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Could SARS-CoV-2 Trigger the Formation of Antinuclear Antibodies?

SARS-CoV-2, Antinükleer Antikorların Oluşumunu Tetikleyebilir mi?

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Abstract

Objective: The effect of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection on autoimmunity in both disease and post-disease stages has not been fully explained. There is not enough information about the evaluation of autoimmune antibodies in convalescent SARS-CoV-2 patients. This study aimed to investigate the presence and types of autoantibodies in post-illness coronavirus disease-2019 (COVID-19) patients and to compare them with indirect immunofluorescence assay (IIF)-antinuclear antibody (ANA) results before SARS-CoV-2 infection.

Materials and Methods: Twenty-four COVID-19 patients with known and reported ANA test results prior to SARS-CoV-2 infection were included in this study. Patients' IIF-ANA, extractable nuclear antigen blot and anti-dsDNA tests were studied three and nine months after SARS-CoV-2 infection.

Results: Three months after SARS-CoV-2 infection, 41.66% of patients had a positive IIF-ANA test. When we compared these results with pre-infection ANA results, 3 patients (12.5%) were variable. The first case was chromosomal granular positive before infection and was found to be homogeneous, and cytoplasm was speckled positive after infection. Additionally, Scl-70, DFS70, and anti-dsDNA were found to be positive. We think that lupus symptoms were triggered after COVID-19. The second case had negative ANA before infection, while the ANA was antinuclear membrane positive (2+) three months after infection. Also, anti-RNP/Sm was detected as positive. The third case had negative ANA before infection, and was detected to have speckled weakly positive ANA three months after infection. However, autoantibody positivity was not detected.

Conclusion: As a result, these data support the idea that SARS-CoV-2 infection may trigger autoimmunity and be associated with the development of autoantibodies.

Keywords: SARS-CoV-2, COVID-19, antinuclear antibodies, autoimmunity

Öz

Amaç: Şiddetli akut solunum yolu sendromu-koronavirüsü-2 (SARS-CoV-2) enfeksiyonunun hem hastalık hem de hastalık sonrası evrelerde otoimmünite üzerindeki etkisi tam olarak açıklanamamıştır. Nekahet dönemindeki SARS-CoV-2 hastalarında otoimmün antikorların değerlendirilmesi hakkında yeterli bilgi bulunmamaktadır. Bu çalışmada, hastalık sonrası koronavirüs hastalığı-2019 (COVID-19) hastalarında otoantikorların varlığını ve tiplerini araştırmak ve bunları SARS-CoV-2 enfeksiyonu öncesi dolaylı immünofloresan (IIF)-antinükleer antikor (ANA) sonuçlarıyla karşılaştırmak amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmaya SARS-CoV-2 enfeksiyonu öncesi bilinen ve bildirilen ANA test sonuçları olan 24 COVID-19 hastası dahil edilmiştir. Hastaların IIF-ANA, ekstrakte edilebilir nükleer antijen blot ve anti-dsDNA testleri, SARS-CoV-2 enfeksiyonundan üç ve dokuz ay sonra çalışılmıştır.

Bulgular: SARS-CoV-2 enfeksiyonundan üç ay sonra hastaların %41.66'sında pozitif IIF-ANA testi saptanmıştır. Bu sonuçlar enfeksiyon öncesi ANA sonuçlarıyla karşılaştırıldığında üç hastada (%12.5) değişkenlik gözlenmiştir. İlk olgu enfeksiyondan önce kromozomal granüler pozitif ve homojen, enfeksiyondan sonra sitoplazma benekli pozitif saptanmıştır. Ek olarak, Scl-70, DFS70, anti-dsDNA pozitif bulunmuştur. Lupus semptomlarının

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COVID-19 sonrası tetiklendiği düşünülmektedir. İkinci olguda, enfeksiyondan önce ANA negatif iken, enfeksiyondan üç ay sonra ANA antinükleer membran pozitif (2+) saptanmıştır. Ayrıca, anti-RNP/Sm pozitif tespit edilmiştir. Üçüncü olgu da enfeksiyondan önce ANA negatif, enfeksiyondan üç ay sonra benekli zayıf pozitif ANA olarak tespit edilmiş, ancak otoantikor pozitifliği tespit edilmemiştir.

