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Investigation of COVID-19 Serology in a Tertiary Care Center

Üçüncü Basamak Sağlık Kuruluşuna Başvuran Hastalarda COVID-19 Serolojisinin İncelenmesi

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ÖZ

Amaç: Hastanemize başvuran hastalarda PCR testi sonrası SARS-CoV-2 spesifik antikor bakılan hastalar retrospektif olarak taranıp, ilimizin serolojik profili hakkında fikir edinmek amaçlanmıştır.
Araçlar ve Yöntem: Laboratuvarımıza Ocak-Haziran 2021 tarihleri arasında, serum örneklerinde anti SARS-CoV-2 IgG ve/veya IgM çalışılan hastalar retrospektif olarak incelenip dahil edildi. Eş zamanlı olarak SARS-CoV-2 PCR testi uygulandı.
Bulgular: 725 hastanın 123'üne IgM veya IgG olmak üzere yalnızca bir istem yapılmış, 602 kişiye ise IgM ve IgG birlikte istenmişti. PCR testi ile anti SARS-CoV-2 IgG ve/veya IgM istemleri arasında 30 gün süre bulunan hastalara bakıldığında ise toplam 304 (%42) hastanın 40 (%13)'ının PCR testi pozitif olarak saptanmıştır, bu hastaların 60%'ının 30 gün içindeki IgM ve IgG iki testi birlikte pozitif olarak bulunmuştur. PCR testi negatif 264 hastadan %64'ünün IgM ve IgG testi negatif bulunmuştur. Sonuçlarımıza göre, testlerin %58'i PCR istemi olmadan istenmiştir. PCR ve seroloji istemleri arasındaki süreler incelendiğinde ilk 7 gün içerisinde 233 (%76.6) seroloji istemi, 8-14 gün içinde 27 (%8.8), 15-21 gün içinde 7 (%2.3), 22-30 gün içinde 37 (%12.3) istem yapıldığı görülmüştür. 117 (%38.5) istemde PCR ve serolojinin aynı anda yapıldığı saptanmıştır.
Sonuç: DSÖ serolojik test istemlerinin PCR testinden sonraki 1. ve 3-4. haftalarda yapılmasını önermektedir. Hastanemizde yüksek oranda uygun olmayan istem yapıldığı görülmekte olup bu durum serolojik test istem algoritma eksikliğini göstermektedir. Serolojik testlerin COVID19 hastalığının tanısında tek başına kullanımı değil; nükleik asit testleriyle birlikte ve onlara yardımcı olarak kullanımı önerilmektedir. Bu durum bize aşı sonrası antikor yanıtını görmek için gereksiz test istemine yol açıldığını düşündürmüştür.

Anahtar Kelimeler: CLIA; COVID19; IgG; IgM; PCR

ABSTRACT

Purpose: This study aimed to screen the patients admitted to our hospital for SARS-CoV-2 specific antibodies after a PCR test and understand the local serological profile.
Materials and Methods: The patients tested for anti-SARS-CoV-2 IgG and/or IgM between January-June 2021 were included in the study. SARS-CoV-2 PCR test was performed simultaneously.
Results: Either IgM or IgG alone was requested in 123 of 725 patients, and IgM and IgG together in 602. The PCR test was positive in 40 (13%) of 304 (42%) patients who had a PCR test after 30 days of the serology request. Of these PCR-positive patients, 60% had IgM and IgG antibodies together, whereas among 204 PCR-negative patients, 64% tested negative for IgM and IgG. 58% of the tests were ordered without a PCR request. The period between PCR and serology testing was as follows: 233 (76.6%) in 7 days, 27 (8.8%) in 8-14 days, 7 (2.3%) in 15-21 days, and 37 (12.3%) in 22-30 days. 117 (38.5%) of the requests were made simultaneously.
Conclusion: WHO recommends that serology testing should be performed after the 1st and 3-4th week of the initial PCR test. The high rate of inappropriate testing demonstrates a lack of algorithms. The use of serological tests is recommended in conjunction with nucleic acid tests but not to be used alone in the diagnosis of COVID-19. Our results demonstrated the high rate of unnecessary requests for serology testing to determine the antibody response against SARS-CoV-2 vaccines.

