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Original Article

Evaluation of Coronavirus PCR and Anti-Coronavirus Antibody Test Results: The First 4 Months of the Pandemic

Coronavirus PCR ve Anti-Coronavirus Antikor Test Sonuçlarının Değerlendirilmesi: Pandeminin İlk 4 Ayı

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ABSTRACT

Objective: We aimed to evaluate the Coronavirus polymerase chain reaction test results sent to our laboratory and the anti-Coronavirus antibody positivity in our region.

Methods: Samples sent to our laboratory for Coronavirus polymerase chain reaction and anti-Coronavirus antibody tests were scanned retrospectively. The polymerase chain reaction and enzyme-linked immunosorbent assay tests were screened within 4 months, and then the people who had both polymerase chain reaction and enzyme-linked immunosorbent assay tests were examined.

Results: Coronavirus polymerase chain reaction test was performed on 31 162 people in 4 months. Of which, 794 (2.5%) of these people were found to be positive. The positivity rate in the first, second, third, and fourth months was 11.2%, 1.2%, 0.7%, and 1%, respectively. Enzyme-linked immunosorbent assay tests were studied from 1163 people over 4 months. Enzyme-linked immunosorbent assay test was found positive in 23 (1.98%) of these people.

Conclusions: As a result, the number of COVID-19 cases could be brought under control in the first month due to our province's good cooperation between the laboratory and field teams. Anti-Coronavirus antibody level in the community was found below expected. There is a need to closely monitor society's immunity and carry out more comprehensive studies, including asymptomatic cases.

Keywords: Antibody, SARS-CoV-2, pandemic, PCR.

ÖZ

Amaç: Çalışmamızda laboratuvarımıza gönderilen Coronavirus PCR test sonuçlarını ve bölgemizdeki anti-Coronavirus antikor pozitifliğini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Laboratuvarımıza Coronavirus PCR ve anti-Coronavirus antikor testi çalışılması için gönderilen örnekler retrospektif olarak taranmıştır. 4 aylık süre içinde PCR ve ELISA testleri kendi içinde taranmış, daha sonra ise hem PCR hem de ELISA testi yapılan kişiler irdelenmiştir.

Bulgular: Dört aylık sürede 31162 kişiye Coronavirus PCR testi yapılmıştır. Bu kişilerden 794'ü (%2,5) pozitif saptanmıştır. Birinci, ikinci, üçüncü ve dördüncü aydaki pozitiflik oranı sırasıyla %11,2, %1,2, %0,7 ve %1 olarak bulunmuştur. Dört aylık sürede 1163 kişiden ELISA testi çalışılmıştır. Bu kişilerin 23'ünde (%1,98) ELISA testi pozitif bulunmuştur.

Sonuç: Sonuç olarak ilimizde laboratuvar ile saha ekipleri arasındaki işbirliğinin iyi olmasından dolayı ve ülke genelinde alınan tedbirlerin de etkisiyle COVID-19 vaka sayısı ilk ay içinde hızla kontrol altına alınabilmiştir. Toplumdaki anti-Coronavirus antikor seviyesi beklenenin altında bulunmuştur. Bu anlamda toplumdaki bağışıklığın yakından takip edilmesi ve asemptomatik vakaları da içerecek şekilde daha kapsamlı çalışmalar yapmaya ihtiyaç vardır.

Anahtar Kelimeler: antikor, SARS-CoV-2, pandemi, PCR.

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INTRODUCTION

In December 2019, a series of cases consistent with viral pneumonia were reported from Wuhan, China. As a result of the sequence analysis of these cases, it was revealed that this disease was caused by the new Coronavirus and named 2019-nCoV.[1] This name was changed as SARS-CoV-2 on February 11, 2020, by "The Coronaviridae Study Group of the International Committee on Taxonomy of Viruses" and has been accepted worldwide.[2] On the same day, the World Health Organization (WHO) declared the name of this disease as "Coronavirus Disease-19" (COVID-19). On March 11, 2020, WHO identified this outbreak as a pandemic.[3] The pandemic has a severe and profound impact not only on the health system but also on socioeconomic activities worldwide. According to the WHO data, more than 40 million people have been infected worldwide by the end of October 2020 due to COVID-19 and more than 1 million have died.[4]

