ORIGINAL ARTICLE

Post-vaccination analysis of anti-spike antibody responses in kidney transplant recipients with and without COVID-19 infection in a tertiary care centre, India

Sanjiv Jasuja1, Vivekanand Jha2,3,4, Gaurav Sagar1, Anupam Bahl1, Shalini Verma5, Neharita Jasuja5 and Jasmeet Kaur6

1Department of Nephrology, Indraprastha Apollo Hospital, New Delhi, India, 2George Institute for Global Health, UNSW, New Delhi, India, 3School of Public Health, Imperial College, London, UK, 4Prasanna School of Public Health, Manipal Academy of Higher Education, Manipal, India, 5Department of Clinical Research, AVATAR Foundation, New Delhi, India and 6Department of Histocompatibility and Transplant Immunology, Dr Lal PathLabs Ltd, National Reference Laboratory, New Delhi, India

Correspondence to: Sanjiv Jasuja; E-mail: sanjivjasuja@yahoo.com

ABSTRACT

Background. To investigate the anti-spike antibody response to vaccination in kidney transplant recipients (KTRs) previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as compared with KTRs with no history of coronavirus disease 2019 (COVID-19) from India.

Methods. SARS-CoV-2 spike immunoglobulin (Ig) G antibody response was measured in 105 post-COVID-19 KTRs with PCR-confirmed SARS-CoV-2 infection who received either no vaccination (cohort 1), a single dose (cohort 2) or two doses (cohort 3) of vaccine and compared with 103 two-dose vaccinated COVID-19-naïve KTRs with no history of COVID-19 (cohort 4).

Results. Out of 103 COVID-19-naïve two-dose vaccinated KTRs, <50% became seropositive with anti-spike antibody titres >50 arbitrary unit/mL subsequent to complete vaccination, the seroconversion rate being comparable in subjects receiving Covishield™ versus Covaxin™ vaccines. However, the seropositive KTRs vaccinated with Covishield™ had higher anti-spike antibody titres as compared with those who received Covaxin™. We observed higher anti-SARS-CoV-2 spike antibody levels in post-COVID-19 KTRs after one dose of vaccine as compared with COVID-19-naïve two-dose vaccinated KTRs. Importantly, the second dose in post-COVID-19 KTRs did not significantly increase anti-spike antibody levels compared with the single-dose recipients.

Conclusions. Our data present that in KTRs with previous SARS-CoV-2 infection, a single dose of vaccine (Covishield™) may be effective in mounting an optimal immune response. In contrast, COVID-19-naïve two-dose vaccinated KTRs respond poorly (<50%) to the current recommendation of a two-dose regimen in India.

Keywords: anti-spike antibody, COVID-19, kidney transplant recipients, previously infected, SARS-CoV-2

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INTRODUCTION

Kidney transplant recipients (KTRs) are at an elevated risk of developing severe coronavirus disease 2019 (COVID-19) [1]. Studies have demonstrated increased morbidity and mortality in transplant patients [1–17]. In the absence of a definitive cure for COVID-19, vaccines are perhaps the most promising option available to control the pandemic. There are several severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines currently available whose immunogenicity and safety have been assessed in various clinical trials [18]. However, no vaccine trial included transplant recipients. Recent investigations demonstrate that even though mRNA vaccines induce robust immune response in non-transplant individuals protecting against severe COVID-19, KTRs develop significantly lower antibody response post-vaccination [19–30]. In contrast, studies evaluating the serologic response of transplant recipients to COVID-19 infection provide conflicting results reporting normal levels of anti-SARS-CoV-2 antibodies in KTRs subsequent to past COVID-19 infection [31–33]. However, the majority of these studies explored the immune response to mRNA vaccines, currently not available in India; similar data following immunization with vaccines approved in India are not available. Importantly, the dynamics of vaccination after natural infection in transplant recipients remain unexplored. In this study, we investigated the spectrum of antibody responses to SARS-CoV-2 in a cohort of KTRs with different vaccination status.