Keywords: CLIA; COVID19; IgG; IgM; PCR

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INTRODUCTION

Coronaviruses are positive-sense single-stranded RNA viruses with an envelope that belongs to the Nidovirales order, Coronavirideae family. Four subgroups have been identified: alpha, beta, gamma, and delta. It has been defined that only alpha and beta groups are pathogenic to humans, and four subtypes (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) are responsible for seasonal upper respiratory infections.[1] On the other hand, the Severe Acute Respiratory Syndrome virus (SARS-CoV), transmitted from bats to humans, has caused acute-severe lower respiratory symptoms and led to epidemics in 2002 in China, which ended after one year. Ten years later, the Middle East Respiratory Syndrome virus (MERS), transmitted from cattle to humans, has caused severe lower respiratory symptoms.[2]

Coronaviruses are susceptible to mutations due to their abundance in nature and animals and their ability to transmit between various species.[1,3] In December 2019, cases of pneumonia with unknown origin occurred in Wuhan, Hubei, China, and it was determined that a new species of Coronavirus is the causative agent of these cases. This virus has been identified as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) due to its similarity with beta coronaviruses by the International Committee on Taxonomy of Viruses (ICTV). The disease caused by SARS-CoV-2 has been named Coronavirus Disease (COVID-19).[4] Due to the increase in the transportation opportunities between countries, it rapidly spread intercontinental, and World Health Organization (WHO) declared this disease a pandemic on 11th March 2020.[5]

There have been over 315 million cases and 5.5 million deaths until today - when the second year of the pandemic has ended. Early diagnosis and isolation strategies are crucial to eliminating the exposure routes, which include direct contact, airborne droplets, and contaminated surfaces.[6] The diagnosis of COVID-19 is made using clinical symptoms, computational tomography, and laboratory findings. Microbiological analysis is needed for a definite diagnosis. These include genomic sequencing, PCR-based methods, and serology.[7] Sequencing is an advanced method used for the first identification of a new pathogen or determining genomic mutations. The gold standard method utilized for nucleic acid amplification test is to detect the viral nucleic acids by real-time reverse transcriptase polymerase chain reaction (rRT-PCR). The results of this test are affected by the pre-analytical process, including sample collection method, sampled area, conditions of transportation, and sampling time. Positive results provide a definite diagnosis due to its high sensitivity. However, negative results could not be used for exclusion, and tests should be repeated if the clinical suspicion continues. Repetitive negative results are common in the late phases of COVID-19. Additional tests are needed in these situations. Serological tests are based on the measuring of antibody responses against the virus. These tests help us understand the transmission dynamics of an infection in a certain population by seroprevalence studies. Their conjunct use with nucleic acid amplification tests but not alone is recommended in the diagnosis of COVID-19.[8-12] They are beneficial in the confirmation of the diagnosis in late phases with a negative PCR test.[8,10,13,14]

This study aims to achieve a better understanding of the serological profile of our city by analyzing retrospective results of SARS-CoV-2 specific antibodies after a PCR test was performed.

MATERIALS and METHODS

This study was approved by the TR Ministry of Health, General Directorate of Health Services (Date: 17.06.2021, Decision No: 2021-06-17T10_22_48) and Amasya University Non-Interventional Research Ethics Committee. (Date: 08.07.2021, Decision No:109).

Patients admitted to Amasya University Sabuncuoğlu Şerefeddin Research and Training Hospital between January-June 2021, whose serum samples were analyzed for anti-SARS-CoV-2 IgG and/or IgM, were included in the study. Anti-SARS-CoV-2 IgG antibodies against N antigen and IgM antibodies against S antigen were detected semi-quantitatively in serum using the chemiluminescence enzyme immunoassay method (Abbott, USA). According to the manufacturer's recommendations, values above 1.4 for anti-SARS-CoV-2 IgG and 1 for anti-SARS-CoV-2 IgM were interpreted as positive and others as negative.

In the meantime, the Bio-speedy SARS-CoV-2 (2019-nCoV) RT-qPCR detection kit (Bioeksen, Istanbul, Turkey) was. The patients' demographic values were determined using medical records.

RESULTS

A total of 1478 requests were performed from 725 patients; 813 were IgG requests, whereas 665 were IgM. Either IgM or IgG test was demanded in 123 patients, whereas both IgM and IgG tests were inquired together in 602 individuals. Repetitive tests were performed on 82 patients. The number of positive results was 352/813 (43.3%) for IgG and 227/665 (34.1%) for IgM.

A total of 304 patients, who had both serology and PCR requests for SAR-CoV-2 in 30 days between January and June 2021, were investigated. 154 of the patients were female and 150 were male. The age range of these patients was 18-94, and the mean age was 53.3 years. 169 of the patients were from outpatient clinics, 109 from inpatient, and 26 from intensive care units.

