Original Article

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Anti-SARS-CoV-2 Antibody Levels in Chronic Kidney Disease Stage 5 Patients on Maintenance Haemodialysis: A Prospective Observational Study from a Government Hospital in Central Kerala, India

Biochemistry Section

BIJU K GOPINATH[1], ZAINAB YASEEN[2], ARUN KARAT[3], USHA SAMUEL[4]

ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) pandemic caused significant mortality among vulnerable populations in India, including patients with Chronic Kidney Disease (CKD) on maintenance haemodialysis. The vaccination was the mainstay of prevention. CKD Stage 5 patients requiring haemodialysis have high mortality and poor immune response to many infections, including postvaccination Hepatitis B and Influenza. Antibody response to COVID-19 vaccination in these patients was lower compared to the general population.

Aim: To estimate Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) IgG antibody level in the blood sample of CKD Stage 5 maintenance haemodialysis patients.

Materials and Methods: A prospective observational study was conducted at the Nephrology department of Government Medical College, Ernakulam, Kerala, India for six months. A total of 55 patients of CKD Stage 5 who were on maintenance haemodialysis were included in the study. The control group consisted of 55 matched close relatives of patients. The anti-SARS-CoV-2 antibody levels were measured in both groups. Blood samples for antibody testing were drawn from the cases, along with routine monthly tests done before a dialysis session. The blood samples were collected simultaneously from the controls, sent to the hospital's central laboratory and processed on the same day. Serum was separated after centrifugation and then stored in small 1 mL Eppendorf tubes/vials and refrigerated at 2-8°C. These serum samples were analysed for anti-SARS-CoV-2 IgG antibody levels in both cases and controls within 24 hours, using the Chemiluminescence Immunoassay (CLIA) method on the VITROS ECiQ Immunodiagnostic system. Relevant data were collected in MS Excel and statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software version 27.0. An unpaired t-test was applied for comparison, with a p-value <0.05 considered statistically significant.

Results: Males predominated in the test/case group (43, 78.1%), while females were more prevalent in the control group (42, 76.4%). The comparison of antibody levels between cases and controls showed higher levels in the control group, with a p-value of 0.65, indicating a lack of significance (p-value >0.05). Interestingly, participants vaccinated with Covishield had significantly higher antibody levels at 188.95 BAU/mL compared to those vaccinated with Covaxin or other vaccines, with a p-value of 0.04.

Conclusion: The antibody response in CKD Stage 5 haemodialysis patients was found to be lower compared to the control group; however, the difference was not significant. In the current study, the antibody response to the Covishield vaccine was high compared to other vaccine. Larger sample size studies should be conducted in future to validate the results of the present study, if similar COVID-19 like situation reoccurs.

Keywords: Coronavirus disease-2019, Covishield, Immunoglobins

INTRODUCTION

The COVID-19 pandemic has ravaged India, with three distinct waves causing significant mortality and morbidity among susceptible populations. COVID-19 vaccination is the mainstay for preventing mortality and morbidity among all population subgroups, covered more than 90% of the adult population in Kerala State, India [1]. CKD Stage 5 haemodialysis patients are a distinct subgroup with high mortality and morbidity with COVID-19 infection [2]. CKD Stage 5 haemodialysis patients show poor immune response to many infections, even after vaccination (such as Hepatitis B and Influenza) [3]. Patients with End-Stage Renal Disease (ESRD), including those on haemodialysis, have been largely excluded from vaccine trials due to safety concerns. Additionally, ESRD patients typically exhibit diminished responses to vaccination compared to healthy individuals because of dysfunction in the adaptive immune system.

One study found that the antibody response to COVID-19 vaccination in kidney transplant and CKD patients on maintenance haemodialysis at nephrology centres was significantly lower compared to controls [4]. Another study conducted in French patients with CKD receiving maintenance dialysis aimed to measure plasma anti-SARS-CoV-2 spike protein S1 immunoglobulin after the second dose and at least three weeks after the third dose of the BNT162b2 vaccine. It was found that there was a considerably increase in antibody levels after a third dose of the BNT162b2 vaccine and it appeared to be as well tolerated as the second dose [5].