MATERIALS AND METHODS

Study design and population

SARS-CoV-2 anti-spike IgG antibody titres were assessed in 208 KTRs, treated at a tertiary care hospital in New Delhi, India between 1 April 2020 and 30 November 2021. Out of the 208 KTRs, 105 KTRs were previously infected with COVID-19 (confirmed with SARS-CoV-2 real-time reverse transcription polymerase chain reaction) and had not received convalescent plasma during treatment. The 105 KTRs were either not vaccinated (referred to as ‘post-COVID-19 non-vaccinated’) or received a single dose (referred to as ‘post-COVID-19 single-dose vaccinated’) or both doses (referred to as ‘post-COVID-19 two-dose vaccinated’) of the approved vaccines, Covishield™ (ChAdOx1-nCOV or AZD1222, Oxford-AstraZeneca, manufactured by Serum Institute of India, Pune, India) and Covaxin™ [BBV-152, manufactured by Bharat Biotech, Hyderabad, in collaboration with Indian Council of Medical Research (ICMR), India] subsequent to their recovery from COVID-19. The remaining 103 KTRs with no history of COVID-19 were fully vaccinated with two doses of either of the approved vaccines (referred to as ‘COVID-19-naïve two-dose vaccinated’). The distribution of study cohorts is summarized in Figure 1. Necessary institutional approvals were secured for carrying out the data analysis and manuscript development.

Data collection

Data were collected retrospectively from the medical records of the hospitals’ or patients’ follow-up submissions. Clinical data collected included demographics (age, height, weight, sex, duration) from transplant to COVID-19, comorbidities, baseline immunosuppression regimen and details of vaccination.

Outcomes

The primary objective of this study was to quantitatively evaluate the SARS-CoV-2 anti-spike IgG antibody response in previously infected KTRs with respect to their vaccination status, comparing with fully vaccinated uninfected KTRs. The secondary outcomes included evaluating the association and correlation of anti-spike antibody levels with comorbidities and other baseline transplant characteristics.

Anti-spike IgG antibody evaluation

Anti-spike IgG antibodies to SARS-CoV-2 were assayed with the AdviseDx SARS-CoV-2 IgG II assay (Abbott Diagnostics, Chicago, IL, USA) using a chemiluminescent microparticle immunoassay intended for the qualitative and semi-quantitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma on the Alinity i system (Abbott Diagnostics, Chicago, IL, USA). The analytical measurement interval is stated as 22–40 000 arbitrary units (AU)/mL, and the positivity cut-off is ≥50 AU/mL (manufacturer defined). According to the manufacturer, the observed limit of quantification on the Alinity i system was 7.2 AU/mL, representing the lowest concentration at which a maximum allowable precision was met. The observed limit of detection (LoD) on the Alinity i system was 4.8 AU/mL and represents the lowest...
**Statistical analysis**

Data were tabulated using Microsoft Excel, imported to SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL, USA) for analysis, and R-software version 3.6.1 was applied for determining the median difference 95% confidence interval. The continuous variables were summarized as mean ± standard deviation (SD) and median (inter-quartile range [IQR]). The qualitative variables were reported with number and percentage.

To compare normally distributed continuous variables among the cohorts, a one-way ANOVA followed by a Tukey’s test was performed. Skewed distributed variables were tested using the non-parametric Kurskal–Wallis followed by the Mann–Whitney U-test, and P-values were adjusted as per the Bonferroni correction. For comparing qualitative variables among the cohorts, the chi-squared test was applied. The unpaired Student’s t-test, the Mann–Whitney U-test and the simple logistic regression were performed to find the association between responders and non-responders with regard to demographic and other clinical variables. We applied Spearman’s correlation to find the strength of association between anti-spike antibody titres and other continuous variables. The Bonferroni correction was applied, keeping the small sample size in consideration and multiple variable testing. P-value <0.05 was considered as significant.

**RESULTS**

Demographics, comorbidities and baseline transplant characteristics of study cohorts

