



## Assessment of Antibody Titers after SARS-CoV-2 Vaccination in Bangladesh: A Single Centre Observational Study

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(Received: 05 January 2026

Revised: 15 February 2026

Accepted: 05 March 2026)

### KEYWORDS

SARS-CoV-2, COVID-19 vaccination, Antibody titers, Vaccine effectiveness, Bangladesh

### ABSTRACT:

**Background and Purpose:** The SARS-CoV-2 virus, which emerged in Wuhan, China in 2019, led to the COVID-19 pandemic, posing a global healthcare challenge. The primary pathology involves viral replication in the early disease stages. COVID-19 vaccines play a crucial role in reducing susceptibility to future infections. Evaluating antibody levels and longevity post-vaccination is essential to assess vaccine effectiveness. This study aims to quantify antibody levels among hospital employees to analyze the immune response to vaccination.

**Methods:** A single-center study was conducted to measure antibody levels among hospital employees. Data were collected from medical records, including the number of days post-vaccination (first and second doses), age, and antibody titers. Antibody titers were analyzed using a commercial Anti-SARS-CoV-2 kit. The study also examined antibody responses in individuals who had spontaneous SARS-CoV-2 infections post-vaccination.

**Results:** There was a positive correlation between age and antibody titer. In COVID-19-positive individuals, a positive correlation was found between antibody titer and the duration between the first and second vaccine doses. In COVID-negative individuals, a negative correlation was observed. Male antibody titers had a skewness of 3.2, while female titers had a skewness of 2.6. Individuals who did not receive both vaccine doses had mean antibody titre 880.74 Au/ml and to those who received both doses 5530.11 Au/ml.

**Conclusions:** COVID-19-positive individuals showed a stronger immune response post-vaccination. Despite waning immunity over time, vaccination led to significantly higher antibody titers in adults, reinforcing the importance of full-dose COVID-19 vaccination.

### INTRODUCTION

The COVID-19 pandemic causing severe acute respiratory Coronavirus-2 (SARS-CoV-2) has infected over 600 million people, and is responsible for more than 6 million deaths across the world. Vaccines are well-known to have potential to improve population immunity, avert severe disease, and alleviate the continuing health crisis [1]. As a result, tremendous hopes are placed on COVID-19 vaccinations to limit the

pandemic. Several vaccines have been developed, approved and immunized that claim protection against COVID-19 variants by eliciting immune responses against the spike antigen of SARS-CoV-2 [2,3,4]. Till 2023, 72% of the world's population has received at least single dose of COVID-19 vaccination [5]. A recent report confirms that 150,049, 129 individuals have received the first dose, 131,182,263 individuals have taken the second dose, 65,672,743 individuals have completed the third dose, and 569,825 individuals have



taken the fourth dose in Bangladesh [6]. These people received vaccines developed by AstraZeneca, Pfizer, Sinopharm, Moderna, Sinovac, Janssen (Johnson & Johnson), Pfizer-PF (Comirnaty). These vaccines showed tremendous efficacy in preventing infection, reducing disease severity and mortality worldwide. Although a considerable part of the population worldwide has survived infection however the remaining people are at risk of SARS-CoV-2 infection. Moreover, people who already received multiple doses of vaccination are still getting infected. The duration of the rise in antibody titers and its longevity against the new coronavirus (SARS-CoV-2) is thought to be a crucial signal for validating the efficacy of a COVID-19 vaccination.

The evaluation of anti-spike protein receptor-binding domain (anti-S-RBD) antibodies represents a useful tool to estimate individual protection against SARS-CoV-2 infection [7]. Infection fatality risk has been very low in 2022, and the majority of Omicron infections seem to be asymptomatic [8]. In populations with substantial prior exposure to SARS-CoV-2, reinfections (the vast majority occurring in the omicron waves) had less than a quarter of the hospitalization risk and one-tenth the mortality risk compared with the primary infections [9].

Hence, the present study was designed to evaluate the antibody response after receiving the first and second dose of the SARS-CoV-2 vaccine. We also checked the association between the antibody titers after the first and second dose and the patient's age or the number of days since vaccination.

