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Correlation of Rapid Antibody and RT-PCR Tests With Clinical and Radiological Findings in COVID-19 Patients Admitted to an Employee Health Outpatient Clinic

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ABSTRACT

BACKGROUND/AIM: To evaluate the results of simultaneous rapid antibody tests and Real-time polymerase chain reaction (RT-PCR) tests in patients diagnosed with coronavirus disease-2019 (COVID-19) retrospectively, and to evaluate the compatibility rates of these results with clinical and radiological findings.

MATERIALS AND METHODS: Between March 31, 2020 and July 31, 2020, simultaneous COVID-19 RT-PCR and COVID-19 rapid antibody assay were applied to the health care personnel who were admitted to a healthcare personnel COVID-19 outpatient clinic with COVID-19 complaints.

RESULTS: A total of 1010 healthcare personnel who were admitted to the healthcare personnel COVID-19 outpatient clinic were included in this study. One hundred and sixty-seven of them (16.54%) were doctors, and 363 (35.94%) were nurses or midwives. The most common symptoms were sore throat (27.92%), cough (25.94%) and weakness (14.75%). Throat nasal swab RT-PCR revealed that a total of 989 (98%) personnel had PCR negative, and 21 (2%) had PCR positive results. Sixteen (1.58%) personnel did not have a registered assay result. Rapid antibody test revealed that 1006 (99.6%) personnel had negative, and 4 (0.4%) personnel had positive results. When the assay results were evaluated with simultaneous computed tomography findings, 990 (98%) did not have any signs suggesting COVID-19.

CONCLUSION: In serological rapid assays used to diagnose COVID-19, specific antibodies in the "window period" are at undetectable levels in the patient's blood. Therefore, false negative results may be obtained. For this reason, serological tests cannot be used as the basic diagnostic tool for COVID-19 infections.

Keywords: Coronavirus, pandemic, antibody tests, RT-PCR

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INTRODUCTION

In December 2019 in Wuhan, China, a new coronavirus (2019-nCoV) was detected in individuals with acute respiratory disease.[1] Coronavirus disease-2019 (COVID-19), (Sars-CoV-2) was declared to be a pandemic by the World Health Organization (WHO) on March 11, 2020.[2] Several studies showed that COVID-19 exhibited tropism against extra pulmonary tissue cells as well as respiratory system epithelial cells in humans and it had the ability to grow on these areas.[3] The symptoms of the infection include pulmonary and extrapulmonary signs such as fever, cough, dyspnea, diarrhea, headache, and conjunctivitis.[4] Real-time-polymerase chain reaction (RT-PCR) has become the standard method for nucleic acid identification to diagnose COVID-19. However, RT-PCR tests also have several limitations. These limitations include; the need for trained personnel and certified laboratories with special, expensive equipment for the assays. However, several studies showed that false negative results may be obtained by these assays. Therefore, simple, highly sensitive assays are required for rapid response, and immediate, accurate diagnosis. immunoglobulin M (IgM) is the defense response of the body during the first virus challenge, and IgG is the memory response associated with prolonged immunity. It is suggested that rapid antibody assays detecting IgM and IgG will be important for the diagnosis and treatment of COVID-19. Rapid antibody assays have been developed to detect the presence of IgM and immunoglobulin G (IgG) within 15 minutes from serum or whole blood taken from finger capillaries.[5]

The aim of this study is to evaluate the results of simultaneous rapid antibody tests and RT-PCR tests in patients diagnosed with COVID-19 retrospectively, and to evaluate the compatibility rates of these results with clinical and radiological findings.

MATERIALS AND METHODS

The Healthcare personnel COVID-19 Outpatient Clinic of University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Department of Infectious Diseases opened for COVID-19 complaints of the hospital personnel in order to facilitate convenient testing for them. Between the dates of March 31, 2020 and July 31, 2020, simultaneous COVID-19 R- PCR and COVID-19 rapid antibody assay for COVID-19 were applied to the health care personnel who were admitted to the healthcare personnel COVID-19 outpatient clinic with COVID-19 complaints.