However, the data on antibody response to COVID-19 infection after vaccination in these groups of patients residing at Kerala is not available in literature. Hence, this study focused on CKD Stage 5 haemodialysis patients to analyse their Anti-SARS-CoV-2 antibody levels postvaccination.

MATERIALS AND METHODS

A prospective observational study was conducted at the Nephrology Centre of Government Medical College, Ernakulam, Kerala, India from August 2022 to February 2023, sample collection, thereafter data analysis up to July 2023. A total of 55 patients with CKD Stage 5 who

were on maintenance haemodialysis during the study period were included in the study. The control group consisted of 55 matched close relatives of patients, after obtaining Ethical Committee approval with IEC number (IEC 105/2022).

Inclusion criteria for cases:

(1) CKD Stage 5 patients, undergoing haemodialysis, for more than three months;

(2) Males and females vaccinated with at least one dose of the COVID-19 vaccine;

(3) Age >18 years;

(4) Those who consented to participate in the study.

Exclusion criteria for cases:

(1) Age <18 years;

(2) Patients who were not vaccinated with COVID-19 vaccine;

(3) Not willing to be the part of the study.

Inclusion criteria for control:

(1) Individuals staying in the same house as the case/patient;

(2) If the case/patient was documented to be COVID-19 positive by Polymerase Chain Reaction (PCR) or antigen test, preferably the corresponding control was documented COVID-19 positive by PCR or antigen test;

(3) Age group matched, age >18 years.

Exclusion criteria for control:

(1) On haemodialysis or suffering from a chronic illness;

(2) Not willing to be part of the study;

(3) Taking immunosuppressant medication;

(4) Non vaccinated were excluded from the study.

Informed written consent was taken from both the test/case and control groups before conducting the study. The anti-SARS-CoV-2 antibody levels were measured in both groups. Venous blood samples were collected simultaneously from both the controls and case/test group and sent to the central laboratory of the hospital in specially blinded labelled blood tubes. The blood samples were processed on the same day and the serum was separated by the centrifugation and then stored in 1 mL Eppendorf tubes refrigerated at 2-8°C. These serum samples were analysed for anti-SARS-CoV-2 IgG antibody levels in cases and controls within 24 hours, by using CLIA on VITROS ECiQ Immunodiagnostic system.

Principle of CLIA method: An immunometric technique was used as a quantitative test to measure the amount of IgG antibodies in serum. The antibodies against coronaviruses focus on trimeric spikes and are mostly formed against the Receptor Binding Domain (RBD). Different types of IgGs have been found depending on their affinity towards RBD epitopes [6].

In this study, the immunoassay technique uses antibodies against the RBD for SARS-CoV and its S1 epitope. This was a quantitative immunoassay that involved a two-stage reaction. In the first stage, antibodies to SARS-CoV present in the sample bind with SARS-CoV spike protein S1 antigen coated on the wells. The unbound sample is removed by washing the automated process inside the analyser. In the second stage, Horseradish Peroxidase (HRP)-labeled murine monoclonal anti-human IgG antibodies are added to the conjugate reagent. The conjugate specifically binds to the antibody portion of the antigen-antibody complex. The excess unbound conjugate is removed by the subsequent wash step. The bound HRP conjugate is measured through a luminescent reaction [6]. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalysed the oxidation of the luminol derivative, producing light. The electron transfer agent (substituted acetanilide) increased the level of light produced and prolonged its emission. The light signals were read by the system. The intensity of light was directly proportional to the concentration (amount) of the SARS-CoV-2 IgG antibody present in the test sample. This whole process is fully automated and carried out by using CLIA on the VITROS ECiQ Immunodiagnostic system. Results were automatically calculated by the VITROS ECI software for the serum SARS-CoV-2 IgG antibody levels for all samples.

STATISTICAL ANALYSIS

The data were collected for all cases and controls, along with anti-CoV-2 IgG antibody values, and entered into the MS Excel data sheet and statistical analysis was done using SPSS software version 27.0. The difference in mean levels of antibody levels between the test group and controls was analysed by unpaired t-test, with a level of significance set at 0.05.