Sonuç: Sonuç olarak bu veriler SARS-CoV-2 enfeksiyonunun otoimmüniteyi tetikleyebileceği ve otoantikor gelişimi ile ilişkili olabileceği fikrini desteklemektedir.

Anahtar Kelimeler: SARS-CoV-2, COVID-19, antinükleer antikorlar, otoimmünite

Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) continues to affect the world and spreads very rapidly due to mutations despite vaccination and isolation practices. SARS-CoV-2 infection can be overcome asymptomatically. On the other hand, it can result in clinical conditions such as adult respiratory distress syndrome, respiratory failure, disseminated intravascular coagulation, disseminated thromboembolism, and even death. In addition, approximately 50-80% of symptomatic patients with recovered SARS-CoV-2 have symptoms of the post-illness syndrome (1,2). Several long-term effects of SARS-CoV-2 infection such as fibromyalgia, chronic fatigue syndrome, autonomic nervous system dysfunction of autoimmune origin, and cognitive impairment have been reported (3). SARS-CoV-2 can also be associated with a wide variety of autoimmune clinical manifestations (4). Cases of SARS-CoV-2 with autoimmune events such as Guillain-Barré syndrome, antiphospholipid syndrome, Kawasaki-like syndrome, and idiopathic thrombocytopenic purpura have been reported (5,6).

Although the etiology is not known exactly, genetic predisposition, viral, bacterial and fungal infections and hormonal factors are thought to be effective in the emergence of autoimmune diseases (7). Viruses such as Epstein-Barr virus, parvovirus B19, herpes virus-6, cytomegalovirus, Rubella virus, hepatitis C and A virus are reported to trigger autoimmune diseases (8-11). It is assumed that viruses trigger the activation of cytokines such as interleukin-1, interleukin-6, TNF-α, interleukin-17, and interleukin-18 in genetically susceptible individuals (12). Antinuclear antibodies (ANA) can be defined in many autoimmune illnesses and viral infections. ANA tests are among the first and most commonly used tests for the detection of autoantibodies today. The target of autoantibodies is usually nuclear antigens (13).

In this study, the types and presence of autoantibodies in post-illness SARS-CoV-2 patients and compared them with indirect immunofluorescence (IIF)-ANA results prior to SARS-CoV-2 were analyzed. Based on these observations, we aimed to investigate whether SARS-CoV-2 contributed to autoimmunity activation and stimulation of autoantibody production.

Materials and Methods

Study Design

This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Samsun Training and Research Hospital (date: 01.04.2021, approval number: GOKA/2021/8/18) and was performed in accordance with the 1964 Declaration of Helsinki. Twenty-four patients with SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) test (+) were included in the study. Bio-Speedy Bioeksen (Istanbul, Turkey) device was used for RT-PCR test. Informed consent forms were obtained from the patients. Serum samples of these patients were taken at the third and ninth months after the disease. The samples were stored in a deep freezer until the study day in the microbiology laboratory of the hospital where the study was conducted.

Patients

Only three of twenty-four patients (20 female, 4 male) were hospitalized for SARS-CoV-2 infection. These twenty-four patients had no oncological disease, history of systemic autoimmune illness, use of biological agents, and hepatitis C or B virus co-infection.