This study was approved by Ethics Committee of University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital with 2020/10-28 approval number.

RT-PCR

From the oro-nasopharyngeal swab samples of the patients, viral nucleic acid isolation kit (Bio-Speedy, Turkey) was applied for viral nucleic acid extraction. Swab samples were obtained in Viral Transport Medium (VTM), and put into a 100 µL R1 tamponade clean micro-centrifuge tube. A 100 µL respiratory sample was added to this tube. The tube was vortexed at 15 sec high speed, then incubated at room temperature for 5 minutes. The tube was centrifuged at maximum speed for 3 minutes (above 10,000 g) and 25 µL supernatant was put into a clean micro-centrifuge tube. Then, 25 µL'R2 tamponade was added and mixed. This 50 µL mixture was used for PCR immediately.

Covidien RT-q-19 PCR Detection Kit (Biospeedy, Turkey) was used.

According to the manufacturers specifications:

1) 10 µL 2X Prime Script Mix

2) 5 µL oligomix

3) 5 µL of Nucleic acid were added.

A total volume of 20 µL was obtained.

For RT-PCR, Rotor gene device (Qiagen, Germany) was programmed according to the company's recommendations.

Rapid antibody test: COVID-19 IgM/IgG rapid antibody diagnosis assay (Hotgen, China) was used in accordance with the manufacturer's recommendations.

The test tape, diluent and sample to be tested were brought to room temperature, after (15–30 min) aluminum foil was opened, and the sample number was written on the plastic face of the tape. 10 µL serum was dropped in the sample section of the tape. Three drops of sample diluent were dropped above it. It was incubated at room temperature for 15 minutes.

If a red or magenta colored line was visible in both parts (T and C line), the test was considered to be positive. If a red or magenta line was visible only in the C line, and there was no color change in the T line, the test was considered to be negative. If there was no color change in the C line, the test was considered invalid and the test was repeated.

Statistical Analysis

All statistical analyses were performed through The Statistical Package for Social Sciences 23.0 (SPSS, Chicago, IL, USA) software. The data collected within the scope of the study were summarized as mean ± standard deviation. Pearson chi-square test was used in the analysis of categorical data. A value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 1010 healthcare personnel who were admitted to the healthcare personnel COVID-19 outpatient clinic of University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital Infectious Diseases were included in this study. Their mean age was 42, the gender distribution was male, 167 of them (16.54%) were doctors, 363 (35.94%) were nurses or midwives. The distribution of the healthcare personnel is shown in Table 1.

When the healthcare staff were questioned about whether they wore masks; it was found that 550 (54.45%) used masks during patient visits, and 460 (45.55%) did not. The analysis of the clinical signs of the patients included in the study showed that the most common symptoms were sore throat (27.92%), cough (25.94%) and weakness (14.75%). The clinical findings of the healthcare personnel are shown in Table 2.

Throat nasal swab RT-PCR revealed that total of 989 (98%) personnel had PCR negative, and 21 (2%) had PCR positive results. Sixteen (1.58%) personnel did not have a registered assay result.

Rapid antibody test revealed that 1006 (99.6%) personnel had negative, and 4 (0.4%) personnel had positive results.

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When the assay results were evaluated with simultaneous computed tomography (CT) findings, 990 (98%) did not have any signs suggesting COVID-19. Fourteen (1.4%) had COVID-19 compatible CT findings. One (0.09%) staff member had ground glass opacity findings suggesting a different viral infection. CT findings of 36 (3.56%) people were not compatible with COVID-19. CT findings could not be obtained in six personnel (Table 3).

A total of 21 (2%) personnel had PCR positive tests. Nine hundred and ninety of 1010 personnel had CT incompatible with COVID-19 findings. Among those patients without CT findings, 859 (87%) had PCR negative, 20 had (2%) PCR positive, three had (0.3%) rapid test positive, and one patient (0.1%) had both PCR and rapid test positive results.

Among the patients with CT findings, none of the health personnel had positive PCR results. One person with CT positive and PCR negative had a positive rapid test. Table 4 presents the correlation between the clinical symptoms and assay results.