The study subjects were categorized into four cohorts based on their COVID-19 infection and vaccination status (Figure 1). A total of 208 KTRs were included in the study, out of which 105 KTRs were infected in the past with COVID-19 and 103 KTRs remained uninfected. Amongst the previously infected 105 KTRs, 57 patients were non-vaccinated (cohort 1: post-COVID-19 non-vaccinated), whereas 18 patients received only one dose (cohort 2: post-COVID-19 single-dose vaccinated) and 30 KTRs received both vaccination doses (cohort 3: post-COVID-19 two-dose vaccinated) of either of the approved vaccines. The 103 uninfected KTRs were fully vaccinated with the recommended two-dose regimen of the approved vaccines in India (cohort 4: COVID-19-naive two-dose vaccinated). Table 1 summarizes the demographics, baseline characteristics, comorbidities and vaccination details of the four study cohorts. No significant difference was observed between the cohorts with respect to mean weight, height, gender and median time interval from transplant to COVID-19 and laboratory investigations. There was a significant difference observed in the average age of the KTRs between the four cohorts. The average age of the post-COVID-19 two-dose vaccinated KTRs (54.70 ± 11.35 years) was significantly higher than the post-COVID-19 non-vaccinated (44.11 ± 12.63 years; \( P = 0.001 \)) and COVID-19-naïve two-dose vaccinated KTRs (45.91 ± 12.21 years; \( P = 0.004 \)).

Nearly all the individuals in the study documented the presence of pre-existing comorbidities. Comorbidities such as diabetes mellitus (DM), hypertension (HTN), chronic liver disease (CLD) and chronic allograft dysfunction were comparable between the cohorts. Interestingly, significantly fewer COVID-19-naïve two-dose vaccinated KTRs reported a history of chronic obstructive airway disease (COAD) (1.9% \( P = 0.001 \)) and vascular disease (1%, \( P < 0.001 \)).

Treatment with mycophenolate mofetil/mycophenolic acid (MMF/MPA) was significantly higher in the post-COVID-19 non-vaccinated cohort (100%) as compared with the COVID-19-naïve two-dose vaccinated cohort (85.4%, \( P = 0.018 \)), whereas treatment with steroids and calcineurin inhibitors (CNIs) was comparable.

Vaccination details of the study cohorts

Details about vaccination are summarized in Table 1. Amongst the 18 post-COVID-19 single-dose vaccinated KTRs, 17 received Covishield™ and only 1 received Covaxin™, whereas out of the 30 post-COVID-19 two-dose vaccinated KTRs, 20 individuals received Covishield™ and 10 received Covaxin™. Amongst the COVID-19-naive two-dose vaccinated cohort, 75 KTRs were vaccinated with Covishield™ and 25 with Covaxin™, 3 KTRs were vaccinated with other anti-SARS-CoV-2 vaccines approved in India.

To assess the immune response elicited upon vaccination against SARS-CoV-2, anti–SARS-CoV-2 spike protein IgG (‘anti-spike antibody’) levels were measured. Individuals with antibody titres >50 AU/mL were considered seropositive. Despite being fully vaccinated, only 50 out the 103 COVID-19-naïve two-dose vaccinated KTRs became seropositive (Figure 2A); more than half of the cohort (53/103; 51.5%) had anti-spike antibody titres < 50 AU/mL (\( P < 0.001 \)). Amongst KTRs with past COVID-19 infection, 96.7% of non-vaccinated patients, 100% of vaccines receiving single-dose vaccination and 94.7% of two-dose vaccinated KTRs were seropositive (Figure 2A). Out of the four KTRs that were non-responsive, three KTRs were non-vaccinated and one subject, despite receiving both doses, did not express antibody >60 AU/mL.

The anti-spike antibody levels were significantly different between the cohorts (\( P < 0.001 \)). Notably, the median antibody titres of the COVID-19 two-dose vaccinated KTRs, inclusive of seropositive and seronegative individuals, [17.1 (IQR 1.6–2125) AU/mL] were significantly lower than KTRs with past COVID-19 infection, irrespective of their vaccination status (\( P < 0.001 \)) (Table 1 and Figure 2B).

Amongst the KTRs with past COVID-19 infection, non-vaccinated patients had lower median antibody titres [745 (IQR 239–3022) AU/mL] as compared with post-COVID-19 single-dose vaccinees [3436 (IQR 661–10 450) AU/mL; \( P = 0.066 \)] or post-COVID-19 two-dose vaccinated KTRs [3706 (IQR 867–10 700) AU/mL; \( P = 0.006 \)]. Interestingly, median antibody titres of post-COVID-19 KTRs vaccinated with a single dose were comparable to those who received two doses (\( P = 1.00 \), Bonferroni adjusted) (Table 1 and Figure 2B).