## MATERIALS AND METHODS

**Study Design:** This cross-sectional, single-center, observational study aimed to assess SARS-CoV-2 antibody titers among hospital employees based on vaccination status and prior infection.

**Study Site:** The study was conducted in the Department of Lab Medicine at Imperial Hospital Limited, Chattogram, Bangladesh.

**Period of Study:** The study was carried out over a 7-month period, from April 2021 to October 2021.

**Sample Size:** A total of 81 hospital employees participated in this study, comprising 77% males and 23% females, with a median age of 37 years. Participants

were categorized into six groups based on COVID-19 history and vaccination status:

Group 1: 14 (COVID-negative, no vaccine), Group 2: 11 (COVID-positive, no vaccine), Group 3: 9 (COVID-negative, one vaccine dose), Group 4: 21 (COVID-negative, two vaccine doses), Group 5: 10 (COVID-positive, one vaccine dose), Group 6: 16 (COVID-positive, two vaccine doses)

**Sampling Technique:** Participants were selected using purposive sampling, and peripheral blood samples were collected via venipuncture in a non-fasting state.

**Data Collection & Laboratory Analysis:** After centrifugation, serum samples were separated and stored at  $-86^{\circ}\text{C}$  for further analysis. SARS-CoV-2 IgG antibodies were detected using the Architect I System (Abbott, Germany), based on a two-step chemiluminescent microparticle immunoassay. All kits, calibrators, and quality control materials were obtained from Abbott Diagnostics through a local distributor.

**Data Analysis:** The study analyzed the correlation between antibody titers and age and observed trends in antibody levels post-vaccination using a linear regression model.

**Ethical Approval:** This study was approved by the Institutional Ethics Committee of Imperial Hospital Limited, Chattogram, Bangladesh. All participants provided written informed consent before enrollment, ensuring confidentiality and voluntary participation. Participants retained the right to withdraw at any time without any consequences. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent amendments.

## RESULTS

### Participant characteristics

The demographic and vaccination data of the participants are summarized in Table 1. In total of 81 subjects, 63 were male and 18 were female whose antibody titers were measured at Clinical Biochemistry Department of IHL. 38 participants received the first dose; 31 participants took the second dose. A total of 18 female participants were included in the study. 09 participants took the first dose and 06 participants took the second



dose of covid-19 vaccine. The median age of the participants was 37 years.

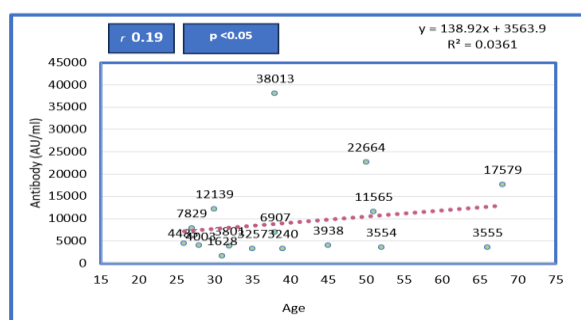
**Table 1. Participants' characteristics up to 1<sup>st</sup> & 2<sup>nd</sup> vaccine doses**

	Total Participants	SARS COVID-2 Positive Before vaccination	SARS COVID-2 Negative Before vaccination	SARS COVID-2 Positive After the 1 <sup>st</sup> dose of vaccination	Participant s took 1st Dose	Participant s took the 2nd Dose
<b>Total</b>	81				38	31
<b>Male, n (%)</b>	63 (77)	28	33	02		
<b>Female, n (%)</b>	18 (23)	06	11	01	09	06
<b>Mean Age</b>	39.38 ± 11.44					
<b>Median Age</b>	37					

### Association antibody titers and age in COVID-19 patients

The scatter diagram of the antibody titers and age is shown for 16 (15 male and 1 female) COVID-19 subjects who had been received double dose of vaccine. We found a mild positive correlation ( $p < 0.05$ ) between antibody titer

and increasing age (Figure 1). It depicts that 3% of the variance of antibody titers can be correlated with increasing age.