IIF Testing

ANA was determined in patient samples collected using the IIF method. For this, HEp-20-10 liver biochip, Euroimmune AG (Luebeck, Germany) kit was used at 1:100 dilution, taking into account the manufacturer's recommendation. The same laboratory specialist evaluated and reported the patients' IIF-ANA tests prior to SARS-CoV-2 infection using an Eurostar III plus fluorescent microscope Euroimmune AG (Luebeck, Germany). The fluorescence intensity of the positive control was accepted as four+. Therefore, the titer intensity values were evaluated as ± (borderline), one+, and four+ in the ×400 lens. In this process, an evaluation was made taking into account the international consensus ANA patterns standards (14).

Anti-dsDNA Testing and Extractable Nuclear Antigen (ENA)

The presence of ENA in ANAs positive samples was examined by a line immunoassay method using the Euroline ANA-profile 1 (IgG) kit, Euroimmun AG

(Luebeck, Germany). Each strip consisted of Sm, nRNP/Sm (U1-nRNP), SS-A, SS-B, recombinant Ro52 (Ro-52, 52 kDa), histidyl-tRNA synthetase (Jo-1), Scl-70, DFS70 antigens and was tested according to the manufacturer's protocol. Anti dsDNA tests were performed with the Chorus dsDNA-G (DIESSE Diagnostica Senese, Italy) kit using the micro-ELISA method. According to the kit package insert, it was evaluated as (>30 IU/mL) positive, (20-30 IU/mL) intermediate, and (<20 IU/mL) negative.

Statistical Analysis

Data were analyzed using SPSS software (version 17, SPSS Inc. Chicago, IL). In the definition, data were determined as mean, standard deviation, frequency and percentage. Pearson chi-square test was used to compare categorical variables.

Results

Twenty-four patients with known ANA test results prior to SARS-CoV-2 infection were screened for the prevalence of ANA. The mean age of all patients was 42.70 ± 10.28 years. Four (16.67%) were male and twenty (83.33%) were female. When the ANA results of the twenty-four patients included in the study were analyzed three months after SARS-CoV-2 infection, 41.66% (n=10/24) of the patient's serum was positive for IIF-ANA. The distribution and titration values of the ANA patterns of the patients were shown in Table 1. We first observed nuclear patterns in the IIF-ANA test, 40% (4/10) were speckled, 20% (2/10) were homogeneous and speckled. In addition, anti-dsDNA was detected in two of the ANA positive patients. We also observed a cytoplasmic pattern in two of the twenty-four patients.

When we compared the ANA results nine months later with the pre-COVID ANA results, we observed that the results of three (12.5%) patients changed. We repeated IIF-ANA, ENA blot and anti-dsDNA testing for these three patients. The results were shown in Table 2.

In the first case, while the IIF-ANA was DFS (1+) before infection, three months after the infection, the IIF-ANA was homogeneous (1+) and cytoplasm was speckled (1+). Also, Scl-70 was positive in the ENA blot test, and Anti dsDNA ELISA was (43.6 IU/mL) positive. After nine months, IIF-ANA was the same pattern, Anti dsDNA was 46 IU/mL, and Anti Scl-70 was negative.

The second case had negative IIF-ANA before infection, three months after the infection, the nuclear membrane was found to be positive (2+). In this case, during acute SARS-CoV-2, aspartate aminotransferase was 51 U/L, alanine aminotransferase was 92 U/L and then decreased to mean values. AMA, ASMA, LKM tests for autoimmune liver disease was studied, and it was negative. However, anti-RNP/Sm antibody was positive in the ENA blot test. In contrast, the ANA test, which was re-run nine months later, was again detected as nuclear membrane (2+), anti-RNP/Sm antibody was found to be negative in the ENA blot test.

The third case had negative IIF-ANA before infection, three months after the infection, IIF-ANA speckled was detected as weakly positive. However, autoantibody positivity was not detected.

Discussion

Different mechanisms are hypothesized to explain how infections might provoke autoimmune reactions. These mechanisms, which can be directly or indirectly induced by infection, are epitope spreading, molecular mimicry, cryptic antigens and bystander activation (15). Similarly, different autoantibodies such as ANA, lupus anticoagulant, anti-β2glycoprotein 1, anti-Ro/SSA and anti-cardiolipin antibody have been detected in patients with SARS-CoV-2 (16-19).

