ORIGINAL ARTICLE

The Persistence of Antibodies in ESRD Patients 12 Months after the BNT162b2 Vaccine Booster Dose.

Ummu Afeera Zainulabid[1], Mohammad Hanif Hashim[2], Abdullah Shamsir Abd Mokti[3], Awla Mohd Azraai[4], Hajar Fauzan Ahmad[5], Ahmad Mahfuz Gazali[5,*]

[1]Department of Internal Medicine, Sultan Ahmad Shah Medical Centre (SASMEC)@International Islamic University Malaysia (IIUM, Pahang, Malaysia.
[2]Hospital Muadzam Shah, 26700, Muadzam Shah, Rompin, Pahang, Malaysia.
[3]Ministry of Health Malaysia, Putrajaya, Malaysia.
[4]Faculty of Medicine, Universiti Teknologi MARA (UiTM) Selangor Branch, Sungai Buloh Campus, Sungai Buloh, Selangor, Malaysia.
[5]Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA), Pahang, Malaysia.

Corresponding Author
Ahmad Mahfuz Gazali
Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA), Lebuh Persiaran Tun Khalil Yaakob, 26300, Kuantan, Pahang, Malaysia.
Email: mahfuz@umpsa.edu.my

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Abstract

End-stage renal disease (ESRD) patients undergoing haemodialysis have been reported to have impaired humoral response after coronavirus disease 2019 vaccination. However, most studies focus on one antibody profile or one-time point only. Here, we longitudinally investigate the antibody response towards severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) in ESRD patients after receiving the primary BNT162b2 vaccine schedule and a booster dose. Neutralising antibodies against SARS-CoV-2 were detected after the first vaccine dose, which increased exponentially after the second vaccine dose, maintaining similar levels even after 12 months of the booster dose in all patients. Immunoglobulin G (IgG) but not immunoglobulin M against Spike protein and receptor binding domain were maintained 12 months after the booster dose, and correlation analyses confirmed the relationship between neutralising antibodies and IgG responses. In conclusion, neutralising and IgG antibodies persist in ESRD patients 12 months after the BNT126b2 booster dose, a novel study finding.

Keywords: *COVID-19; immunoglobulin; vaccine.*

Introduction

End-stage renal disease (ESRD) is a medical term used to describe the condition of further progression of chronic kidney disease when left untreated. In ESRD, the kidney's function ceases permanently, and chronic dialysis treatment is required for the patient to maintain the physiological function [1]. ESRD patients may experience impaired immune dysfunction, as demonstrated by lower antibody response towards severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) compared to healthy controls after coronavirus disease 2019 (COVID-19) vaccination [2].

COVID-19 is caused by SARS-CoV-2 infection and was declared a pandemic globally. One reason why COVID-19 has been successfully contained is the introduction of vaccines to prevent severe disease and hospitalisation. The earliest vaccine approved was BNT162b2, manufactured by Pfizer. Malaysia started administering the BNT162b2 vaccine to its population on Feb 25, 2021 [3].

Recent research on COVID-19 has focused on vaccine correlates of protection. The most important correlate of protection is neutralising antibodies towards SARS-CoV-2 [4]. Another study showed that antibody titres against the Spike (S) protein of SARS-CoV-2 are an essential marker of protection from infection in ESRD patients [5]. In addition, another study demonstrated that antibodies against the receptor binding domain (RBD) of SARS-CoV-2 are vital in neutralising various SARS-CoV-2 variants, such as alpha, beta, gamma, delta, epsilon, and omicron [6].

Therefore, our study aims to perform a longitudinal study to investigate the humoral response among Malaysian ESRD patients.

Materials and methods

Study design and cohort

The International Islamic University Malaysia Research Ethics Committee (IREC) approved the study design with the IREC 2021-168 code. In this prospective, single-centre study, five long-term ESRD patients from a hospital in Pahang who had received two primary doses and a booster dose of the mRNA vaccine BNT162b2 (BioNTech) were selected. Only patients meeting the ESRD diagnostic criteria were included in the study (estimated glomerular filtration rate of <60 ml/mon/1.73 m^2 that is present >3 months with or without evidence of kidney damage). Patients below 18 years, non-Malaysian, and pregnant individuals were excluded from the study. A phlebotomist withdrew blood samples from each subject's antecubital fossa or forearm. Five milliliters of blood was collected into plain tubes. After centrifugation, patient sera were frozen at -80°C for analysis of SARS-CoV-2 antibodies. All patients reported no COVID-19 infection before and during the sample collection. The demographics of ESRD patients and blood withdrawal times are listed in Table 1.