SARS-CoV-2 is an enveloped, positive polarity, 30 kb genome, and single-stranded ribonucleic acid (RNA) virus.[2] The virus's mutation rate is relatively low, thanks to its RNA editing mechanism. The genome encodes structural (spike [S], envelope [E], membrane [M], nucleocapsid [N]) and non-structural proteins. The virus enters the cell by binding to the angiotensin-converting enzyme 2 (ACE2) receptor.[5]

SARS-CoV-2 is the seventh Coronavirus (HCoV) known to infect humans. Of these viruses, HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43 are endemic. These four viruses are seasonal and tend to cause mild respiratory disease. The other two viruses are the more virulent zoonotic Middle East respiratory syndrome Coronavirus (MERS-CoV) and severe acute respiratory syndrome Coronavirus type 1 (SARS-CoV-1). SARS-CoV-2 is genetically most similar to SARS-CoV-1, and both of these viruses belong to the subgenus *Sarbecovirus* within the genus *Betacoronavirus*.[6]

In order to determine the spread characteristics of the virus, it is necessary to determine the seroprevalence of SARS-CoV-2 in society. In addition, these serological tests can be an indicator of immunity after infection.[7] Most people who have had SARS-CoV-2 disease have been found to have antibodies that neutralize the virus.[8,9] When and how antibodies to SARS-CoV-2 appear has just begun to be identified. Accordingly, IgM antibodies reach detectable levels 5 days after the onset of the symptom, IgG antibodies 5-7 days after the onset of the symptom, and IgA 3-6 days after the onset of the symptom. Depending on the method applied, detection of antibody positivity can last up to 14 days.[7] But it is clear that more research is needed on this issue.

This study aimed to evaluate the Coronavirus polymerase chain reaction (PCR) test results sent to our laboratory and the anti-Coronavirus antibody positivity in our region.

METHODS

Study Design

Medical Microbiology Laboratory of our university is among the first 16 laboratories authorized to diagnose COVID-19 in Turkey in March 2020, and in this study, Coronavirus PCR and anti-Coronavirus antibody (including IgG and IgA) testing between March 21, 2020 and July 21, 2020, were used. Samples sent from Edirne, Tekirdağ, and Kırklareli provinces were scanned retrospectively. During the 4 months, PCR and enzyme-linked immunosorbent assay (ELISA) tests were scanned in themselves, and then people who had both PCR and ELISA tests were examined.

Polymerase Chain Reaction

Diagnosis, monitoring, and treatment of COVID-19 are based on guidelines published by the Turkish Ministry of Health COVID-19 Scientific Committee.[10] For the reverse transcription-polymerase chain reaction test used in the diagnosis of severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), combined nasooropharyngeal swab or sputum samples were taken from patients who met the criteria specified in the guidelines, and samples were transferred to our laboratory within no later than 12 hours. Real-time PCR experiments were applied to the patient's sample. RNA was isolated using vNAT solution (Bioeksen, Istanbul, Turkey). For all reactions, Montania 4896 (Anatolia Geneworks, Turkey) and Rotor-Gene Q (Qiagen, Antwerp, Belgium) instruments and Biospeedy SARS-CoV-2 RT-qPCR kit (Bioeksen) were used. The kit targets the RdRp (RNA-dependent RNA polymerase) gene fragment. The data were analyzed using Rotor-Gene Q Software.

Enzyme-Linked Immunosorbent Assay

Community screening was carried out within the scope of the study organized by the Ministry of Health in our province and aimed at determining the level of antibodies in the community and detecting asymptomatic cases. For this purpose, blood samples were taken from individuals in the community for anti-Coronavirus antibody testing. The blood samples taken were delivered to our laboratory within 12 hours. The serum of the blood samples reaching our laboratory was separated by centrifugation at 3000 rpm for 5 minutes. Cobas e601 (Roche Diagnostics, Mannheim, Germany) was studied using the Elecsys Anti-SARS-CoV-2 kit (Roche Diagnostics, Rotkreuz, Switzerland) to measure antibody levels in patient serums.

Ethics Committee Approval

This study's ethics committee approval was obtained from the Scientific Research Ethics Committee of Trakya University (Date: December 21, 2020, Decision no: TUTF-BAEK 2020/411).

Statistical Analysis

Descriptive statistics for the categorical variables were presented as counts and percentages. The SPSS V21 statistical program was used for all statistical computations.

RESULTS

Coronavirus PCR tests were performed on 31 162 people in 4 months. Of which, 794 (2.5%) of these individuals were positive (Table 1). The positivity rates in the first, second, third, and fourth months were 11.2%, 1.2%, 0.7%, and 1%, respectively.