Forty (13%) of the patients had positive PCR results. Twenty-four (60%) of these PCR-positive patients had both IgG and IgM antibodies, whereas 15% had IgG and 7% had IgM. However, 18% of patients were PCR positive, but IgM or IgG antibodies could not be detected. 264 (87%) of patients were PCR negative, 20% had both IgM and IgG antibodies, 10% had IgG, and 6% had IgM. Among 264 PCR-negative patients, 64% tested negative for IgM and IgG (Table 1).

Table 1. COVID-19 PCR and antibody tests

	SARS-CoV-2 PCR		
SARS-CoV-2 Antibody	**Positive (13%)**	**Negative (87%)**	**Total (100%)**
IgG Positive	6 (15%)	27 (10%)	33 (11%)
IgM Positive	3 (7%)	16 (6%)	19 (6%)
IgG and IgM Positive	24 (60%)	52 (20%)	76 (25%)
IgG and IgM Negative	7 (18%)	169 (64%)	176 (58%)
Total	40 (13%)	264 (87%)	304 (100%)

The time range between PCR and serology requests was evaluated. 233 (76.6%) of the tests were in the first 7 days, 27 (8.8%) in 8-14 days, 7 (2.3%) in 15-21 days, and 37 (12.3%) in 22-30 days. 117 (38.5%) of them were demanded simultaneously.

DISCUSSION

It is obvious that microbiologic tests have gained importance and awareness among the public due to this pandemic because of the use of rRT-PCR as a gold standard method in diagnosis. It provides a definite diagnosis due to its high sensitivity. However, repetitive negative results can be obtained in the late phases of the disease. In these cases, serological tests take over as alternative methods. Serological tests are based on the detection of antigen or antibody testing. In this study, antibody presence is evaluated.

There are various types of serological tests based on their methodologies, such as Enzyme-Linked Immunosorbent Assay (ELISA), Lateral flow immunoassay (LFIA), and Chemiluminescence Immunoassay (CLIA). These tests detect the antibodies against certain peptide structures of the virus. There are four structural peptides of SARS–CoV–2: envelope (E), spike (S), membrane (M), and nucleocapsid (N). S peptide is found to have a role in specific antibody response,[15] N peptide in triggering an immune response in the host.[16] N peptide is shown to demonstrate more cross-reactions than S peptide against other coronaviruses.[17,18] This implies that tests that use S peptide or its fragments may cause fewer cross-reactions.[18] To conclude, the peptide to which the antibody binds is important because it defines its sensitivity and purpose of use. In our study, a commercial kit based on the semiquantitative CLIA method that detects IgG antibodies against N antigen and IgM antibodies against S antigen has been utilized.

Various commercial kits for antibody detection against SARS-CoV-2 have been developed during the COVID-19 pandemic. The commercial kit used in this study detects the IgM antibodies against S antigen, which demonstrates the patients' encounter with the virus. However, it does not provide information on the time that passed. As a result, it is not sufficient for the diagnosis of acute infection. Furthermore, S antigen is used in studies on neutralizing antibodies and vaccines.[19-21] The IgG antibodies against N antigen detected by the kit in this study might establish an immune response in the host but could not give information on neutralizing antibodies. Hence, patients that have IgG antibodies might still be RNA positive.[22] Serological tests cannot demonstrate viral clearance or spread

of live viruses; as a result, they cannot replace molecular tests.[22]

To prevent the spread of an infectious pathogen, it is crucial to isolate the contacts, determine the local propagation rate, establish the attack rate, and demonstrate the mortality rates, as well as the detection of patients.[23] Establishing the seroprevalence in a society, the size of asymptomatic patients, the vulnerable groups, and the risk factors can guide the choice of preventive measures. For this reason, serological tests can be preferred to scan the specific groups, as they are cost-effective and rapid methods. Scanning with serology testing can guide the authorities to estimate the size of vulnerable individuals and prevalence in high-risked groups, such as healthcare workers, and to determine the seropositive patients who can volunteer for plasma treatment.[23] This study aims to provide information on the local serological profile of COVID-19 in Amasya. In our study, either IgM or IgG test was demanded in 123/725 (16.9%) patients. Whereas both IgM and IgG tests were inquired together in 602/725 (83.1%) individuals. IgM antibodies are known to be formed earlier than IgG. Conjunctive testing of IgM and IgG is recommended, especially in the acute phase, to prevent the risk of false negatives.[24-26]

