, and frequency) were used. In addition, tables and 2×2 cross tables are provided. To measure the power and performance of the test, its specificity and sensitivity were analyzed and calculated. Cohen's Kappa Test was applied to measure the accuracy, compatibility, and reliability of the test.

RESULTS

The SARS-CoV-2 results of samples taken from a total of 185 individuals were evaluated with the RADT and RT-qPCR methods. Accordingly, 46.5% of the samples used in the study belonged to men and 53.5% to women. The age distributions of the participants are given in Table 1. Seventeen participants were under the age of 20 (9.2%), 60 participants were between the ages of 21–30 (32.4%), 52 participants were between the ages of 31–40 (28.1%), 37 participants were between the ages of 41–50 (20%), and finally 19 participants were identified as over 51 years old (10.3%) (Table 1).

Out of 185 samples, 63 (34%) tested positive by RT-qPCR and RAD, and 85 (45%) tested negative by both methods. Discordant results were found in 37 patients who were false negative (21%) (Table 2).

In general, the specificity and sensitivity for the detection of SARS-CoV-2 infection in the antigen test were found 63% and 100%, respectively (Table 3). The accuracy of the antigen test was found to be 80% (Cohen's K=0.690, 95%, p<0.001).

When the data of the patients with false negative results were examined; 54% of the participants were female and 45% were male and the mean age was 34.08. In addition, when the vital status of the participants who showed discordant results in is examined, it is observed that the patient's vital status was normal in 100%, and none of the participants were hospitalized (Table 4). Considering the contact of the participants, 32% were found to be exposed to COVID. About 67% of participants showed symptoms of COVID-19. While tiredness was the most common symptom with 27% of these symptoms, joint pain was observed afterward.

The cycle threshold (Ct) values, age, sex, and symptom display characteristics of individuals, showing that discordant results between RADT and the RT-qPCR are given in Table 5. It was determined that the mean Ct value of samples with discordant results was 26.71 (17–36.2).

DISCUSSION

Given the ongoing COVID-19 pandemic, diagnostic testing for SARS-CoV-2 is critical to limit the spread of the virus in the community and to manage patients' treatment and follow-up appropriately.[6,7] Our study included 185 (86 females and 99 males) cases who came to the hospital for testing on suspicion of COVID-19. In the study, it was determined that there were 100 positive and 85 negative patients in total according to the q-RT-PCR test, which was the control group. When the results were compared with the antigen test, it was found that 148 results were Ag negative PCR negative and 37 results were Ag negative PCR positive. When the demographic distribution of the study group was examined, it was determined that the majority of the participants were men, and were mostly between the ages of 21–40.

The WHO recommends the use of kits with a sensitivity of ≥80% and a specificity of more than 97% in its guidelines on the use of rapid antigen kits.[1] Möckel et al.[8] analyzed 473 patients. In the adult cohort, the sensitivity of the RAD test was 75.3% and the specificity was 100% with a SARS-CoV-2 prevalence of 32.8%; the positive predictive value was 100%, and the negative predictive value was 89.2%. In the

Table 1. Ages of the subjects participating in the study

Age range	n	%
20>	17	9.2
21–30 age	60	32.4
31–40 age	52	28.1
41–50 age	37	20.0
51<	19	10.3
Total	185	100

n: Number

Table 2. Summary of the results of the SARS-CoV-2 rapid antigen test compared to RT-qPCR

RT-qPCR	SARS-CoV-2 rapid antigen test results		
	Positive	Negative	Total
Positive	63	37	100
Negative	0	85	85
Total	63	122	185