Measurement of SARS-CoV-2 antibodies using enzyme linked-immunosorbent assay (ELISA) kits

Sera samples were tested for anti-SARS-CoV-2 antibodies in triplicate measurements. Neutralising antibodies were determined using the SARS-CoV-2 Surrogate Virus Neutralization Test Kit (Cat. No. L00847-A, Lot. No. A220801, Genscript, Piscataway, New Jersey, USA). The kit is semi-qualitative; anything less than 30% inhibition is considered negative for neutralising antibodies. Calculation was performed using the following formula: -

$$\text{Inhibition} = \left(1 - \frac{\text{Optical Density (OD) value of sample}}{\text{OD value of negative control}}\right) \times 100\%$$

IgM and IgG against S protein were measured using Human SARS-CoV-2 Spike Protein IgM (Cat. No. E-EL-E603, Lot. No. NM124F487633) and IgG ELISA (Cat. No. E-EL-E602, Lot. No. NM14T6263894) kits, respectively (Elabscience, Houston, Texas, USA). Furthermore, IgM and IgG towards RBD of S1 were measured using

Human SARS-CoV-2 S1 RBD IgM (Cat. No. CSB-EL33242HU, Lot. No. B07223408) and IgG Antibody ELISA (Cat. No. CSB-EL33241HU, Lot. No. B07223407) kits, respectively (Cusabio, Houston, Texas, USA). A result was considered positive if the OD value was more than 0.35 for Elabscience ELISA kits and 2.1 times the negative control value for Cusabio ELISA kits. All the procedures were according to the recommendations of the corresponding kit manufacturers.

Statistical analyses

The Kruskal-Wallis test was employed to compare differences in neutralising antibodies between four blood withdrawals, followed by Dunn's multiple comparison test to evaluate differences between the groups. Additionally, Spearman's correlation test was utilized to demonstrate the relationship between IgM, IgG and neutralising antibodies. All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

Results

High concentration of neutralising antibodies against SARS-CoV-2 after BNT162b2 booster dose among ESRD patients

At baseline, three out of five ESRD patients demonstrated detectable levels of neutralising antibodies towards SARS-CoV-2 (Fig. 1A). After the first and second doses, all patients had detectable neutralising antibodies (Fig. 1A). The sera of ESRD patients after the first dose demonstrated a mean of 53.3% of SARS-CoV-2 inhibition (baseline vs first dose, p=0.9999), followed by a mean of 95.3% of SARS-CoV-2 inhibition after the second dose (Fig. 1B) (baseline vs second dose, p=0.0385).

Furthermore, 12 months after the booster dose, the inhibition of SARS-CoV-2 or neutralising antibodies towards SARS-CoV-2 were maintained at similar levels compared to after the second vaccine dose (Fig. 1A and 1B) (baseline vs 12 months after booster dose, p = 0.0005).

IgGs were maintained in all patients 12 months after the booster dose

The ELISA kit used to measure IgM and IgG was a semi-qualitative kit; therefore, OD values are shown in Fig. 2. Since patients' responses are inconsistent among individuals, each antibody profile is shown in Fig. 2: A) IgM against S protein, B) IgM against RBD, C) IgG against S protein, and D) IgG against RBD. Most patients had undetectable levels of IgM and IgG at baseline and after the first dose. In contrast, most patients display IgM and IgG levels of positivity after the second dose.

Further investigation revealed that two of five patients maintained the levels of IgM against S protein and RBD after 12 months of booster dose compared to the second dose, respectively, as shown in Fig. 2(A) and (B). In contrast, all patients maintained IgG levels against S protein and RBD after 12 months of booster dose compared to the second dose, respectively, as shown in Fig. 2(C) and (D).

Neutralising antibody strongly correlates with IgG against S protein and RBD

The Spearman correlation test confirmed the relationship between neutralising antibodies and other antibodies against SARS-CoV-2. Neutralising antibodies against SARS-CoV-2 correlate with IgG S protein ($R = 0.887$, $p < 0.0001$) and IgG RBD ($R = 0.9023$, $p < 0.0001$), but not with IgM S protein ($R = 0.3053$, $p = 0.1906$) and IgM RBD ($R = 0.4421$, $p = 0.051$).

Discussion

In summary, all patients displayed similar neutralising antibody profiles: low or undetectable at baseline, above cut-off during the first dose, high levels after the second dose and being maintained 12 months after the booster

dose. Our results align with Bergamaschi *et al* [7], which reported similar neutralising antibody profiles in healthy controls. Another study indicated that end-stage renal disease affected neutralising antibodies only six months post-booster dose but not 10 and 12 months after a booster dose, which aligns with our study [8]. Furthermore, a Japanese study of healthy controls demonstrated the persistence of neutralising antibodies 12 months post-COVID-19 and started to decline after 24 months post-infection [9]. However, the IgM profile did not show similar trends. Most patients display high levels of IgM towards S protein and RBD after the second dose, but the concentrations of IgM were undetectable in most patients 12 months after the booster dose. IgM is a primary antibody secreted in response to SARS-CoV-2; therefore, it may be possible that the IgM concentration may decline after the second vaccine dose. In contrast, all patients had high levels of IgG toward S protein and RBD, similar to a study which reported maintained levels of anti-S protein antibodies [10] and RBD [11] 12 months after the booster dose. A correlation analysis was performed because neutralising antibodies and IgG demonstrated high levels after the booster dose. Neutralising antibodies strongly correlate with IgG against S protein and RBD, which aligns with Benning *et al* [12]. The persistence of antibodies among ESRD patients 12 months after the booster is a novel finding of the study.