The results of 449 patients whose PCR test was positive became negative in the subsequent PCR test. When the negation days were calculated, the mean was 14 days (the lowest was 1, the highest was 91), and the median was 12.

The ELISA test from 1163 people was studied over 4 months. The ELISA test was positive in 23 (1.98%) of these individuals. During this period, there were 1134 people whose both PCR and ELISA tests were studied, and the ELISA test was positive in 20 of them.

Among the patients who studied the PCR test and found positive, 6 patients then studied the ELISA test and found positive. When these 6 patients were evaluated, the antibody test was observed to be positive 35 days (minimum 19 days, highest 82 days, and median 25 days) after the PCR test was performed. Fourteen other patients who tested positive for ELISA were found to have a negative PCR test.

Table 2. Patients Whose Enzyme-Linked Immunosorbent Assay Test Was Found to Be Negative after the Polymerase Chain Reaction Test Became Positive

Patients	Number of Days Between Two Tests (n)
1	1
2	1
3	2
4	2
5	3
6	29
7	45

When 7 patients were evaluated with a PCR test, and the result was positive, then ELISA test and the result was negative; ELISA test was found to be negative after 11.9 days (lowest 1 day, highest 45 days, and median 2 days) (Table 2).

DISCUSSION

All developments related to this disease, which started in Wuhan in December 2019 and spread rapidly around the world, have been carefully followed by our department. After the declaration of this situation as a pandemic by WHO on March 11, 2020, the following months were planned as a Medical Microbiology Laboratory, and necessary arrangements were made (closing non-emergency tests, sending tuberculosis tests to another center, and

Table 1. Distribution of PCR Test Results by Provinces and Months

		PCR (–) (n)	PCR (+) (n)	PCR (+) (%)	Total (n)
Edirne	First month	2171	260	10.7	2431
	Second month	3578	48	1.3	3626
	Third month	6169	29	0.5	6198
	Fourth month	7322	74	1.0	7396
Total Edirne		19 240	411	2.1	19 651
Kirklareli	First month	1647	185	10.1	1832
	Second month	4001	45	1.1	4046
	Third month	2709	35	1.3	2744
	Fourth month	2340	26	1.1	2366
Total Kirklareli		10 697	291	2.6	10 988
TEKİRDAĞ	10 days	431	92	17.6	523
Total		30 368	794	2.5	31 162

PCR, polymerase chain reaction.

Table 3. Target Gene Regions Used by Some Countries for Coronavirus Polymerase Chain Reaction Testing[11]

Country	Target Gene Region
China	*ORF1ab* and *N*
France	*RdRP* (two target areas)
United States	*N* (three target areas)
Germany	*RdRP*, *E*, *N*
Thailand	*N*

E, Envelope; N, nucleoprotein; ORF, open reading frame; RdRP, RNA-dependent RNA polymerase.

using the tuberculosis laboratory as a Coronavirus PCR laboratory, employing technicians on a rotating basis, etc.) with the hospital management and the Provincial Health Directorate.[4] The first Coronavirus PCR test study was carried out on March 21, 2020, and this study continued with an increasing number of samples in the following days.

Early laboratory diagnosis of SARS-CoV-2 infection can help with clinical management and epidemic control. Diagnostic tests for the disease include the detection of the virus itself (viral RNA or antigen) or antibodies formed against the virus. The target gene regions of the Coronavirus PCR test are the *E*, *RdRP*, *N*, and *S* genes.[5] As of July 2020, the gene regions used by some countries are given in Table 3.[11] Kits that detect *RdRp*, *ORF1ab*, *N*, and *E* genes are available in Turkey. Coronavirus PCR tests in our lab target the *RdRp* gene.

After a person is infected, the average time for symptoms to appear (incubation period) is 5-6 days, ranging from 1 to 14 days. The virus can be detected from upper respiratory tract samples 1-3 days before symptoms begin. SARS-CoV-2 concentration in the upper respiratory tract is the highest at symptom onset and decreases in the following days. In some patients, viral RNA can be detected for only a few days, while in some patients, it can be detected for several weeks, possibly months. The long-term presence of viral RNA does not necessarily mean long-term infectiousness.[5] In our study, it took 91 days for the PCR test to become negative in one patient.