RESULTS

[Table/Fig-1] shows the age and gender distribution of study participants. It was observed that males were more in number among cases/patients, while females were more in the control group. Males were more in number with age greater than 50 years among the cases in study population.

Gender	Age group (years)	Cases	Controls
Male	≤50	9	7
	>50	34	6
Female	≤50	4	19
	>50	8	23
Total		55	55

[Table/Fig-1]: Demographic distribution of study participants.

[Table/Fig-2] shows the minimum to maximum value of Anti-SARS-CoV IgG antibody levels detected by CLIA, with a mean of 183.68 BAU/mL among a total of 110 study population. Further, the antibody levels were compared among cases and controls in [Table/Fig-3]. However, mean Anti-SARS-CoV Ab levels were higher in the healthy control group; however, no significant difference was observed between the two groups. The mean Anti-SARS-CoV IgG antibody level in study participants vaccinated with the Covishield vaccine was higher at 188.95 BAU/mL compared to those vaccinated with Covaxin or other vaccines, with a p-value <0.05, suggested that the difference was statistically significant.

Variable	Minimum value (BAU/mL)	Maximum value (BAU/mL)	Mean±SD
Anti SARS-CoV IgG antibody	2	200	183.68±47.23
Age (years)	18	76	50.36±12.77

[Table/Fig-2]: Anti-SARS-CoV antibody levels in study population.

Variables	Number	Mean Anti-SARS-CoV Ab (in BAU/mL)±SD	p-value
Case	55	181.62±52.92	0.65
Control	55	185.74±41.15	
Male	56	181.25±51.52	0.58
Female	54	186.20±41.39	
PCR+ve	58	178.95±54.24	0.26
PCR-ve	52	183.95±37.76	
Positive h/o COVID-19 in family	70	187.59±41.32	0.65
No h/o COVID-19 in family	40	176.85±56.02	
Covishield vaccination	98	188.95±39.19	0.04
Covaxin and other vaccination	12	157.51±63.06	

[Table/Fig-3]: Comparison of Anti-SARS-CoV antibody among study population. Unpaired t-test; p≤0.05- statistically significant

The comparison of Anti SARS-CoV-2 antibody levels among the cases and controls have been depicted [Table/Fig-4]. The antibody levels were higher in the cases with a family history of COVID-19 when compared to the cases with no family history (p-value=0.05). The cases and controls who took second vaccination dose showed significantly higher antibody levels when compared to the individuals who did not take the second vaccine dose (p-value=0.01 and p-value=0.001, respectively).

Variables		Number	Mean±SD	p-value
Case	<=50 years	13	162.93±69.62	0.14
	>50 years	42	187.41±46.11	
Control	<=50 years	26	188.42±40.93	0.53
	>50 years	29	183.35±41.92	
Case	Males	43	176.49±58.98	0.17
	Females	12	200±000	
Control	Males	13	197±10.82	0.26
	Females	42	182.26±46.29	
Case	Vaccinated <=1 year	20	167.76±70.04	0.14
	>1 year	35	189.55±39.11	
Control	Last vaccine <=1 year	19	184.15±47.48	0.83
	>1 year	36	186.58±38.08	
Case	COVID-19 in family yes	33	192.92±33.35	0.05
	COVID-19 in family no	22	184.68±70.76	
Control	COVID-19 in family yes	37	182.83±47.27	0.45
	COVID-19 in family no	18	191.72±24.31	
Case	PCR+ve	32	179.08±58.49	0.67
	PCR-ve	23	185.17±45.09	
Control	PCR+ve	26	178.80±49.66	0.24
	PCR-ve	29	191.96±31.24	
Case	Vaccine Covishield	49	186.96±43.86	0.67
	Vaccine Covaccine and others	6	200±000	
Control	Vaccine Covishield	49	190.93±34.25	0.006
	Vaccine Covaccine and others	6	143.35±67.86	
Case	Second dose taken	45	188.25±43.37	0.01
	Not taken	10	136.16±87.82	
Control	Second dose taken	52	190.71±32.17	0.001
	Not taken	3	99.60±86.96	

