Evaluation of hemodialysis patients and hemodialysis health workers with COVID-19 IgM and IgG antibody test; a multicenter study from Eskisehir, Turkiye

**ABSTRACT**

**OBJECTIVE:** Hemodialysis (HD) patients are a population at high risk for exposure to the severe respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Undiagnosed mild or asymptomatic SARS-CoV-2 infection in HD patients can make these patients a potential source of infection. In our study, we aimed to evaluate the entire spectrum of SARS-CoV-2 infection with the IgM and IgG rapid antibody kit in HD patients and healthcare providers working in HD unit.

**METHODS:** 633 HD patients and 134 health workers from all dialysis centers (three private and three public) in Eskisehir were included in the study. Blood samples obtained from participants were allowed to clot for 30 min at room temperature at 15°C using a serum separator tube. Then it was centrifuged at 1000 g at 2–8°C for 15 min. The supernatant was collected and the samples were stored at -20°C until use. Serum samples stored at the end of the study were studied with the A.B.T.™ Biotechnology COVID-19 Rapid IgG-IgM Diagnostic Test. Routine examination was measured by standard methods. All participants were evaluated by serological analysis of IgG and IgM antibodies against the SARS-CoV-2 recombinant antigen.

**RESULTS:** Two symptomatic HD patients (0.27%) were diagnosed with SARS-CoV-2 infection by real-time reverse-transcription-polymerase-chain - reaction test and chest tomography. In 15 (2.36%) of 633 asymptomatic patients, antibody was positive against the SARS-CoV recombinant antigen (IgG in 13, both IgG and IgM in 2), while no antibodies were detected in 134 health workers.

**CONCLUSION:** We have shown that most HD patients with SARS-CoV-2 experience the disease asymptomatically, and that antibody testing plays an important role in identifying patients with asymptomatic infection.

**Keywords:** Antibody test; COVID-19; health workers; hemodialysis.

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On March 11, 2020, the World Health Organization declared the corona Virus Disease 2019 (COVID-19) as a pandemic, and many countries were affected by this disease, which is highly contagious, and there were many casualties [1].

Although the entire population is susceptible to the virus, the elderly and those with underlying diseases show a more serious clinical course and a higher death rate [2].

Hemodialysis (HD) patients are a population at high risk of exposure to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, because patients often provide transportation to the dialysis unit by public transport and are treated collectively in dialysis units. Limited and conflicting information is available on the true prevalence and clinical course of SARS-CoV-2 infection in HD patients. Probably because of the impaired T-cell immune response and poorer ability to develop cytokine storm, it has been suggested that symptoms are usually mild and the rate of progression to severe disease is lower in these patients [3, 4]. On the other hand, there are also some studies reporting that symptoms may be milder, atypical, or similar compared to the general population with more severe clinical consequences [5–8].

Undiagnosed COVID-19 infection in asymptomatic patients or in those with mild clinical course can make these patients a potential source of infection. On the other hand, health-care providers working at dialysis center are exposed to a high risk of infection because they are in close contact with these HD patients.

Although positive nucleic acid testing with real-time reverse-transcription-polymerase-chain-reaction (RT-PCR) remains the standard reference test for the definitive diagnosis of COVID-19, because of problems in sample collection, lack of symptoms, and limited availability of RT-PCR, some existing or previous infections may not be detected [9, 10].

Detection of specific SARS-CoV-2 antibodies in blood samples appears to be a good choice for the rapid, simple, and highly sensitive diagnosis. During viral infections, immunoglobulin M is known to provide the first line of defense before the formation of adaptive, high-affinity IgG responses, which is important for long-term immunity and immunological memory [8]. Because IgM antibodies indicates recent exposure and IgG antibodies indicates earlier exposure to SARS-Cov-2 virus, rapid detection of both IgM and IgG antibodies will be useful in the diagnosis and treatment of COVID-19 disease and also in identifying the asymptomatic carriers with the potential of spreading the virus [11].

