Rev Nefrol Dial Traspl. 2021;41(3):159-65

Antibody production against COVID-19 in hemodialysis patients

Producción de anticuerpos contra COVID-19 en pacientes en hemodiálisis

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ABSTRACT

Introduction: Dialysis patients may have a very high prevalence and death rate for COVID-19. The aim of this study is to show the level of antibody against SARS-CoV-2 in hemodialysis and staff working in the same dialysis center. **Methods:** Anti-SARS-CoV-2 IgG antibodies were studied in 156 hemodialysis patients and 27 staff. After a 5-minute resting period, blood pressure was measured and then subsequent to an approximately 12-hour fasting period, blood sample were drawn for biochemistry parameters and anti-SARS-CoV-2 IgG antibodies. **Results:** Three of hemodialysis patients were diagnosed with COVID-19 in March and their PCR tests were positive. The symptoms of these patients were extreme fatigue and muscle weakness. Anti-SARS-CoV-2 IgG antibodies tests were performed on all patients and staff in July. In total, 13/156 (8.3%) patients were diagnosed as COVID-19 based on anti-SARS-CoV-2 antibodies. The other 10 patients were asymptomatic. The staff and 143 hemodialysis patients had IgG (-). Hemodialysis patients who had anti-SARS-CoV-2 IgG (+) antibodies had decreased level of haemoglobin and high levels of C-reactive protein and alkaline phosphatase. **Conclusions:** Antibody tests are particularly important for detecting people with COVID-19 who have few or no symptoms. It has also been seen that the spread of infection in the dialysis center can be prevented by very strict precautions.

KEYWORDS: COVID-19; SARS-CoV-2; prevalence; renal dialysis; hemodialysis; antibodies; immunoglobulin G

RESUMEN

Introducción: Los pacientes que se realizan diálisis tienen una prevalencia y una tasa de mortalidad muy alta en lo que respecta a la COVID-19. El objetivo de este estudio fue mostrar la concentración de anticuerpos contra el SARS-CoV-2 en los pacientes de hemodiálisis de un centro y en el personal que trabaja en ese lugar. **Material y métodos:** Se investigaron los anticuerpos IgG contra el SARS-CoV-2 en 156 pacientes que reciben hemodiálisis y en 27 trabajadores. Se midió la tensión arterial luego de 5 minutos de reposo y, luego de un ayuno de aproximadamente 12 horas, se extrajeron muestras de sangre para determinar variables bioquímicas y la concentración de anticuerpos IgG contra el SARS-CoV-2. **Resultados:** En marzo,

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Acknowledgments: The authors would like to thank our self-sacrificing staff and our patients who want to contribute to this study.

Ethics: A written informed consent of the patient for publication has been obtained. This study had been approved by the Education Research Hospital's ethical committee with the following approval number and date: 2020/131, 23.07.2020. All participants included in the study were informed about the study and their written permissions were obtained.

Conflict of interest: No conflict of interest.

Funding: There is no funding support.

Recibido: 09-12-2020
Corregido: 07-02-2021
Aceptado: 19-02-2021

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a través de pruebas PCR, se les diagnosticó COVID-19 a tres pacientes en hemodiálisis. Los síntomas de estos pacientes fueron fatiga extrema y debilidad muscular. En julio se realizaron pruebas de anticuerpos IgG contra el SARS-CoV-2 a todos los pacientes y al personal. En total, de los 156 pacientes, 13 (8,3 %) fueron diagnosticados con COVID-19 según los anticuerpos contra el SARS-CoV-2. Los otros 10 resultaron asintomáticos. El personal y 143 pacientes en hemodiálisis tuvieron resultados negativos para IgG. Los pacientes en HD con resultados positivos para IgG contra el SARS-CoV-2 presentaron niveles disminuidos de hemoglobina y niveles altos de proteína C reactiva y fosfatasa alcalina. **Conclusiones:** Las pruebas de anticuerpos son particularmente importantes para detectar personas con COVID-19 que tengan pocos síntomas o ninguno. También se ha visto que es posible prevenir la propagación de la infección en el centro de diálisis mediante precauciones muy estrictas.