RT-qPCR: Reverse transcriptase-polymerase chain reaction

pediatric cohort, the sensitivity was 72.0%, the specificity was 99.4% with a prevalence of 12.4%; the positive predictive value was 94.7% and the negative predictive value was 96.2%.[8] According to Krüttgen et al.,[9] they determined the sensitivity and specificity of the RAD test. The specificity was determined as 96%. Grouped their studies as <25, 25–<30, 30–<35, and ≥35 based on the Ct, and the test sensitivity was 100%, 95%, 44.8%, and 22.2%, respectively.[9] In this study, we evaluated the performance of the SARS-CoV-2 antigen test in this context by comparing it with RT-qPCR. In this assessment, the SARS-CoV-2 kit shows an excellent accuracy of >80% and a specificity of 100% when using a Ct value of ≤35. The highest sensitivity rate was found in samples with a Ct value of ≤20, which corresponds to a higher viral load. At Ct ≤20, sensitivity was 85%> and specificity was 100%. As might be expected, diagnostic sensitivity was largely dependent on viral load. Therefore, careful local evaluation of analytical and clinical performance is vital before performing any rapid antigen SARS-CoV-2 test in routine COVID-19 diagnosis.

In another study comparing RT-PCR and SARS-CoV-2 rapid antigen kit on 381 patients in Italy, it was determined that the accuracy was 80–86%, the sensitivity was 63.9–71.1%, and the specificity was 99.4%. They reported that the sensitivity was 97% when the Ct value was <20, 50.3–80.6% in the

Table 3. Sensitivity and specificity of Roche SARS-CoV-2 rapid antigen test

	%
Sensitivity	63.0
Specificity	100.0
Positive predictive value	100.0
Negative predictive value	69.7
Accuracy (correctly classified)	80.0

Table 4. Information on patients with false negative results

	%
Female	54.1
Male	45.9
Average age	34.08
Patient vital status	
Outpatient	100
Inpatient	0
Contact status with COVID 19 patient	32.43
COVID-19 symptom	67.6
Tiredness	27.0
Joint pain	16.2
Cough	10.8
Anosmia	2.7
COVID-19 Symptom none at all	32.4
Ct Average	26.71

Ct: Cycle threshold

range of Ct 25–30, and decreased to 4% in the case of Ct >35.[10] Similar to our study, it was observed that the decrease in viral load decreased the sensitivity of the rapid antigen test. In a similar study conducted in Japan, the SARS-CoV-2 rapid antigen kit showed 77% accuracy, 70% sensitivity, and 100% specificity.[11] Differences may be due to different factors such as analytical performance of rapid antigenic tests, viral load, sample quality and how it is processed, and heterogeneity of the administered group.

Albert et al.[12] examined the correlation of RT-PCR and RADT results with Ct values and SARS-CoV-2 RNA viral loads. They took samples from 412 patients. As a result of RT-PCR and RADT studies, 43 tests (10.4%) were found positive by both methods, and 358 test results (86.9%) were found negative. Inconsistent results (RT-PCR+/RADT–) were obtained as a result of the study of 11 tests (2.7%) performed with both methods. Examining the results of patients with RT-PCR+/RADT

Table 5. Information of patients regarding discordant results

Number	Age	Gender	Contact status	COVID-19 symptom	Ct value
1	66	M	Negative	Positive	20.00
2	56	F	Negative	Positive	29.00
3	52	M	Positive	Positive	34.13
4	50	M	Negative	Positive	25.00
5	49	M	Negative	Negative	20.29
6	47	M	Negative	Negative	29.00
7	47	M	Negative	Negative	26.80
8	46	M	Negative	Positive	26.58
9	44	F	Negative	Positive	17.00
10	44	F	Negative	Negative	25.13
11	42	F	Negative	Negative	17.00
12	41	F	Negative	Negative	26.44
13	41	F	Positive	Negative	30.99
14	39	M	Positive	Positive	21.00
15	38	F	Negative	Negative	25.00
16	37	F	Negative	Negative	26.90
17	34	F	Negative	Negative	23.33
18	30	M	Negative	Positive	29.81
19	30	M	Positive	Positive	21.00
20	29	M	Positive	Positive	32.67
21	29	F	Negative	Negative	28.90
22	28	M	Negative	Negative	25.00
23	26	M	Negative	Positive	27.38
24	26	F	Negative	Positive	30.81
25	26	M	Negative	Positive	23.00
26	25	F	Negative	Positive	31.43
27	24	F	Negative	Positive	29.36
28	24	M	Positive	Positive	29.00
29	24	F	Negative	Positive	26.00
30	23	F	Negative	Negative	25.77
31	22	M	Positive	Positive	36.2
32	22	F	Positive	Positive	23.00
33	21	F	Negative	Negative	29.00
34	20	F	Positive	Positive	28.30
35	20	M	Negative	Positive	30.66
36	20	F	Positive	Positive	29.49
37	19	F	Positive	Positive	28.17