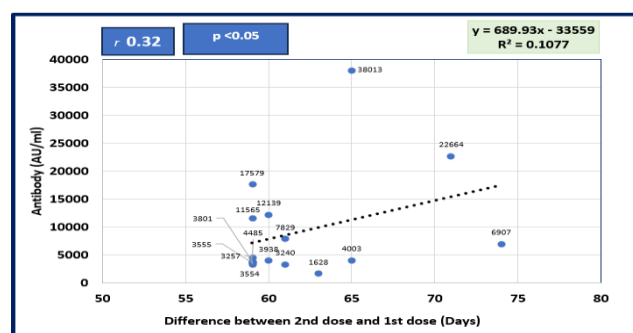
**Fig. 1 Scattergram of age and Antibody titer and correlation analysis in COVID-19 positive subjects with double dose of vaccination.**

### Correlation of antibody titer with duration of vaccine doses in COVID-19 patients

The scatter diagram of the antibody titers and duration between the 1<sup>st</sup> and 2<sup>nd</sup> doses of vaccination is shown for 16 COVID-19-positive patients (15males and 1 females). A positive association ( $p < 0.05$ ) was found between the

antibody titer and vaccine duration from 1<sup>st</sup> to 2<sup>nd</sup> dose among the subjects who have been COVID-19 positive and taken double doses of vaccine. 10% of the titer variance can be attributed to the difference of days between

two doses of vaccine. It shows that titres are clustered around 60 to 65 days after 1<sup>st</sup> dose of vaccine



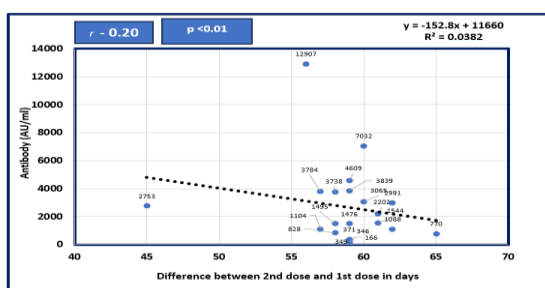
**Fig. 2 Scattergram of the antibody titer and duration between the 1<sup>st</sup> and 2<sup>nd</sup> doses of vaccination and**



**correlation analysis in COVID-19 positive subjects with double dose of vaccination.**

**Correlation of antibody titer with duration of vaccine doses in COVID-19 negative patients**

Figure 3 represents a negative correlation of the antibody titers and duration between 1<sup>st</sup> dose to 2<sup>nd</sup> dose among 21 subjects (16 males and 5 females) who have not been COVID-19 positive and taken double doses of vaccine. 3% of the titer variance can be attributed to the difference of days between two doses of vaccine. It shows that titers are clustered around 55 to 63 days after 1<sup>st</sup> dose of vaccine.



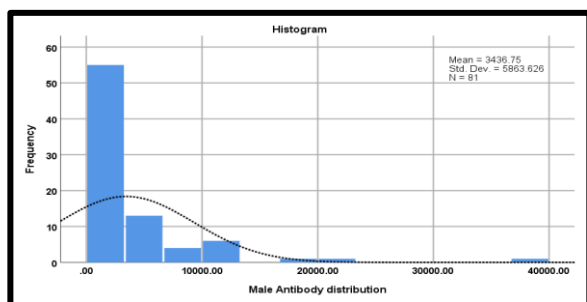
**Fig. 3 Scattergram of the antibody titer and duration between the 1<sup>st</sup> and 2<sup>nd</sup> doses of vaccination and correlation analysis in COVID-19 negative subjects with double dose of vaccination.**

**Antibody Titer among male and female participants**

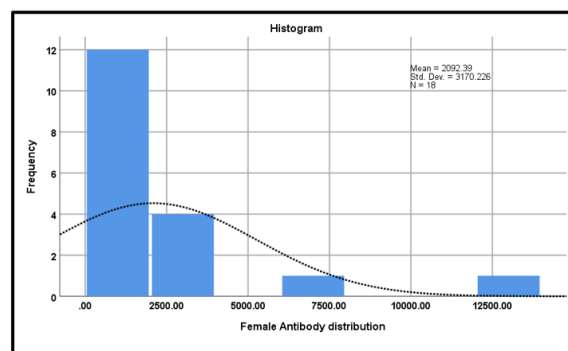
The histogram of the male and female antibody titer out of 81 participants is shown for is shown in figure 4. The