PALABRAS CLAVE: COVID-19; SARS-CoV-2; prevalencia; diálisis renal; hemodiálisis; anticuerpos; inmunoglobulina G

INTRODUCTION

Coronavirus disease 2019 (COVID-19), a pandemic that affects the world population, causes serious acute respiratory syndrome in humans, usually with symptoms of fever, cough, shortness of breath, myalgia and fatigue.[1]

Older age and concomitant hypertension, diabetes, cardiovascular disease, lung disease, neutrophil and organ dysfunction are risk factors for COVID-19.[1-3] Dialysis patients may have high prevalence and death rate for COVID-19, since they combine older age, malnutrition, cardiovascular disease, diabetes, lung disease and less efficient immune system.[3-6]

End stage renal disease is accompanied by systemic inflammation and immune deficiency.[7-8] Immune deficiency causes a severity of microbial infections and poor response to vaccination while systemic inflammation contributes to atherosclerosis and cardiovascular disease. Immune deficiency is due to depletion of antigen presenting dendritic cells, T and B lymphocytes, decreased function and impaired phagocytic ability of monocytes and polymorphonuclear neutrophils. Therefore, patients with chronic renal failure and hemodialysis (HD) respond poorly to standard vaccination procedures and antibody response levels may be low.[7-8]

Considering the transmission rate of COVID-19, an infected patient or staff also increases the likelihood of infection of many patients undergoing dialysis in the same environment due to the following conditions; the crowded environment of dialysis centers consisting of many patients, nurses, doctors and support staff at the same time, dialysis patients are taken from their homes before the session with 10-15 person vehicles and brought to the center and they are transported to their homes with the same vehicle after the session. Thrice weekly dialysis may increase the risk of infection spread among patients and staff. However, these patients should continue their dialysis treatment. Not every infected person may be symptomatic. There may be many asymptomatic carriers in the community [1]. IgM and IgG antibodies become detectable within days and weeks after infection.[9] Serological tests are important in predicting the prevalence of infections, including asymptomatic patients.[9] The determination of antibodies may enable confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases.[9] There is no clear data to measure the levels of antibodies against SARS-CoV-2 in patients with chronic renal failure and HD patients.

The aim of this study is to show the level of antibody formation against SARS-CoV-2 in HD and staff working in the same dialysis center.

MATERIAL AND METHODS

In March 2020, a dialysis center in Kayseri had 156 HD patients and 27 staff. All the patients were performed for 4-5 hour/session thrice weekly. March 10, 2020 since the first cases seen in Turkey, measures have been tightened. Three of 156 HD patients were infected with SARS-CoV-2. The first COVID-19 patient was diagnosed on March 23 who had only very intense fatigue and muscle weakness. Soon, the second and third diagnosed patients appeared on April 13 and 23 respectively. These two patients presented with the same symptoms and they

 ISSN 0326-3428

were immediately hospitalized. Patient's PCR test were positive. These three patients were discharged from the hospital on April 14, May 18, and April 28, respectively.

The study was conducted on patients having an SARS-CoV-2 infection (3 male) and 153 (64 females and 89 male) other HD patients and all staff (8 females, 19 male) on July. Anti-SARS-CoV-2 IgG antibodies were studied in 156 HD patients and 27 staff. Three groups were created in the study: 1st group: COVID-19 antibody test positive; 2nd group: COVID-19 antibody test negative; 3th group: Healthy staff in the same dialysis unit.

After a 5-minute resting period, blood pressure was measured and then subsequent to an approximately 12-hour fasting period, blood sample were drawn for biochemistry parameters such as haematocrit, serum calcium, phosphate, albumin, parathyroid hormone levels and Anti-SARS-CoV-2 IgG antibodies. Dialysis efficacy expressed as Kt/V urea.

Serological (IgG) test was performed using enzyme immunoassay (ELISA). COVID-19 ELISA test systems (EUROIMMUN, Medizinische Labordiagnostika AG) was worked in accordance with the manufacturer's instructions. The sensitivity in samples taken between day 10 and day 20 amounted to 75.0% for the Anti-SARS-CoV-2 ELISA (IgG), after more than 20 days, the sensitivity of this ELISA amounted to 93.8%. The results are interpreted as follows: Ratio <0.8 negative; Ratio ≥ 0.8 to <1.1: borderline; Ratio ≥1.1: will be considered positive.

This study had been approved by the Education Research Hospital's ethical committee with the following approval number and date: 2020/131, 23.07.2020. All participants included in the study were informed about the study and their written permissions were obtained.

STATISTICAL ANALYSES

All statistical analysis was performed using the SPSS version 22.0 packet program. The results were shown as mean ± standard deviation (SD). Shapiro-Wilk test will be performed to check the normality assumption of the data. Student t test will be used for continuous variables when the data were normally distributed, otherwise we used Mann-Whitney U test, categorical variables were compared by the chi-square test. Logistic regression analysis was performed for the variables found significant. A p value 0.05 was accepted as statistically significant.