Table 1. Demographic characteristics and distribution of autoantibody patterns of ANA positive patients

Patient no	Age/Gender	Post-COVID-19 ANA pattern (titer)	Pre-COVID-19 ANA pattern (titer)	Anti-dsDNA
1	54/F	Homogeneous (1+) cytoplasm speckled (1+)	DFS (±)	+
2	47/F	Nuclear membrane (2+)	Negative	-
3	49/F	Speckled (1+)	Speckled (1+)	-
4	48/F	Speckled (1+) and discrete cytoplasmic dots	Speckled (1+)	-
5	58/F	Homogeneous (±) Speckled (±)	Homogeneous (±) Speckled (±)	-
6	47/F	DFS (±)	DFS (±)	-
7	38/F	Speckled (1+)	Speckled+	-
8	42/F	Homogeneous (±) Speckled (±)	Homogeneous (±) Speckled (±)	-
9	40/F	Speckled (1+)	Negative	-
10	45/F	Nucleolar (1+)	Nucleolar (±)	+

ANA: Antinuclear antibody, DFS: (Nuclear dense fine speckled), F: Female, COVID-19: Coronavirus disease-2019

In different studies conducted in patients with SARS-CoV-2, the prevalence of ANA was found to be between 18% and 57.5% (19-27). In addition, in these studies, patients had no prior clinical record of the presence of antibodies. Most of these studies used IIF for ANA detection. On the other hand, in two of these studies, immunochemical methods were used for serum ANA detection (20,24). The variability in the prevalence of ANA in these studies may be due to characteristics such as sample sizes, different assay methods, and demographic, environmental, or genetic factors of the study population.

Firstly, in the study of Zhou et al. (20) with 8 severe and 13 critical COVID-19 patients, the prevalence of ANA was found to be 50%. In a study conducted with 156 COVID-19 patients in Turkey, it was found that ANA was positive in 40 patients (25.6%). Of these 156 patients, 18 were hospitalized. Of these 18 hospitalized patients, only 4 (22.2%) were found to be ANA positive (21). In another study, it was reported that the prevalence of ANA was 35.6% in patients hospitalized for SARS-CoV-2 pneumonia (17). In addition, Pascolini et al. (22) found that 45.4% (15 of 33 patients) of COVID-19 patients responded to at least one autoantibody and especially 33.3% of patients (11 patients) were found to have ANA reactivity.

In studies on the prevalence of ANA in COVID-19 patients, the lowest prevalence was found in the study of Peker et al. (27) with the rate of 18%. In the study of Peker et al. (27), the mean age of patients with positive ANA test was 62.08 years. In addition, as in the others, there was no previous clinical record of the presence of antibodies in the patients included in the study, and the IIF-ANA test was studied from samples collected from patients hospitalized for acute COVID-19. In our study, only three of 24 patients were hospitalized, the total positive rate of ANA was 41.6%, and the mean age of patients with positive ANA test was 46.8 years. Additionally, we detected different patterns in three patients (12.5%) compared to the pre-COVID ANA results.

Although diverse patterns of ANA have been detected at the onset of SARS-CoV-2 infection, the presence of patterns for mainly nuclear antigens has been reported. Pascolini et al. (22) detected 36.3% speckled, and 36.3% nucleolar staining among ANA-positive cases. Yumuk and Okumus (21) detected 10.0% mixed, 12.5% mitotic, 22.5% cytoplasmic and 55.0% nuclear patterns in forty ANA test positive cases. Similarly, in our study, we mainly observed nuclear patterns, such as 40% speckled and 20% homogeneous speckled staining among ANA test positive cases.