Considering the rapid spread of the virus, high transmission rate, and the need for urgent diagnostic verification, it is recommended that patients with suspected COVID-19 should be tested for IgM and IgG antibodies specific to SARS-CoV-2, regardless of RT-PCR results [12].

In this study, we aimed to evaluate the entire spectrum of SARS-CoV-2 infection with the IgM and IgG rapid antibody kit in HD patients and healthcare workers from a total of six HD units.

**MATERIALS AND METHODS**

Data collected between September 1 to October 1, 2020, from patients and health workers in all dialysis centers (three public and three private centers) in Eskisehir were included in this multicenter, retrospective, and observational study. The study included the patients over the age of 18 years who are on standard HD treatment and the medical professionals (doctors, nurses, and auxiliary staff) working at the same HD center. Those who were not willing to participate in the study were excluded from the study. A total of 871 people (732 patients and 139 health workers) were included in the study. Of the 732 patients treated for HD in dialysis centers, two (0.27) were found to have symptomatic infections. Of the remaining asymptomatic patients, 633 (86.5%) agreed to participate in the study and 99 patients (13.5%) refused to participate in the study. There were 139 health workers, of whom 134 (96.4%) agreed and 5 (3.6%) refused to participate in the study.

The study was approved by the Ethics Committee of Eskisehir Osmangazi University with the decision number of E-80558721-050.99-86913. It was also found to be appropriate to conduct by the Scientific Research
Commission of the Ministry of Health. A clinical trial approval form was received from all participants so that we could use their data. The study was financially supported by Eskisehir Osmangazi University Scientific Research Project Unit (project code: 2020–11054).

Before the first case was detected in the country, medical staff and patients at all dialysis centers were given regular training on monitoring respiratory and gastrointestinal symptoms, hand washing, mask use, and social distance by measuring body temperature every day. Patients were not allowed to change dialysis centers at random, and food intake was prohibited during the dialysis procedure and patient transfer.

Since the first case was detected in the country in March 2020, health workers at six centers have used full personal protective equipment, including a surgical mask, waterproof apron, protective glasses, and a hat. At all centers, patients wore surgical masks when coming to the unit, during the dialysis process and when moving from the unit to their homes.

Data Collection
Data collected from all centers by reviewing electronic health records and patient files of dialysis units. Two independently trained doctors in the research team collected and checked the data. In case of incomplete or erroneous data, direct contact was made with the relevant doctors or other medical staff to determine epidemiological and symptom data. Data on demographic characteristics, clinical characteristics, computed tomography and laboratory tests, smoking habits, and etiologic cause of kidney disease were recorded.

Symptomatic patients and health workers diagnosed with COVID-19 disease by RT-PCR test of the samples obtained from nasopharyngeal creeps and chest tomography were recorded. Patients and healthcare workers who were asymptomatic screened with IgG and IgM antibodies against the recombinant antigen of the virus.

For antibody-positive patients, laboratory results during antibody testing were used.

COVID-19 Rapid IgG-IgM Diagnostic Test
Preparation and collection of samples
Serum samples from HD patients were collected by trained medical personnel from dialysis centers.

Using a serum separator tube, samples were clotted at room temperature at 15°C for 30 minutes. It was then centrifuged for 15 min at 2–8°C at 1000 g. The supernatant was collected and the samples were stored at −20°C until use.

Serum samples collected from patients were studied with A.B.T.”™ Biotechnology COVID-19 Rapid IgG-IgM Diagnostic Test (REF: T01-01-25).

This kit is a rapid chromatographic immunoanalysis for qualitative detection of COVID-19-specific IgG and IgM antibodies in human whole blood, serum or plasma and is used to help the scientific research of primary and secondary COVID-19 infections.

ABT COVID-19 Rapid IgG-IgM Diagnostic Test is a qualitative test that detects antibodies to 2019 nCoV in blood, serum or plasma.

COVID-19 IgG/IgM quick test cassette work
Before performing the test, the frozen samples were thoroughly mixed after being completely dissolved at room temperature.

A drop (~5 µl) of the sample was dripped into the well above the test cassette with a disposable sterile pasteur pipette included in the kit.