Amongst the vaccinated cohort, the median time interval for assessment of the serological response past vaccination was comparable. Anti-spike antibody tests were conducted for post-COVID-19 single-dose vaccinated cohort at median 47 days (IQR 28–84.8), post-COVID-19 two-dose vaccinated cohort at median 44.11 ± 12.63 years; \( P = 0.001 \) and COVID-19-naïve two-dose vaccinated KTRs (45.91 ± 12.21 years; \( P = 0.004 \)).
Table 1. Demographics and baseline characteristics of COVID-19-naïve and post-COVID-19 KTRs based on their vaccination status

| Post-COVID-19 KTRs | COVID-19-naïve | P-value (F-test/chi-squared test) | Multiple group comparison (Tukey’s test)/Bonferroni adjustment |
|--------------------|----------------|----------------------------------|---------------------------------------------------------------|
|                     | Non-vaccinated | Single-dose vaccinated | Two-dose vaccinated | N = 57 | n = 18 | n = 30 | N = 103 |                         |                          |
| **Demographics**    |                |                                |                      |         |         |         |         |                          |                          |
| Age (years), mean (SD) | 44.11 (12.63) | 47.83 (14.57) | 54.70 (11.35) | 45.91 (12.21) | 0.002a | • Post-COVID-19 two-dose vaccinated versus post-COVID-19 non-vaccinated: P = 0.001 |                          |
| Height (m), mean (SD) | 1.68 (0.10) | 1.67 (0.09) | 1.68 (0.09) | 1.66 (0.09) | 0.511a |                          | NA |
| Weight (kg), mean (SD) | 66.51 (13.7) | 74.6 (16.11) | 30 (70.1) | 65.86 (12.82) | 0.056a | • Post-COVID-19 two-dose vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.004 | NA |
| Gender, n (%)         |                |                                |                      |         |         |         |         |                          |                          |
| Male                 | 40 (70.2) | 13 (72.2) | 22 (73.3) | 70 (68.0) | 0.941b | • Post-COVID-19 two-dose vaccinated versus post-COVID-19 non-vaccinated: P = 0.001 | NA |
| Comorbidities, n (%)  |                |                                |                      |         |         |         |         |                          |                          |
| Any                  | 51 (89.5) | 18 (100.0) | 29 (96.7) | 91 (88.3) | 0.276 | • Post-COVID-19 two-dose vaccinated versus post-COVID-19 non-vaccinated: P = 0.001 | NA |
| DM                   | 30 (52.6) | 9 (50.0) | 16 (53.3) | 45 (43.7) | 0.663b | • Post-COVID-19 two-dose vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.004 | NA |
| HTN                  | 48 (84.2) | 17 (94.4) | 28 (93.3) | 86 (83.5) | 0.376b | • Post-COVID-19 two-dose vaccinated versus post-COVID-19 non-vaccinated: P = 0.001 | NA |
| CLD                  | 5 (8.8) | 0 (0.0) | 2 (6.7) | 6 (5.8) | 0.647b | • Post-COVID-19 two-dose vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.004 | NA |
| COAD                 | 2 (3.5) | 1 (5.6) | 6 (20.0) | 1 (1.0) | 0.001b | • Post-COVID-19 two-dose vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.004 | NA |
| Vascular disease (CAD/PVD) | 5 (8.8) | 4 (22.2) | 10 (33.3) | 2 (1.9) | <0.001b | • Post-COVID-19 two-dose vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.004 | NA |
| Chronic allograft dysfunction |                |                                |                      |         |         |         |         |                          |                          |
| Baseline transplant characteristics, n (%) |
| Duration from transplant to COVID-19 onset (weeks), median (IQR) | 301 (116.5–477) | 243 (112–411) | 199 (112–329) | NA | 0.451c | • Post-COVID-19 non-vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.018 | NA |
| Baseline immunosuppression |
| Steroid | 57 (100.0) | 18 (100.0) | 30 (100.0) | 103 (100) | e | • Post-COVID-19 non-vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.018 | NA |
| CNI      | 56 (98.2) | 18 (100.0) | 30 (100.0) | 101 (98.1) | 0.560b | • Post-COVID-19 non-vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.018 | NA |
| MMF/MPA  | 57 (100.0) | 15 (83.3) | 29 (96.7) | 88 (85.4) | <0.008b | • Post-COVID-19 non-vaccinated versus COVID-19-naïve two-dose vaccinated: P = 0.018 | NA |
| **Vaccination details** |                |                                |                      |         |         |         |         |                          |                          |
| Type of vaccine |
| Covishield | NA | 17 (94.4) | 20 (66.7) | 75 (72.8)d | 0.096 | • COVID-19-naïve two-dose vaccinated versus post-COVID-19 non-vaccinated, single-dose and two-dose vaccinated groups: P < 0.001 | NA |
| Covaxin   | NA | 1 (5.6) | 10 (33.3) | 25 (24.3)d | 0.096 | • COVID-19-naïve two-dose vaccinated versus post-COVID-19 non-vaccinated, single-dose and two-dose vaccinated groups: P < 0.001 | NA |
| Anti-spike antibody (AU/mL), median (IQR) | 745 (239–3022) | 3436 (661–10 450) | 3706 (867–10 660) | 17.1 | P = 0.001c | • COVID-19-naïve two-dose vaccinated versus post-COVID-19 non-vaccinated, single-dose and two-dose vaccinated groups: P < 0.001 | NA |
| Seropositivity |
| Anti-spike antibody titre >50 AU/mL | 54 (94.7) | 18 (100.0) | 29 (96.7) | 50 (48.5) | P < 0.001 | • COVID-19-naïve two-dose vaccinated versus post-COVID-19 non-vaccinated, single-dose and two-dose vaccinated groups: P < 0.001 | NA |
Table 1. Continued.