Table 1. Distribution of healthcare personnel

Position	Number	%
Doctor	167	16.53
Nurse/midwife	363	35.94
Cleaning staff	204	20.20
Computing/data entry	30	2.97
Other	246	24.36

Table 2. Clinical signs of the healthcare personnel

Symptom type	Present (%)	Absent (%)
Weakness	**14.75**	**85.24**
Lack of appetite	1.48	98.52
Fever	7.22	92.73
Muscle and joint pain	12.97	87.03
Sore throat	**27.92**	**72.08**
Nasal congestion	8.42	91.58
Cough	**25.94**	**74.06**
Dry cough	7.82	92.18
Productive cough	2.18	97.82
Headache	11.88	88.12
Changes in consciousness	0.20	99.80
Nausea	1.78	98.22
Vomiting	0.60	99.40
Diarrhea	3.56	95.84
Loss of taste	1.48	92.92
Loss of smell	0.69	98.71
Dyspnea	9.21	90.19
Significant values are shown in bold.		

DISCUSSION

Since the beginning of the pandemic, medical companies and research institutes have been trying to develop assays to detect this viral infection and immunity against COVID-19. RT-PCR is considered as the gold standard for the diagnosis of the COVID-19 infection. However, this assay requires certified laboratories, expensive equipment, materials and trained personnel. Rapid tests detecting specific antibodies in blood samples may be used 3–6 days after the onset of symptoms to detect increased IgM antibodies and 8–10 days after to detect increased IgG antibodies.[6] These assays are cheap, easy to use and rapid, do not require equipment, and the results may be obtained in 15 minutes. They are easily used for screening health workers, allowing them to return to their work quickly. A disadvantage of quick tests is the probability of cross reactions with other coronaviruses (SARS-CoV, MERS-CoV).[7]

In PCR positive but rapid test negative sample results, the first thing to remember is that the absence of antibodies or their levels may be so low in the beginning of the infection. Secondly, the antibody response level of individuals' immunities may be different. Antibody response may not be observed if the immune system is suppressed. Thirdly, IgM response decreases during the second week, and may be negative when the test is applied.[5]

In Italy, 525 health care personnel were screened with rapid antibody assay, and six of them (1.1%) had positive IgM bands. Three of these cases had COVID-19 contact history. None of them had COVID-19 symptoms in these six cases, and their COVID-19 RT-PCR results were negative.[8] In a different study on 3300 patients, rapid assay was positive in six cases (0.2%).[9] In our study, 1006 (99.6%) health care personnel had negative rapid antibody assay, and four had positive results (0.4%). One of four (0.1%) had both positive PCR and rapid assay.

The results of molecular assays detecting viral RNA may be affected by accurate sampling, sample quality, transfer and storing conditions, and these may cause false negative results. If PCR does not detect the virus, we should keep in mind that the infection may be in very early or late period, and the viral load may be very low.[10-12]

Since SARS-CoV-2 is transmitted through airborne droplets, direct contact with COVID-19 patients put health care personnel at high infection risk especially if protective equipment is missing. In Italy, infection rates reported in health care workers were around 25%.[13-15]

The infection incidence of health care workers at a medical faculty was very high, 63% at the end of the first week and 22% at the end of the second week of the pandemic, among all COVID-19 cases diagnosed in the hospital. The first cases of this hospital were detected at those clinics where COVID-19 case were not expected, and one of these cases was a health care staff member. Therefore, it was suggested that health care personnel were not protected. With the precautions taken in the following weeks, this ratio decreased to 8.7% by the end of the 11th week.[16]

Table 3. Comparison of CT findings with RT-PCR results

CT findings not compatible with COVID-19	CT negative-RT-PCR negative	CT negative-RT-PCR positive	CT negative- RT-PCR positive-rapid test positive	CT positive-RT-PCR positive rapid test positive
990 (98%)	859 (87%)	20 (2%)	1 (0.01%)	1 (0.01%)
CT: computed tomography, COVID-19: coronavirus disease 2019, RT-PCR: real-time polymerase chain reaction.				