Ct: Cycle threshold; F: Female; M: Male

– in the study, the Ct values were significantly higher, and SARS-COV-2 RNA loads were significantly lower ($p<0.001$) compared to the RT-PCR+/RADT+ samples. In their study, they showed that there were inconsistent results between RT-PCR and RADT methods when the SARS-CoV-2 RNA viral load was <5.9 log10 copies/mL and the RT-PCR Ct>25.[12] In our study, out of 185 patients, 63 (34%) tested positive by RT-qPCR and RADT, and 85 (45%) tested negative by both

methods, showing discordant results (RT-qPCR+/RADT–) in 37 patients (21%). It was determined that the mean Ct value of those with discordance results was 26.71 (17–36.2). As anticipated, the overall RADT sensitivity was directly dependent on the RT-PCR Ct values (SARS-CoV-2 RNA loads).

RADTs are less sensitive than RT-qPCR.[13–15] Therefore, it may be difficult to detect in the very early or later stages of SARS-CoV-2 infection using RADTs. However, sometimes even detecting the virus by RT-qPCR is difficult. For example, in cases where different types of mutations are seen, RT-qPCR may not be able to detect all variants.[11,16] Performing both PCR and Ag tests will increase the sensitivity even more to complete the deficiencies of each test used and to provide an accurate diagnosis of COVID-19.

Aydin et al.[17] studied 35,443 patients and found the mean age to be 50.6±22.3. Of this total, 16,902 (47.7%) were female and 502 (1.4%) were foreign nationals. In terms of symptoms, fever in 18,958 (53.50%) cases, cough in 18.359 (51.86%) cases, shortness of breath in 21.121 (59.60%) cases, and all three symptoms in 9.619 cases. In our study, 54% of the participants were female and 45% male, with a mean age of 34.08. About 67% of the participants showed COVID-19 symptoms. The most common symptom was fatigue with 27% of these symptoms, followed by joint pain.

Albert et al.[12] as a result of their study in symptomatic patients (n=412) attending primary health care centers, it was shown that COVID-19 patients proven by RT-PCR are less likely to be contagious when negative with RADT. Therefore, it can be thought that false-negative RADT results may be insignificant in terms of public health. This laboratory diagnostic approach, which can be applied in non-hospitalized patients, will certainly alleviate laboratory workloads in terms of time, cost, and laboratory personnel to be assigned in terms of RT-PCR tests.[12] In our study, discordant results were found in 37 patients which were false negative (21%). Covid symptoms were observed in 26 (70.2%) of these 37 patients, while fatigue (27%) and joint pain (16.2%) were the main symptoms. From a public health perspective, it may be recommended to compare RADT and RT-PCR tests in hospitalized patient groups with more severe symptoms.

CONCLUSION

The results highlight that COVID-19 detection with the SARS-CoV-2 RADT kit has the potential to present as an alternative diagnostic method in patients with high viral load, especially in the early and infective stages of infection and also in outpatients with mild symptoms.

Disclosures

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Ethics Committee Approval: The study was approved by the İstanbul Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee (No: 80929729-000-11896, Date: 08/07/2021).

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