RESULTS

The general information and biochemical findings of the groups have been presented in **Table 1**. The mean age of the HD patients was 65.5 ± 12.3 years (27–90 years), and 58.9% were male. Mean age of the staff 32.4 ± 9.2 years and 70.3% were male and their antibody levels 0.46 ± 0.2. The groups were divided into two because the results of all staff were negative. Group 1: anti-SARS-CoV-2 IgG (+) Ab HD and Group 2: anti-SARS-CoV-2 Ab (-).

In total, 13/156 (8.3%) patients were diagnosed as COVID-19 based on anti-SARS-CoV-2 antibodies. Only 3 of these patients had symptoms of extreme fatigue and muscle weakness and their PCR test were positive. The other 10 patients were asymptomatic.

The incidence of COVID-19 in HD patients was 8.3% in this dialysis center. None of the patients died between March and July 2020.

There were not statistically significant difference between patients groups in length of dialysis, Kt/V, interdialytic weight gain, age, gender, access to HD and blood pressure ($p>0.05$). Hemoglobin levels ($p<0.05$) were significantly decreased in the anti-SARS-CoV-2 IgG (+) antibodies group compared to IgG (-) group. C-reactive protein ($p<0.05$) and alkaline phosphatase levels ($p<0.05$) were higher in anti-SARS-CoV-2 IgG (+) antibodies group than IgG (-) group.

Antibody level was significantly higher in anti-SARS-CoV-2 IgG (+) antibodies group compared to the IgG (-) group ($p<0.001$). Other laboratory analysis was not significantly different between the two groups.

There was no statistically significant difference between the two groups in consumption of angiotensin converting enzyme inhibitor/ angiotensin receptor blockers (ACEI/ARB), β-bloker, D vit and in terms of concomitant diseases.

The results of logistic regression analysis are given in **Table 2**. We observed that HD patients who had anti-SARS-CoV-2 IgG (+) antibodies had decreased levels of hemoglobin (OR: 1.80

Table 1. Comparison of parameters related to hemodialysis patients

Variables	Anti-SARS-CoV-2 Antibodies IgG (+) n (13)	IgG (-) n (143)	p
Age, (years)	63.5±13.7	65.7 ±12.2	0.539
Gender n (%)			
Female	4 (30.8)	60 (42.0)	0.317
Male	9 (69.2)	83 (58.0)	
Hemodialysis Access n (%)			
A-V fistula	8 (61.5)	81 (57.4)	0.508
Catheter	5 (38.5)	60 (42.6)	
Duration of HD (months)	30.8±31.4	38.0±35.3	0.474
Systolic blood pressure	108.5±15.7	106.2±14.4	0.595
Diastolic blood pressure	66.2±10.4	63.8±9.3	0.385
Interdialytic weight gain	2.0±1.3	1.4±1.1	0.068
Kt/V	1.6±0.3	1.6±0.3	0.839
Laboratory analysis			
Hemoglobin, g/dl	9.8±1.3	10.8±1.3	0.010
WBC, 10^3/µl	7.3±2.2	7.9±5.7	0.674
Platelets, 10^3/µl	213.8±71.1	214.4±78.5	0.980
Total protein, mg/dl	69.4±4.9	69.2±5.6	0.884
Serum albumin, mg/dl	37.5±4.3	38.8±3.7	0.232
Calcium, mg/dl	8.7±0.7	8.9±0.6	0.428
Phosphorus, mg/dl	4.6±1.0	4.7±1.1	0.629
FBS, mg/dl	168.5±157.1	142.9±62.8	0.238
ALP, U/l	167.1±71.7	129.2±68.0	0.022
ALT, U/l	14.0 ±2.2	12.9±11.5	0.851
Parathyroid hormone,pg/ml	210.24±132.7	211.6±157.1	0.977
Serum CRP, mg/l	50.4±75.3	15.5±29.5	0.029
Iron, µg/dL	39.1±20.2	48.7±27.1	0.215
Potassium, mmol/l	4.9±0.6	5.0±0.7	0.867
Anti HBs (yes/no)	11(84.6) / 2 (15.4)	95 (66.9) /47 (33.1)	0.158
Anti-SARS-CoV-2 antibodies IgG,	5.9±4.9	0.3±0.1	0.000
Concomitant diseases n (%)			
Coronary heart disease	3 (23.1)	32 (22.4)	0.593
Diabetes	6 (46.2)	75 (52.4)	0.441
COPD	1 (7.7)	7 (4.9)	0.510
Asthma	1 (7.7)	5 (3.5)	0.412
Hypertension	3 (23.1)	9 (6.3)	0.064
Medicines used n (%)			
ACE/ARB	-	3 (2.1)	0.769
B-blocker	6 (46.2)	80 (55.9)	0.347
CCB	1 (7.7)	6 (4.2)	0.463
Eritropoetin	11 (84.6)	94 (65.7)	0.139
D-vit	-	2 (1.4)	0.840
IV ıron	13 (100.0)	127 (88.8)	0.231