Table 4. The correlation between clinical symptoms and assay results				
	CT positive	RT-PCR positive	Rapid test positive	CT & rapid test & RT-PCR positive
Sore throat (280)	2 (0.7%)	16 (5.7%)	-	-
Cough (260)	4 (1.5%)	21 (8%)	1 (0.4%)	-
Weakness (146)	5 (3.4%)	15 (10.3%)	-	-
CT: computed tomography, RT-PCR: real-time polymerase chain reaction.				

Since February 24, 2020, it was reported that 3387 (4.4%) of 77,262 cases in China were health care personnel, and this was considered to be a high rate, and its causes were investigated.[17,18] In our study, PCR positivity was 2% in health care personnel, which was very low compared to other studies. We suggest that the importance given to the use of personal protective equipment and the hygiene measures had been effective.

COVID-19 infection primarily involves the respiratory tract, so imaging methods such as direct radiography and CT may give supportive information for diagnosis. The value of direct radiograph is low as a diagnostic tool and is insufficient to detect pulmonary signs. However, CT may detect parenchyma findings even in asymptomatic subjects.[19]

In the early periods of COVID-19, CT may not detect any findings. A negative result in the thorax CT may indicate the condition without any parenchymal findings associated with the infection.[20]

Thorax CT is more sensitive in the diagnosis of COVID-19 pneumonia in comparison to RT-PCR assay, however, CT findings such as ground glass densities in parenchyma, and consolidation areas are not specific to COVID-19. Therefore, the diagnosis should be supported by clinical signs and laboratory results, and it is important to be confirmed by RT-PCR assay.[21]

In serological rapid assays used to diagnose COVID-19, specific antibodies in the "window period" are at undetectable levels in the patient's blood. Therefore, false negative results may be obtained. Therefore, serological tests cannot be used as the basic diagnostic tool for COVID-19 infections.

It is suggested that COVID-19, which may cause severe acute respiratory infection, infects people via droplets. If the infected person coughs, sneezes or talks, the virus is transmitted by respiratory fluids directly to the mucosa, and it may infect the other person. Additionally, if a person touches his eyes, nose or mouth after touching an infected surface, infection may occur. Hand-washing is a basic of viral infection control. Protective clothing such as masks, aprons and gloves should be worn to prevent infection.

Limitations of the Study

We had some limitations for our study. Firstly, the required training was given to the personnel who would take the samples during the sampling phase for the COVID-19 RT-PCR test. However, the same person did not perform all PCR and rapid antibody tests. This situation may have affected the quality of testing. Secondly, a limited number of antibody tests were sent to our hospital. Therefore, antibody tests could not be performed on every patient who underwent PCR test. We think that if the number of antibody tests were higher, it would be more suitable in defining large populations. These are considered as the limitations of our study.

CONCLUSION

The most important points in the fight against COVID-19 are the early detection of infected people, follow-up of contacts and isolation of diagnosed patients. Although rt pcr is the most used method for early diagnosis, new methods have been tried to be developed because it requires a well-equipped molecular microbiology laboratory and equipment. Simultaneous rapid antibody tests, which are among these other methods, are thought to replace rt pcr tests for early diagnosis. However, in the light of the results we obtained from our study; It has been observed that in the "window period", specific antibodies may be at undetectable levels in the patient's blood, which may cause false negative results. Therefore, it has been concluded that serological tests cannot be used as a basic diagnostic tool for COVID-19 infections.

MAIN POINTS

- The most important points in the fight against COVID-19 are the early detection of infected people, follow-up of contacts and isolation of diagnosed patients.

- When simultaneous rapid antibody tests and Real-time polymerase chain reaction (RT-PCR) tests are compared; rapid antibody test appears to be superior in terms of ease of use and accessibility.

- However, rapid antibody tests may cause false negative results in patients who fall within the window period.

- RT-PCR tests are still the gold standard in the diagnosis of COVID-19 infections.

ETHICS

DISCLOSURES

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