Then, two drops of the buffer solution containing bovine (included in the kit) were dropped on it.

The specificity of the test for IgG is 98% and 96% for IgM, and the accuracy is 98.6% for IgG and 92.6% for IgM.

Complete blood count was measured by mindray method, CRP by turbidometric method, and ferritin by ecria method (Roche auto analyzer, Switzerland).

Statistical Analysis
Shown as continuous given mean±standard deviation categorical data are given as percentage (%). Shapiro-Wilk test was used to investigate the compatibility of the data to normal distribution. Independent sample t-test analysis was used for groups with two groups in the comparison of groups showing normal distribution. In comparing the groups that do not conform to normal distribution, the Mann-Whitney U test was used for groups with two groups. Pearson Chi-Square, Pearson’s Exact Chi-Square and Fisher Exact Chi-Square analyzes were used in the analysis of the cross tables created. Logistic Regression analysis was performed to determine the risk factors. IBM SPSS Statistics 21.0 (IBM Corp., 2012 Published. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used for the analysis. P<0.05 value was accepted as the criterion for statistical significance.
RESULTS

Of the two symptomatic HD patients (0.27%) diagnosed with COVID-19 by RT-PCR test and chest tomography, one died and the other recovered. The remaining 633 asymptomatic patients and 134 health workers were evaluated by serological analysis of IgG and IgM antibodies to the SARS-CoV recombinant antigen. In 15 (2.36%) of asymptomatic 633 patients, antibodies against SARS-CoV recombinant antigen was detected (IgG in 13 and both IgG and IgM in 2 patients). On the other hand, none of the 134 health workers (0%) showed positive antibody testing. Although patients who were positive for IgM antibody had no symptoms, all these patients continued to undergo dialysis in special departments allocated in their own dialysis centers. Demographic, clinical, and laboratory characteristics of patients classified according to antibody test results are summarized in Table 1. Patients with positive and negative antibodies shared similar clinical characteristics, such as the etiology of end-stage kidney disease, the presence of co-morbidities, and the count of leukocytes and lymphocytes. It was found that patients with positive antibodies had less arteriovenous fistula use and more tunneled catheter use than patients with negative antibodies (p=0.0156). Patients with positive antibodies have a lower hemoglobin value (9.99±1.49 g/dL vs. 10.8±1.52 g/dL, p=0.0483) but a higher platelet value (250±92.2 10³/µL vs. 203±66.8 10³/ µL, p=0.0485), C reactive protein value (10.7 [4.14–26.5] mg/L vs. 1.98 [0.4–7.0] mg/L, p=0.00118) and ferritin value (1290±649 ng/ml vs. 806±536 ng/ml ± p=0.0053) than those with negative antibodies (Table 1).

No antibodies were detected in any of 134 health workers (0%), and the demographic characteristics of health workers are summarized in Table 2.

In logistic regression analysis, vascular access type, ferritin, and platelet values were independent risk factors for COVID-19, with an odds ratio (95% confidence intervals) of 0.357 (0.193–0.661), 0.999 (0.998–0.999), and 0.993 (0.986–0.999), respectively (Table 3).

DISCUSSION

In our study, asymptomatic cases were detected with antibody testing in 2.36% of patients on HD treatment, while no antibody positivity was found in health workers working at these centers. The majority of HD patients with SARS-CoV-2 infection were asymptomatic with subclinical disease. Many HD patients may be vulnerable to SARS-CoV-2 infection due to the uremic condition and the prevalence of accompanying co-morbid diseases. The collective treatment of HD patients in dialysis centers, transportation to the unit in groups and staying relatively close to each other during treatment session suggest that if these patients become infected, they may play an important role in the transmission of the virus. Therefore, it is critical to immediately identify individuals with SARS-CoV-2 infection in this particular group of patients and take measures to limit the spread of the virus.