[Table/Fig-4]: Comparison of Anti-SARS Ab among cases and controls.
Unpaired t-test; p≤0.05- statistically significant

DISCUSSION

Anti-SARS-CoV Ab levels were studied in 60 haemodialysis CKD stage 5 cases and 60 healthy controls in the present study. The results indicated no significant differences in the mean anti-SARS-CoV antibody levels between the cases and the controls. A study was conducted by Simon B et al., on 81 dialysis patients and 80 control subjects. They were tested for antibody responses after receiving two doses of the vaccine [7]. The results showed that the dialysis patients had a significantly lower titre count than the control group (the median was 171 U/mL for the dialysis patients and 2500 U/mL for the controls). However, in this study, the mean anti-SARS-CoV-2 IgG antibody level was 181.62 BAU/mL among haemodialysis cases and 185.74 BAU/mL in the healthy control group.

A retrospective cohort study included patients to assess the sero-response to SARS-CoV-2 vaccines among maintenance dialysis patients over six months and observed that these patients had low antibody titres. Immunity to SARS-CoV-2 vaccines decreases over time among patients on maintenance dialysis. Hence, additional vaccine doses should be considered for this population, either routinely or after further investigation [8]. De Vriese AS and Reynders M, conducted a similar study but found that the intensity and timing of the antibody response were the same in both the dialysis cases and the non dialysis control group [9]. A cross-sectional study conducted in the US showed that the seroprevalence of IgG Ab was 5.8% among maintenance dialysis patients, who are at high-risk of infections [10]. Agur T et al., conducted a prospective cohort study on haemodialysis CKD patients to estimate antibody response and found a significant difference in response to mRNA COVID-19 vaccine after the first and second doses [11]. Billany RE et al., studied seroprevalence to S1 spike protein following vaccination against COVID-19 in patients receiving haemodialysis. Neutralising antibodies against the RBD of the S1 spike protein were detected in 75 patients (79.8%), while 19 patients (20.2%) showed no detectable antibodies [12].

Another study from the ERA-EDTA Registry indicates high mortality due to COVID-19 in dialysis and kidney transplant patients across Europe. In dialysis patients, mortality rates varied significantly across age groups, with 28-day mortality for patients older than 75 years reaching as high as 31.4%. They suggested the need to focus on these patients to prevent mortality [13]. Vaccination has a protective role even in maintenance dialysis patients [14]. However, many studies have found disturbances of acquired immunity in the case of haemodialysis patients compared to the normal population [15-18]. The study by Simon B et al., observed that haemodialysis patients showed a highly diminished antibody response after COVID-19 mRNA vaccination compared to the healthy controls [7]. Similar results were obtained in the systematic review conducted by Notarte KI et al., [19], which also observed that postvaccination the antibody titre were lower when compared to the healthy individuals [19].

Therefore, robust vaccination strategies are needed to address waning immune responses in vulnerable populations like CKD stage 5 cases on maintenance haemodialysis.

Limitation(s)

The small sample size was the main limitation of the study. Hence, studies with a larger sample size can be conducted in future.

CONCLUSION(S)

The antibody response to COVID-19 vaccination in CKD Stage 5 haemodialysis patients was found to be lower compared to the healthy control groups when measuring anti-SARS-CoV IgG Ab levels. The Ab response to the Covishield vaccine was high compared to other vaccine. To the best of our knowledge, studies on the antibody response to COVID-19 infection and vaccination in the Kerala population have not been studied hence this study was conducted to analyse their SARS-CoV-2 IgG Ab levels.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor (CAP), Department of Nephrology, Government Medical College, Kozhikode, Kerala, India.
2. Assistant Professor, Department of Biochemistry, Government Medical College, Ernakulam, Kerala, India.
3. Assistant Professor, Department of Nephrology, Government Medical College, Ernakulam, Kerala, India.
4. Professor, Department of Nephrology, Government Medical College, Ernakulam, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Zainab Yaseen,
Assistant Professor, Department of Biochemistry, Government Medical College, Ernakulam, Kochi-683503, Kerala, India.
E-mail: drzainab09@gmail.com

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