A series of measures were taken across the country, with the first case seen in our country on March 11, 2020. Banning flights to high-risk countries, closing border crossings, decommissioning face-to-face training in schools, banning prayer with congregants in mosques, playing football competitions without spectators, imposing a curfew on people over 65, reducing the number of passengers on buses by half, and so on, are steps taken to reduce the rate of virus spread.[12] When we look at the PCR test results in our laboratory, there is a high rate of PCR test positivity in the first month, and in the following months, this rate drops dramatically. We think that the measures taken throughout the country were effective in this decrease.

In our laboratory, the Coronavirus PCR test is studied uninterruptedly. Samples are accepted around the clock, and samples usually arrive at the laboratory in the afternoon and evening. The Medical Microbiology Laboratory is studying incoming samples without waiting, and this study continues until morning. As soon as the results are obtained, they are entered into the Laboratory Information Management System, and patients with positive results are notified to filiation teams by phone. Filiation teams also reach these patients and isolate them without wasting time. Another reason why the high positive rate observed in the first month fell in the following months was that the Medical Microbiology Laboratory and the teams in the field work in a coordinated manner, thereby timely intervention of patients.

June and July 2020 "New Coronavirus (COVID-19) Disease Seroprevalence Study" by the Ministry of Health determined many households in each province. One person from these households was included in the study, and blood samples received were sent to our laboratory and screened for antibodies. As a result of this study, anti-Coronavirus antibody positivity was 1.98% in our province. As of July 2020, the Coronavirus PCR test positivity rate is 2.1% for our province. Although the rates are close to each other, when asymptomatic cases and patients with negative PCR tests are considered, it is thought that either the sensitivity of the ELISA test is low or the antibody levels are not high enough in people who have had the disease. However, more work needs to be done to conclude this issue.

The Elecsys Anti-SARS-CoV-2 (Roche Diagnostics) kit is a kit for detecting IgA and IgG. It is reported that the sensitivity is 99.5% on the 14th day after PCR test positivity and 100% on the 16th day.[7] When six patients with positive PCR and positive ELISA tests in their follow-ups were evaluated, it was observed that antibody positivity continued on the 19th at the earliest and the 82nd day at the latest.

When 7 patients whose PCR test was positive and ELISA test was negative in their follow-up were evaluated, it was observed that the ELISA test was performed within 3 days after the PCR test positivity in 5 of these patients. It was thought that antibody levels would not reach detectable levels during this period, and this was considered normal. The ELISA test was performed on the 29th and 45th days after the PCR test positivity in the other two patients. One of these patients was a 41-year-old female patient who did not have any comorbidity. The patient

had a history of contact with a COVID-19 patient 6 days ago. The patient, who did not have any complaints other than a cough, was followed up in the hospital for 2 days. The patient who had no fever in the follow-ups and no lesion was detected in the computed tomography, was quarantined, and discharged. The PCR test performed on the ninth day after hospitalization was negative. The anti-Coronavirus antibody test performed on the 29th day after hospitalization was reported as negative. In a healthy adult with a healthy immune system, the inability to detect antibodies in the fourth week after the positive PCR test indicates that either the levels of antibodies in some patients are not high enough to be detected or the test's sensitivity is low.

The other patient, whose PCR test was positive and ELISA test was negative, was a 54-year-old male patient who applied to the emergency department with complaints of fever and cough. The patient has comorbid diseases such as non-Hodgkin lymphoma, chronic viral hepatitis B, cardiac arrhythmia, and chronic kidney failure. The patient, who was considered immunosuppressive due to comorbid diseases, had a history of hospitalization many times before. Anti-Coronavirus antibody test was found negative on the 45th day after the positive PCR test of the patient. The fact that the patient was immunosuppressive suggested that he could not sufficiently form anti-Coronavirus antibodies.

Since the study was not planned prospectively, it was impossible to reach a definite judgment about whether the immune status against Coronavirus developed in the patients or after how many days. The limitation of the study is that antibody levels are not regularly monitored in patients with COVID-19.

As a result, due to the excellent cooperation between the laboratory and field teams in our province and the measures taken throughout the country, the number of COVID-19 cases was quickly controlled in the first month. The increased testing needs were met seamlessly by the Medical Microbiology Laboratory. The anti-Coronavirus antibody level in the population was lower than expected. There is a need for close follow-up of immunity in the community and more comprehensive studies, including asymptomatic cases.

Ethics Committee Approval: Ethics committee approval for this study was obtained from the Scientific Research Ethics Committee of Trakya University (Date: December 21, 2020, Decision no: TUTF-BAEK 2020/411).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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