Despite maintaining high levels of neutralising antibodies and IgG, it is worth noting that these antibodies may work against the wild type of SARS-CoV-2. A recent study demonstrated that neutralisation towards recent SARS-CoV-2 variants such as XBB.1, BQ.1.1, and BA.4/5 was significantly reduced compared to neutralisation against the wild type [13]. Therefore, a better alternative should be recommended for immunocompromised patients such as ESRD patients. The bivalent vaccine, combining the wild type and the latest strain, should be encouraged as the virus continuously mutates [14]. In addition, the intranasal vaccines towards SARS-CoV-2 show good promise in preventing infection in the nasal cavity, where the virus may enter the body [15].

The research has several limitations: low sample number (n=5), no control population, and the ELISA kits used were qualitative measurements, not quantitative.

Conclusion

Neutralising antibodies, IgG against S protein, and RBD of SARS-CoV-2 could be detected in ESRD patients, especially after the second vaccine and 12 months after the booster dose of BNT162b2. This shows that the antibody induced by vaccination could persist in ESRD patients, even 12 months after the booster dose, a novel study finding.

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Authors' declaration

The manuscript was not presented at any meeting. The authors declared no conflict of interest.

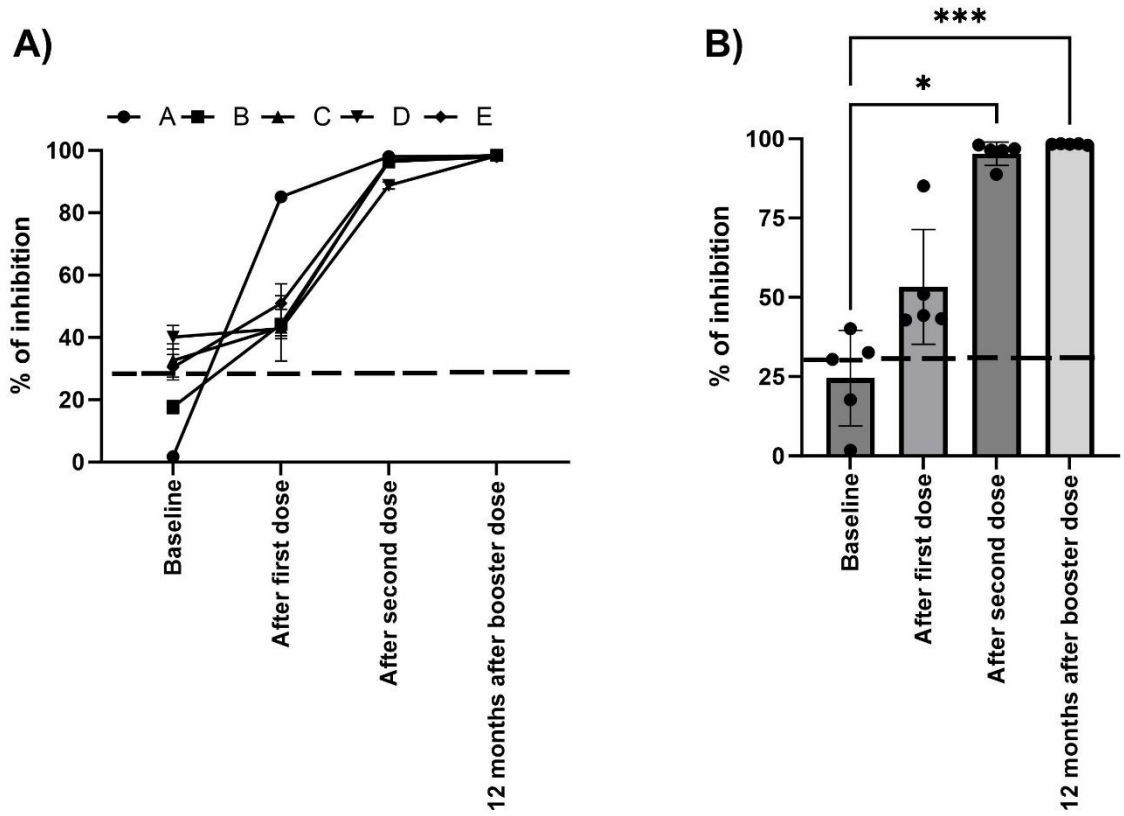

Figure 1. Inhibition of SARS-CoV-2 of ESRD patients. Each dot represents the mean. The dotted line represents seronegativity based on 30% of inhibition. A) demonstrates individual inhibition of each ESRD patient connected to a line, while B) shows the cumulative percentage of inhibition of all ESRD patients. * indicates a p-value of 0.0385, and *** indicates a p-value of 0.0005 of post-test comparisons.