In the early stage of the outbreak (in January 2020), COVID-19 frequency was reported to be 16% in HD patients and 12% in health workers in a series of 230 people reported from Wuhan [13]. Also in Wuhan, it was reported that the frequency of COVID-19 was 2.1% in a multi-centered study involving 7154 patients in 65 HD units where strict measures were taken as of the end of January 2020, and that there were no new cases in the units after February 26 as a result of the measures taken [14]. Universal screening of symptoms and pulmonary CT combined with RT PCR testing were undoubtedly important measures to help identifying and isolating patients infected with SARS-CoV-2, especially in the early stage of the outbreak. However, some infected patients who are asymptomatic, do not have typical radiology characteristics, or have low levels of virus load may be overlooked. In addition, it is difficult to distinguish chest CT manifestations of COVID-19 from those due to uremic status or other common respiratory pathogens in HD patients. In the study by Tang et al. [15], the chest CT of 11.6% of the HD patients with no COVID-19 infection showed frosted glass or patchy opacities suggestive of COVID-19. Another study also reported signs of lung infection including frosted glass opacities on chest CT in more than 30% of seronegative patients, suggesting that these imaging techniques may not be specific markers for SARS-CoV-2 infection [16].

There are little data on the complete clinical spectrum of COVID-19 in HD patients. It has been reported that half of SARS-CoV-2 infections in 1027 patients in 5 dialysis centers in Wuhan are subclinical, these patients are not identified by chest CT and RT PCR, and antibody tests can aid to evaluate the overall prevalence of SARS-CoV-2 infection in HD patients and to understand the diversity of its clinical course [15]. On the other hand, an Iranian study suggested that SARS-CoV-2 infection can affect the vast majority of HD patients asymptotically and of the 32 seropositive HD patients (IgG and/or IgM
| Characteristics                  | Patients without infection (n=614) | Patients with infection (n=15) | p     |
|----------------------------------|-----------------------------------|-------------------------------|-------|
| Hospital no. 1 (%)               | 19.2                              | 6.7                           |       |
| Hospital no. 2 (%)               | 0.7                               | 0                             |       |
| Hospital no. 3 (%)               | 32.4                              | 0                             |       |
| Hospital no. 4 (%)               | 12.4                              | 0                             |       |
| Hospital no. 5 (%)               | 26.1                              | 66.7                          |       |
| Hospital no. 6 (%)               | 9.3                               | 26.7                          |       |
| Age (yr), mean (SD)              | 63.4±13.1                         | 61.7±8.79                     | 0.396 |
| Gender, male, n (%)              | 53.9                              | 46.7                          | 0.769 |
| BMI, kg/m², mean (SD)            | 24.9±4.82                         | 26.6±4.77                     | 0.176 |
| Smoker (%)                       |                                   |                               | 0.458 |
| Never                            | 64.5                              | 80.0                          |       |
| Current                          | 13.5                              | 6.7                           |       |
| Former                           | 22.0                              | 13.3                          |       |
| Etiology of ESKD, n (%)          |                                   |                               | 0.912 |
| DKD                              | 32.4                              | 33.3                          |       |
| Hypertension                     | 37.5                              | 46.7                          |       |
| Hereditary renal disease         | 4.2                               | 0                             |       |
| Glomerulonephritis               | 3.1                               | 0                             |       |
| Other                            | 8.6                               | 6.7                           |       |
| Unknown                          | 14.2                              | 13.3                          |       |
| Dialysis years, mean (SD)        | 5.48±4.99                         | 4.41±2.52                     | 0.908 |
| Comorbidities, n (%)             |                                   |                               |       |
| Hypertension                     | 65.5                              | 66.7                          | 1.00  |
| Diabetes                         | 35.2                              | 40.0                          | 0.91  |
| CHD                              | 10.4                              | 13.3                          | 1.00  |
| COPD                             | 4.7                               | 0                             | 0.811 |
| Cancer                           | 3.3                               | 0                             | 1.00  |
| Use of ACEI, n (%)               | 1.8                               | 6.7                           | 0.683 |
| Use of ARB, n (%)                | 6.5                               | 6.7                           | 1.00  |
| Hemodialysis access, n (%)       |                                   |                               | 0.0156|
| AVF                              | 95.3                              | 80.0                          |       |
| TCC                              | 4.2                               | 20.0                          |       |
| AVG                              | 0.5                               | 0                             |       |
| Type of transport to hemodialysis, n (%) |                       |                               | 0.595 |
| Special vehicle                  | 17.4                              | 26.7                          |       |
| Service                          | 81.1                              | 73.3                          |       |
| Public transport                 | 1.5                               | 0                             |       |
| Laboratory finding               |                                   |                               |       |
| Hemoglobin/dL, mean (SD)         | 10.8±1.52                         | 9.99±1.49                     | 0.0483|
| Leukocytes, 10³/uL, mean (SD)    | 7.16±4.45                         | 7.61±2.06                     | 0.183 |
| Lymphocyte, 10³/uL, median (Q1-Q3) | 1.43 (1.1–1.86)                   | 1.64 (1.26–1.91)              | 0.312 |
| Platelet 10³/µL, mean (SD)       | 203±66.8                          | 250±92.2                      | 0.0485|
| CRP, mg/L, median (Q1-Q3)        | 1.98 (0.4–7.0)                    | 10.7 (4.14–26.5)              | 0.00118|
| Ferritin ng/ml, mean (SD)        | 806±536                           | 1290±649                      | 0.0053|