Histogram shows that there is a skewed distribution of male antibody titre with skewness 3.2. (figure 4A). The

Histogram shows that there is a skewed distribution of female antibody titre with skewness 2.6 (figure 4B). This is extremely skewed data with the increased value of lower titer



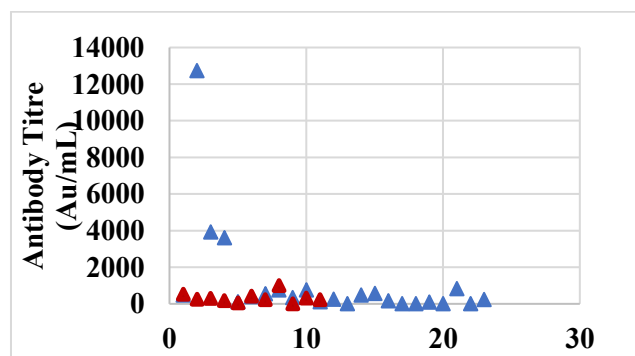
**Fig 4A: Histogram of male antibody titer**



**Fig 4B: Histogram of female antibody titer**

**Distribution of antibody titer who have not taken the double dose of vaccine**

Figure 5 shows the scatter distribution of antibody titer (Au/ml) among COVID-19 positive and COVID-19 negative participants who have not taken the double dose of vaccine.

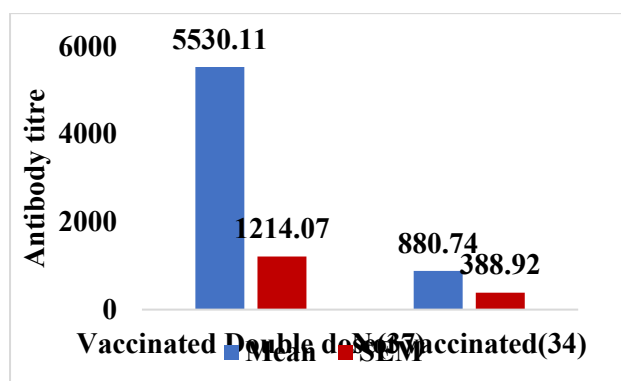


**Fig. 5 Scatter distribution of Antibody titer in Patients who have not taken a double dose of vaccine**

[Red colours are covid positive and blue colours are covid negative]

**Antibody titer distribution in vaccinated and not vaccinated subjects**

Antibody titer distribution in vaccinated and non-vaccinated subjects is shown in figure 6. The mean titer in subjects taken double doses of vaccine is 5530.11±1214.07Au/ml and in non- vaccinated subjects is 880.74±338.92Au/ml.



**Fig. 6: Antibody titer distribution in vaccinated and non-vaccinated subjects**

## DISCUSSION

The present study found that young patients with COVID-19 had greater levels of SARS-CoV-2 antibodies. A direct relationship was seen between the level of antibodies and the time interval between the first and second doses of the COVID-19 vaccination in individuals who tested positive for the virus. Therefore, we hypothesized that elevated levels of SARS-CoV-2 antibodies were a consequence of both experiencing symptoms of COVID-19 and receiving the COVID-19 vaccine.

The present study showed that SARS-CoV-2 antibody titers were boosted by the first or 2<sup>nd</sup> dose of vaccine (figure 2). Typically, when pathogens like viruses infiltrate the human body, our innate and adaptive immune systems shield us from viral invasion and reproduction. Cellular immunity plays a significant role in clearing viruses within the context of adaptive immunity. Nevertheless, there is a debate about the contribution of humoral immunity to the elimination of viruses. Typically, it serves to prevent and shield against novel viral infections, forming the foundation for the development of vaccines against viruses [10]. There is a mild positive correlation of antibody titre with the increase in age (Figure 1). It is seen in this study that young adults who have taken double doses of vaccine showed higher antibody titre than older patients. In a study conducted by Park JH et al. (2022), it was shown that older patients with COVID-19 who had pneumonia and needed oxygen support had higher levels of SARS-CoV-2 antibody titers [11].