CRP: C-reactive protein; WBC: white blood cell count, FBS: fasting blood sugar, ALP: alkaline phosphatases. ALT: alanine aminotransferase. COPD: chronic obstructive lung disease, CCB: calcium channel blocker.

ISSN 0326-3428

Table 2. Factors associated with HD patients in the anti-SARS-CoV-2 IgG (+) antibodies group

Variables	Odds ratio	95% CI	*p*
C-reactive protein	0.988	0.978-0.999	0.034
Alkaline phosphatases	0.992	0.984-0.999	0.025
Hemoglobin	1.800	1.133-2.860	0.013

95% CI: 1.13-2.86), high levels of C-reactive protein (OR: 0.98 adjusted 1.01; 95% CI: 0.97-0.99) and alkaline phosphatase levels (OR: 0.99 adjusted 1.00; 95% CI: 0.98-0.99).

DISCUSSION

The diagnose of COVID-19 in HD patients is quite difficult because disease can be asymptomatic.[3,6] Therefore, the virus-specific antibody detection for COVID-19 could be important in surveying for asymptomatic patients.[6] Anti-SARS-CoV-2 IgG antibodies were studied in 156 patients and 27 staff in this study. Anti-SARS-CoV-2 IgG antibodies was positive in 13 of 156 patients and 143 patients and 27 staff were negative. Ten of the 13 HD patients who tested positive for anti-SARS-CoV-2 antibodies had no symptoms. It was thought that these patients could be protected from severe cytokine storms by their impaired immune systems.[2,3,10]

The risk of SARS-CoV-2 infection in elders and concomitant diseases is increased.[3-6] In our study, there were no significant difference between IgG (+) and IgG (-) groups in terms of age and underlying disease.

No patient died in our study. In some studies, the mortality rate was 16.2%, 10.4% and 30% respectively.[3,6,10] The most important difference of our study from the other two studies is the frequency of hypertension.[10-11] In this center, strict volume control is performed in the treatment of hypertension. The interdialytic weight gain of our patients is 1.45 kg. We think that hypervolemia causing hypertension and congestion in the lung may increase mortality and morbidity in COVID-19 HD patients.