SD: Standard deviation; BMI: Body mass index; ACEI: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin receptor blocker; AVF: Arteriovenous fistula; AVG: Arteriovenous graft; BMI: Body mass index; CHD: Coronary heart disease; COPD: Chronic obstructive pulmonary disease; CRP: C reactive protein; DKD: Diabetic kidney disease; ESKD: End stage kidney disease; TCC: Tunneled-cuffed catheter.
positive), 16 showed concurrent evidence of COVID-19 with alternative methods but 16 had no evidence of infection, indicating the importance of serological testing. In 1542 HD patients in Hubei and Guangdong region of China, SARS-CoV-2 infection was detected in 5 (0.32%) patients by RT-PCR test and in 51 (3.3%) by serological test, and serological test was reported to detect 10 times more SARS-CoV-2 cases compared to the RT-PCR test [16]. The data of our study are similar to the data of latter study and 2 (0.27%) cases were detected by RT-PCR and 15 (2.36%) cases by serological test, approximately 9 times more patients were detected by serological test. Another study from China reported a higher SARS-CoV-2 seroprevalence of 3.3% and 1.8% in asymptomatic HD patients and healthcare workers, respectively, compared to other populations, with decreasing seropositivity in other cities with increasing distance to the epicenter of epidemic [17].

Most of the studies on SARS-CoV-2 seroprevalence have been reported from China, and further studies are needed to determine whether these results are generalizable to other populations and geographic locations, and to what extent seroprevalence has changed with the progression of the COVID-19 pandemic.

In our study, higher CRP and ferritin levels were found in patients with SARS-CoV-2 infection compared to those without. These findings may suggest an immune response similar to that of the general population in infected HD patients. In the regression analysis, it was found that the strongest determinant of SARS-CoV-2 infection was the vascular access type, namely the use of arteriovenous fistula was less and the use of tunnel cuffed catheters was higher in patients with positive antibodies compared to those with negative antibodies. It is not known whether the type of vascular access has an impact on the SARS-CoV-2 infection or the severity of the disease. In the study of Tang et al. [15], similar to our study, it was found that the use of tunnel cuffed catheters was higher and the use of arteriovenous fistula was less in patients with SARS-CoV-2 infection.