The performance of the serological test kits depends on the host factors, immune status of the host, viral incubation period, and the sampling time. The immunosuppressive individuals may not form antibodies, or the amount of antibody response can be in the non-detectable range. The viral incubation period changes interpersonal due to the encountered amount of viral load. In a study, it was demonstrated that the viral incubation period affected the seroconversion time. Seroconversion was detected on the 10th day in the group of patients with an incubation period lasting less than five days; on the contrary, seroconversion was detected on the 7th day in the group of patients with an incubation period lasting over five days.[25] False-negative results can be obtained from mild symptomatic, asymptomatic, or patients in early stages due to the small amount of antibody response.[22] The sampling time is another important factor affecting the results. WHO recommends that samples for serology testing should be obtained after three weeks from the onset of the disease if only one sample is to be taken. However, the ideal collection time of samples is recommended as the first and 3rd-4th weeks of the infection.[27] Because the primary immune response develops in 10-14 days, several studies have recommended not to test before these initial two weeks.[25,26,28] There are various studies with different outcomes in seroconversion studies. There are studies reporting that IgM titer can be detected 10-12 days after the symptom onset and IgG titer 12-14 days after initial IgM detection.[11,25] In another study, it was reported that IgM antibodies could be detected in 3-42 days and IgG antibodies in 5-47 days.[29] Haveri et al. investigated the antibody responses on the 4th, 9th, and 20th days of symptom onset and detected no antibody response on the 4th day. On the 9th and 20th days, anti-SARS–CoV–2 IgM were detected as 80 and 320, respectively, and IgG as 80 and 1280.[30] In our study, patients who had 30 days between PCR and anti-SARS-CoV-2 IgG and/or IgM test requests were included. Only 42% of tests fit these criteria. 58% of the serology tests were requested without a PCR test. The recommendations for serology testing are to perform them together with PCR. These results indicate the algorithms for test requests should be re-evaluated. 76.6 % of the serology tests were within the first week. Half of these tests were simultaneously requested. Only 14.6% of the tests were after two weeks. These results demonstrate that only very few COVID-19 serology testing was performed according to the recommendations of WHO.

Zhao et al. reported the sensitivity of PCR tests as 66.7% and serological tests as 33.8% in the first week of the disease. However, the sensitivity of PCR decreased to 33.8%, and serology testing increased to 90% in the second week. As a result, researchers suggested the conjunctive use of PCR and serology testing in the diagnosis of COVID-19.[11] In our study, the patients who had SARS-CoV-2 antibody testing after a PCR examination were evaluated. Out of 40 PCR-positive patients, 60% had IgM and IgG antibodies together, whereas among 264 PCR-negative patients, 64% tested negative for IgM and IgG. Only 304 out of 725 patients were tested for both PCR and ELISA in 6 months period. These results demonstrated the unnecessary testing for evaluating the antibody responses after vaccine administration since in the period the study was conducted, the

vaccine administration for COVID-19 was initiated, prioritizing healthcare workers and older age. In this retrospective study, vaccination status and the presence of symptoms were not examined. These are the limitations of our study. Various serology tests were developed during the pandemic. The aim of serology testing should be planned carefully. In national guidelines of the Ministry of Health and international guidelines of WHO, serology testing before /or after vaccination was not recommended.

In our study, 40 out of 304 patients (13%) had a positive PCR test, and 82% of them were IgM- and/or IgG-positive. Among the 264 individuals that had a negative PCR result, 95 (36%) were found as seropositive. These patients were considered to be in the late phase of the disease, mild/asymptomatic cases, or vaccinated. In conclusion, it is crucial that the results of COVID-19 serology testing should be interpreted with considering the clinical symptoms, sampling time, and contact history. In addition, clinicians should keep in mind that serological tests, which are performed in the recommended period of the disease, are meaningful in understanding the dynamics of COVID-19.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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Ethics Committee Permission

This study was approved by the TR Ministry of Health, General Directorate of Health Services (Date: 17.06.2021, Decision No: 2021-06-17T10_22_48) and Amasya University Non-Interventional Research Ethics Committee. (Date: 08.07.2021, Decision No:109).

Authors' Contributions

Concept/Design: PO, TÜA, FMS. Data Collection and/or Processing: PO, TÜA, FMS. Data analysis and interpretation: PO. Literature Search: PO. Drafting manuscript: PO. Critical revision of manuscript: PO, TÜA, FMS. Supervision: FMS.

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