In our study, the first case whose IIF-ANA result changed three months after the COVID-19 infection was found to be homogeneous cytoplasm speckled and anti-dsDNA 43.6 IU/mL positive. The case was followed up in the rheumatology department for SLE with these results. In control examination performed nine months after the infection, ANA was positive in a homogeneous pattern,

Table 2. IIF-ANA and ENA Blot results of patients whose IIF-ANA patterns were changed

	Pre-COVID-19 ANA IFA	**Post-COVID-19 ANA IFA**	**Pre-COVID-19 ENA blot**	**Post-COVID-19 ENA blot**
Case 1	Nuclear dense fine speckled (1+)	Homogeneous (1+) cytoplasm speckled (1+)	RNP/Sm (-) Sm (-) SS-A (-) SS-B (-) Scl-70 (-) Jo-1 (-) dsDNA (-) DFS70 (+)	RNP/Sm (-) Sm (-) SS-A (-) SS-B (-) Scl-70 (+) Jo-1 (-) dsDNA (+) DFS70 (+)
Case 2	Negative	Nuclear membrane positive (2+)	RNP/Sm (-) Sm (-) SS-A (-) SS-B (-) Scl-70 (-) Jo-1 (-) dsDNA (-) DFS70 (-)	RNP/Sm (+) Sm (-) SS-A (-) SS-B (-) Scl-70 (-) Jo-1 (-) dsDNA (-) DFS70 (-)
Case 3	Negative	Speckled (1+)	RNP/Sm (-) Sm (-) SS-A (-) SS-B (-) Scl-70 (-) Jo-1 (-) dsDNA (-) DFS70 (-)	RNP/Sm (-) Sm (-) SS-A (-) SS-B (-) Scl-70 (-) Jo-1 (-) dsDNA (-) DFS70 (-)

IIF: Indirect immunofluorescence, ANA: Antinuclear antibody, COVID-19: Coronavirus disease-2019, ENA: Extractable nuclear antigen

and anti-ds DNA was still positive. During this period, the patient was re-evaluated. Due to photosensitivity, arthralgia in hands, morning stiffness, and weakness, SLE was diagnosed, and 400 mg/day hydroxylchloroquine treatment was started. The patient's morning stiffness and fatigue regressed. Similarly, cases of SLE diagnosed following COVID-19 have been reported in the literature (1,28). Although the etiology of SLE is unknown, both endogenous and exogenous factors have affected its pathogenesis. Infectious agents play an important role in the pathogenesis of SLE (29). Viruses such as parvovirus B19, retrovirus, cytomegalovirus, human immunodeficiency virus type 1 and Epstein-Barr virus have been implicated in the development of SLE (30). Possibly in COVID-19 patients, the cross-reacting epitope between the virus and the host leads to both humoral and cellular auto reactivity. This mechanism plays a vital role in the pathogenesis of SLE (31). As the pathogenesis of COVID-19 is understood, it is thought that it may trigger autoimmunity or exacerbate existing autoimmune diseases in genetically predisposed individuals (32). We think that lupus symptoms were triggered after COVID-19 in this first patient.

In a study of Fujii et al. (19), anti-SSA/Ro antibody positivity was detected in two patients with SARS-CoV-2. Fujii et al. (19) hypothesized that it was unclear whether elevation of anti-SSA/Ro antibody was a cause or consequence of aggravated SARS-CoV-2 pneumonia, further aggravating SARS-CoV-2 pneumonia due to the autoimmune response in both patients. In our study, while the ANA IFA test of the second case was negative before COVID-19, three months after the infection, the ANA IFA result was nuclear membrane positive (++), and the ENA blot test positive for anti-RNP/Sm antibody. The patient was investigated in terms of connective tissue diseases in the rheumatology department. It was seen that the patient had Hashimoto's thyroiditis and was stable under treatment for a long time. Furthermore, the ENA blot test became negative nine months after the infection but the nuclear membrane positivity continued. It is known that ANA positivity becomes positive in other organ-specific autoimmune diseases or infections other than systemic rheumatic diseases (33). We thought that positive ANA results in our patient might be associated with autoimmune thyroiditis, and ENA positivity might be associated with auto-reactivity triggered by acute COVID-19 infection. Another consideration of the coexistence of infection and positive ANA tests is that antibodies are not ultimately responsible for the onset of autoimmune illness and are a transient phenomenon.