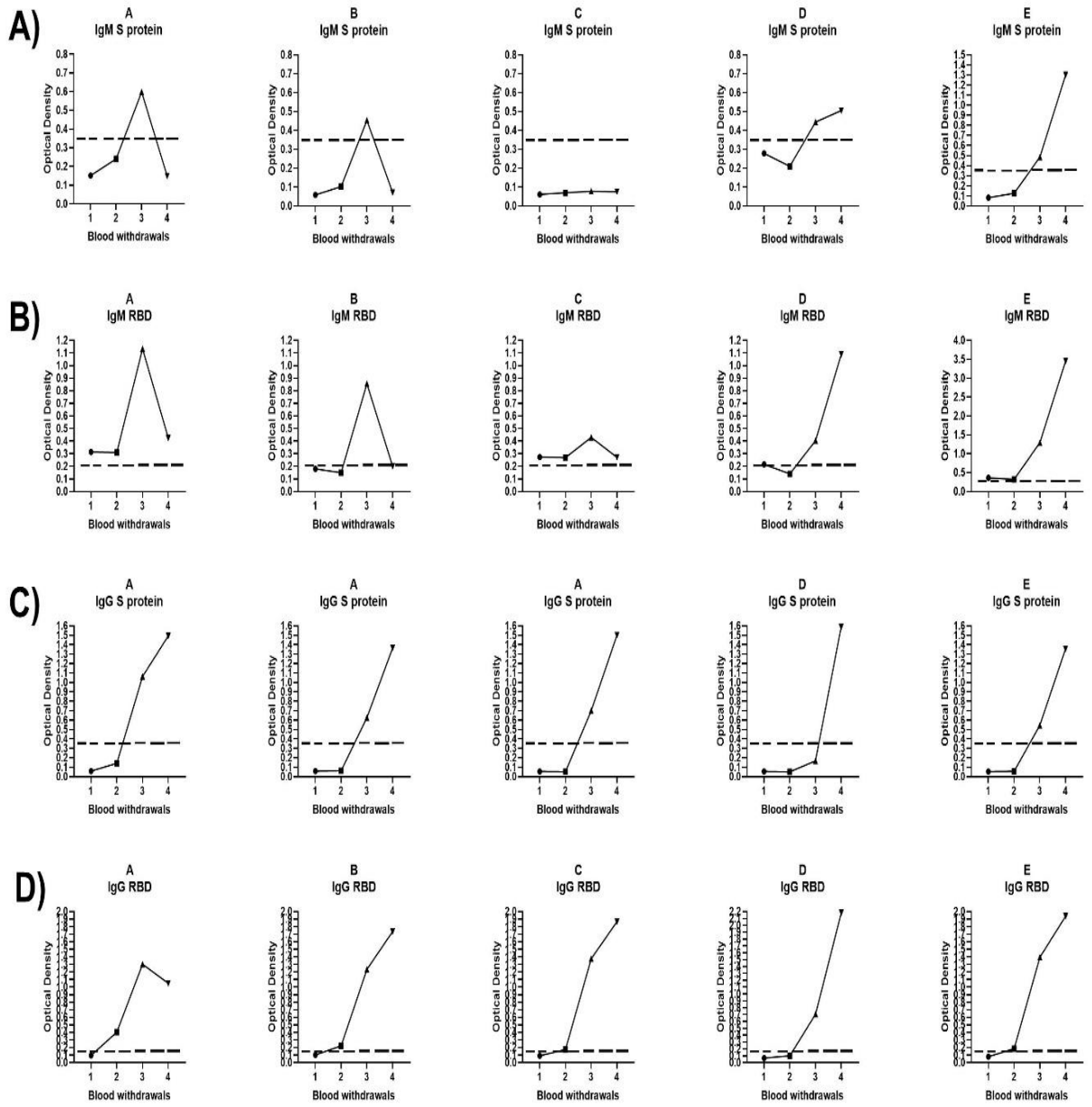

Figure 2. Antibodies profile of ESRD patients. Each dot represents the mean. The dotted line represents seronegativity based on OD values. The figure demonstrates A) IgM against S protein, B) IgM against RBD, C) IgG against S protein, and D) IgG against RBD.

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