### Table 2. Demographic characteristics of 134 healthcare workers

| Hospital no. 1 (%) | 17.1 |
|-------------------|------|
| Hospital no. 2 (%) | 19.4 |
| Hospital no. 3 (%) | 19.4 |
| Hospital no. 4 (%) | 9.0  |
| Hospital no. 5 (%) | 17.2 |
| Hospital no. 6 (%) | 17.9 |
| Gender, male, n (%) | 30.6 |
| Age (yr), mean (SD) | 36.7±9.13 |
| BMI, kg/m², mean (SD) | 24.9±3.49 |
| Smoker, (%) | 67.1 |
| Never | 67.1 |
| Current | 28.3 |
| Former | 4.5  |
| Comorbidities, (%) | 2.2  |
| Diabetes | 2.2  |
| Hypertension | 0  |
| CHD | 0  |
| COPD | 0  |
| Type of transport to hemodialysis, (%) | 41.0  |
| Special vehicle | 41.0  |
| Service | 40.3 |
| Public transport | 18.7 |

SD: Standard deviation; BMI: Body mass index; CHD: Coronary heart disease; COPD: Chronic obstructive pulmonary disease.

### Table 3. Univariate and multivariate logistic regression analysis of variables associated with the positivity of SARS-CoV-2

| Variables      | Univariate analysis | Multivariate analysis |
|----------------|---------------------|-----------------------|
|                | OR (95% CI) Wald p  | OR (95% CI) Wald p    |
| Vascular access| 0.342 (0.194–0.602) | 0.357 (0.193–0.661)   |
| Ferritin, ng/ml| 0.999 (0.998–0.999) | 0.999 (0.998–0.999)   |
| Platelet, 10⁹/µL| 0.992 (0.986–0.998) | 0.993 (0.986–0.999)   |
| Hemoglobin, g/L| 1.404 (1.024–1.926) | 1.404 (1.024–1.926)   |
| CRP, mg/L      | 0.986 (0.972–1.001) | 0.986 (0.972–1.001)   |

CRP: C reactive protein; OR: Odd ratios; CI: Confidence interval.
It is known that the long-term use of central venous catheters for vascular access in HD patients is an important pro-oxidative factor triggering the inflammation [18]. It is difficult to distinguish whether the elevation of ferritin and CRP is due to catheter-related inflammation or inflammation due to SARS-CoV-2 infection. This mechanism needs to be investigated more detailed.

In a meta-analysis, thrombocytopenia was significantly associated with the severity of COVID-19 disease; however, it suggested very high heterogeneity between studies; A greater decrease in platelet counts was reported, particularly in patients who died [19]. Conversely, Qu et al. [20] showed that among 30 patients hospitalized with COVID-19, those presenting with a peak in platelet count during the course of the disease had worse outcomes. Qu et al. [20] suggested that a high platelet/lymphocyte ratio may be indicative of a more pronounced cytokine storm due to increased platelet activation. In our study, although it was within the normal reference range, we found a higher platelet value in those who had SARS-CoV-2 infection. While low platelet count during acute infection may be a sign of disseminated intravascular coagulation and a poor prognosis [21], we think that the clinical significance of platelet value in previous infection should be investigated further.

In our study, no SARS-CoV-2 infection was found in healthcare workers. There may be more than one reason for this. First, although HD patients have to visit dialysis centers regularly even if there is a suspicion of infection, healthcare professionals have no such necessity. Second, health-care professionals have used full personal protective equipment since the first case was detected in the country; however, dialysis patients may have provided insufficient protection with because of using only surgical masks.

Major strengths of our study are that it is the first study with the highest number of patients to show cumulative SARS-CoV-2 infection in HD patients and healthcare workers outside of China, the use of a single test kit, and the homogeneity of the results.

However, we also have some limitations. First, the small number of seropositive cases prevented us from conducting a more detailed analysis among subgroups. Second, because of the cross-sectional design of the study, we were unable to monitor the dynamic changes in antibody titer in infected patients. In fact, it will be important to know the quantitative titer of antibodies that can provide immunity to future infections in an asymptomatic patient. Third, the sensitivity of the serological test depends on the timing of antibody production after infection. Namely, testing before onset or after termination of antibody production may give a false negative result, so serological testing may underestimate the prevalence of SARS-CoV-2 infection.

In conclusion, we showed that most HD patients with SARS-CoV-2 experience the disease asymptomatically and antibody testing has an important role in determining patients with asymptomatic infections.

Ethics Committee Approval: The Eskisehir Osmangazi University Clinical Research Ethics Committee granted approval for this study (date: 27.08.2020, number: 02).

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