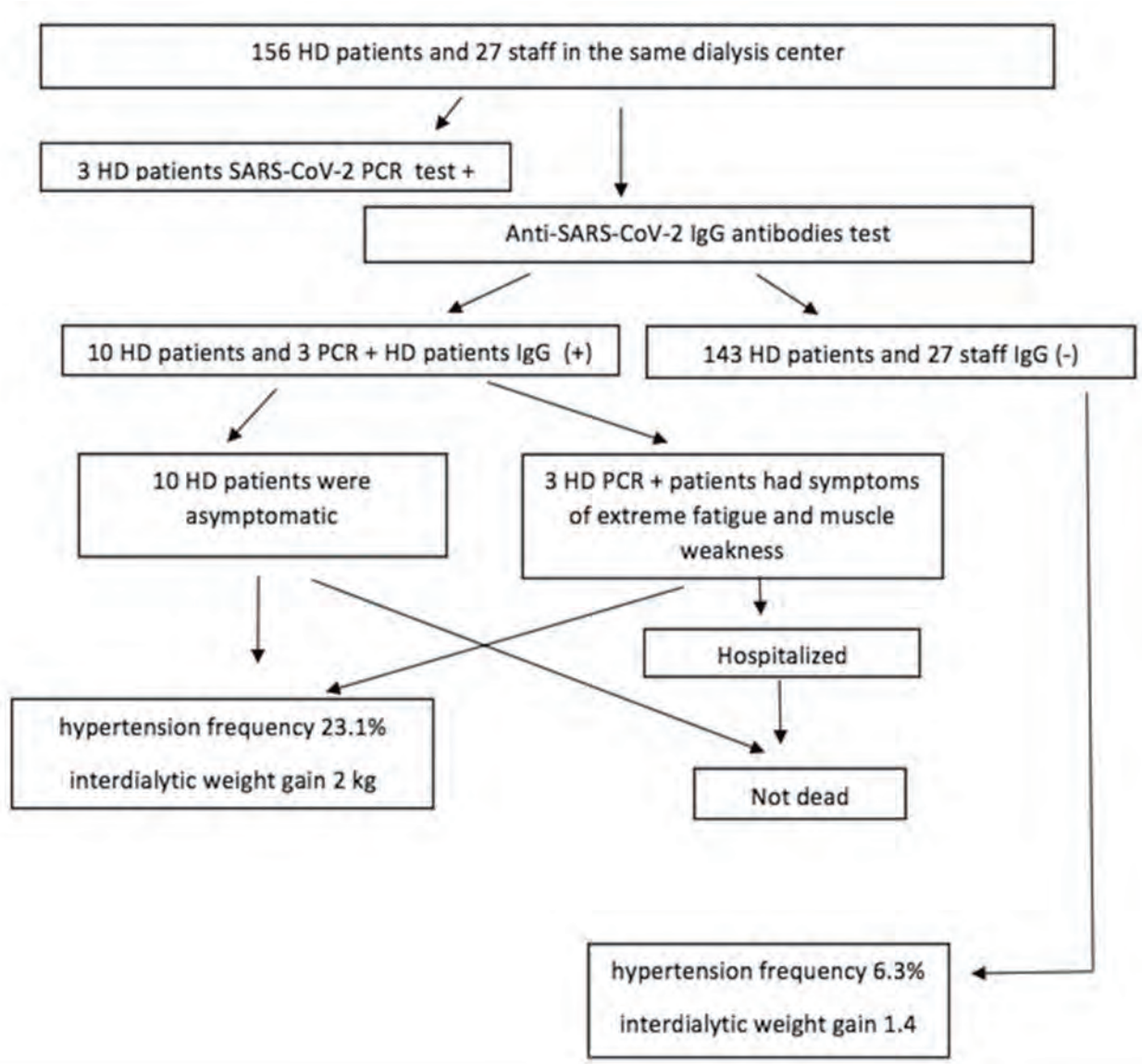
Concomitant hypertension is also known to be a risk factor for COVID-19.[1-3] In this dialysis center, the incidence of hypertension was 23.1% in patients with IgG (+), while it was 6.3% in patients with IgG (-). According to two studies, the rate of hypertension is presented as 97% and 93%, respectively.[10-11]

Hemoglobin levels ($p<0.05$) was significantly decreased, C-reactive protein and alkaline phosphatase levels were higher in the anti-SARS-CoV-2 IgG (+) antibodies HD patients. We think that the reason for the high level of C-reactive protein and low hemoglobin level is the inflammation caused by SARS-CoV-2. In this study, alkaline phosphatase was found significantly higher in HD patients with anti-SARS-CoV-2 IgG (+) antibodies in parallel with other studies.[11-12] We think that this elevation of alkaline phosphatase may be due to hepatic involvement.

Another important point of this study is the focus on infection control strategies. It is noteworthy that although 13 HD patients in our study had anti-SARS-CoV-2 IgG (+) antibodies, the staff constantly in close contact with them and 143 HD patients had IgG (-).

According to our observations, our patients did not have classic symptoms. The following decisions were made by evaluating the first case in our country on March 10, as soon as the following measures were implemented in our own dialysis center; training of all medical staff and patient on prevention and control of COVİD-19; all staff members and patients were required to take body temperature and observe respiratory symptoms every day, all workers took protective measures and effective precautions, including wearing masks, hats, hand hygiene and gowns.[1,5,13-14] In addition to all these warnings, patients with large families were asked to use masks and isolate them at home, frequent ventilation of the dialysis unit, providing isolation with curtains between patients and strict supervision of them and repeated staff training. Then the risk of infection was under control and no new cases were observed.

Figure 1. Flow chart of the study



HD: hemodialysis; **PCR+:** PCR positive patients; **IgG (+):** Anti-SARS-CoV-2 IgG antibodies test positive; **IgG (-):** Anti-SARS-CoV-2 IgG antibodies test negative

The recent study reported zero infections or nosocomial transmissions in 413 health care workers' caring for 42 confirmed COVID-19 infection cases.[5]

CONCLUSIONS

There are many asymptomatic cases of COVID-19 in the community. The disease spreads seriously through these people. This becomes even more important in HD patients with a suppressed immune system.

These measures should not be limited to the dialysis center. It should also be done in patient's relatives and the environment where the patient lives.

Limitation in this study; it was conducted in a single centre and with a small number of patients. We think that a sufficient number of antibody positive and negative cases should be examined.

 ISSN 0326-3428

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