The third case had negative IIF-ANA before COVID-19, three and nine months after the COVID-19, IIF-ANA speckled was detected as weakly positive. However, autoantibody positivity was not detected. In the evaluation made by the rheumatology department, it was decided to follow-up the case with a repeat test one year later.

In one case in our study, the ds-DNA test was positive after COVID-19. This patient was diagnosed as spondylarthritis before the COVID-19 pandemic and was in remission with NSAIDs. Her ANA test was positive before COVID-19, but ENA and ds-DNA were negative. Although she was ds-DNA positive after COVID-19, she did not have any lupus symptoms, but her hip pain and fatigue were increased. NSAIDs were enough for pain management. However, she was evaluated in the rheumatology department and was recommended to follow-up for SLE. We think ds-DNA positivity was triggered by COVID-19 infection.

Since ANA positivity can be seen in healthy population and in other clinical conditions, positive results must be interpreted together with the clinic. It has been reported that ANA positivity can be detected at a titer of 1/40 at a rate of 25-30%, 1/80 at a rate of 10-15% and at a titer of ≥1/160 at a rate of 5% in healthy controls (34,35). In our study, we compared the ANA test results before and after COVID-19. ANA positivity was detected in 12.50% of healthy individuals after COVID-19.

There is no consensus on appropriate definitions for cases where symptoms of COVID-19 persist beyond the acute phase of infection. A review by Aiyegbusi et al. (36) summarizes the available evidence regarding symptom prevalence, complications, and management of long-term COVID. According to this review, the 10 most commonly reported symptoms of long-term COVID-19 were: Fatigue 47%, dyspnea (shortness of breath) 32%, myalgia (muscle pain) 25%, arthralgia 20%, headache 18%, cough 18%, chest pain 15%, odor change 14%, taste change 7% and diarrhea 6%. Similarly, in our study, we observed arthralgia and fatigue in the hands in our first case. Beyond persistent symptoms, patients with long-term COVID-19 may have disease-related clinical complications and these complications are currently not well understood (37,38). According to the data we obtained in our study, even if there are no symptoms in post-COVID-19 patients, it may be useful to follow-up with autoantibody tests.

Study Limitations

The small number of patients was a limitation of our study. However, knowing the IIF-ANA results of the patients included in the study before COVID-19 reveals the difference of our study from other studies. In addition, our study sheds light on the long-term effects of this disease, thanks to tests performed three and nine months after the COVID-19 illness. Whitehead et al. (34) also reported that the recommended pilot trial sample size could be 24 in their study called Statistical Methods in Medical Research.

Conclusion

As a result, these data support the idea that SARS-CoV-2 infection may trigger autoimmunity and may be effective in the development of autoantibodies. Therefore, it might be beneficial to follow-up patients with IIF-ANA and autoantibody tests after COVID-19 infection. However, our study needs to be confirmed in larger scale studies and longer-term follow-up data to define the role of autoantibodies due to COVID-19 and their association with autoimmunity activation.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Samsun Training and Research Hospital (date: 01.04.2021, approval number: GOKA/2021/8/18) and was performed in accordance with the 1964 Declaration of Helsinki.

Informed Consent: Informed consent forms were obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B., H.S.B., Concept: M.B., E.B., Design: M.B., E.B., A.K., R.A., Data Collection or Processing: M.B., E.B., H.S.B., A.K., R.A., Analysis or Interpretation: M.B., H.S.B., A.K., Literature Search: M.B., H.S.B., A.K., Writing: M.B., A.K., R.A.

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