A negative correlation of antibody titre with the duration between 1<sup>st</sup> and 2<sup>nd</sup> doses of vaccine in COVID-negative patients and taken double doses of vaccine (Figure 3).

Vaccines that are specifically created to trigger immune responses that provide protection are the major solution for controlling the COVID-19 pandemic caused by SARS-CoV-2. Specifically, mRNA vaccines have demonstrated excellent efficacy when given in two doses with a gap of three or four weeks between them [12,13]. There is increasing evidence that neutralizing responses are a correlate of protection [14,15,16]. It was observed in (Figure 7) that subjects who had not been vaccinated had a low titre even after covid positive. These findings correlate with the observations by Baden, L. R. et al. (2021) [13]. The mean antibody titre had been higher at 5530.11 Au/ml in double-vaccinated subjects than in non-vaccinated (880.74 Au/ml) subjects (Figure 8).

The current breakthrough of COVID-19, which is mostly caused by the Omicron variation of concern (VOC), is believed to be due to the decline in antibodies after SARSCoV-2 vaccination and the significant number of mutations in the spike protein of the Omicron VOC compared to the spike protein of the original vaccine. Nevertheless, administering further vaccine booster doses that significantly enhance the levels of antibodies specific to the wild-type virus can offset the decreased specificity for a minimum of three months. Consequently, it is advisable to administer frequent boosters, particularly to individuals at high risk, such as hemodialysis patients. In prior investigation on the general population, we found that convalescent and vaccinated patients had stronger immune responses, both in terms of antibodies and immune cells, against the alpha, beta, and delta variants of concern (VOCs) compared to vaccinated persons who had not been infected [17]. This study explained the increased titre level after COVID-19 positive after a double dose of vaccine and recommended taking a booster dose for enhancement of cellular immunity.

## CONCLUSION

This study evaluated SARS-CoV-2 antibody titers among hospital employees in Bangladesh, confirming that double-dose vaccination significantly enhances immunity, with vaccinated individuals showing higher titers (5530.11±1214.07 Au/ml) compared to non-vaccinated (880.74±338.92 Au/ml). A mild positive



correlation ( $p < 0.01$ ) was observed between antibody levels and age in COVID-19-positive individuals, while vaccine interval positively influenced titers in this group ( $p < 0.05$ ). Conversely, COVID-19-negative individuals showed a negative correlation between titers and vaccine dose intervals, indicating prior infection boosts vaccine-induced immunity. Gender-based analysis revealed skewed antibody distributions (male skewness: 3.2, female skewness: 2.6). Given the decline in antibody levels over time and the emergence of variants like Omicron, the study highlights the importance of full-dose vaccination, timely booster doses, and further research on long-term immunity to strengthen public health strategies.

#### ABBREVIATIONS

1. SARS-CoV-2 – Severe Acute Respiratory Syndrome Coronavirus 2
2. COVID-19 – Coronavirus Disease 2019
3. PRNT – Plaque Reduction Neutralization Test
4. RT-PCR – Reverse Transcription Polymerase Chain Reaction
5. IgG – Immunoglobulin G
6. CMIA – Chemiluminescent Microparticle Immunoassay
7. WHO – World Health Organization
8. ELISA – Enzyme-Linked Immunosorbent Assay
9. SEM – Standard Error of Mean
10. VOC – Variant of Concern
11. CI – Confidence Interval
12. p-value – Probability Value (Statistical Significance)
13. ML – Machine Learning
14. Au/ml – Arbitrary Units per Milliliter (Measurement Unit for Antibody Titer)
15. IHL: Imperial Hospital Limited

#### ACKNOWLEDGEMENT

The authors would like to acknowledge the support of Clinical Biochemistry Department of Imperial Hospital Limited in facilitating this research.

#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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