| Post-COVID-19 KTRs                  | Non-vaccinated N = 57 | Single-dose vaccinated n = 18 | Two-dose vaccinated n = 30 | Two-dose vaccinated N = 103 | P-value (F-test/chi-squared test) | Multiple group comparison (Tukey’s test/Bonferroni adjustment) |
|------------------------------------|------------------------|-----------------------------|---------------------------|-----------------------------|----------------------------------|---------------------------------------------------------------|
| Duration from COVID-19 onset to anti-spike antibody test (weeks) | 12.0 (10.0–31.5)       | 18.0 (10.8–44.3)            | 35.0 (25.8–43.0)          | NA                          | $P < 0.001^c$                      | Post-COVID-19 non-vaccinated versus post-COVID-19 two-dose vaccinated: $P < 0.001$ |
| Duration from last vaccine dose to anti-spike antibody test (days) | NA                     | 47.0 (28.0–84.8)            | 65.5 (24.0–95.5)          | 54.0 (29.0–120.0)            | 0.584$^d$                        | NA                                                            |

CAD/PVD, coronary artery disease/peripheral vascular disease; TLC, total leucocyte count; and Hb, haemoglobin. NA, not applicable because not significant in overall comparison (F-test/chi-squared test).

aOne-way ANOVA followed by Tukey’s test (The Tukey’s/Bonferroni is applicable only when the overall effect was significant).

bChi-squared/exact chi-squared test.

^cMann–Whitney U-test with Bonferroni corrected P-values.

dIn group ‘COVID-19-naïve two-dose vaccinated’, three patients received vaccine other than Covishied and Covaxin.

eData/P-value could not be computed.

65.5 days (IQR 24.0–95.5) and COVID-19-naïve two-dose vaccinated cohort at median 54 days (IQR 29–120) past last vaccine dose. For post-COVID-19 non-vaccinated cohort, the anti-spike antibody was assessed at median 12 weeks (IQR 10–31.5) from onset of COVID-19.

Demographics, comorbidities and baseline transplant characteristics of responders versus non-responders in fully vaccinated uninfected KTRs

Out of the 103 COVID-19-naïve two-dose vaccinated subjects, 50 seropositive individuals with anti-spike antibody titre >50 AU/mL were considered as ‘responders’ and the remaining subjects with anti-spike antibody titre <50 AU/mL were identified as ‘non-responders’. The demographics, baseline transplant characteristics, comorbidities and laboratory investigations, along with vaccination details are documented in Table 2. The average age (48.28 ± 12.34 years) of non-responders was significantly higher, whereas average weight (63.22 ± 10.63 kg) was lower than the responders (age: 43.40 ± 11.67 years, $P = 0.042$; weight: 68.55 ± 14.41 kg; $P = 0.038$). No significant difference was observed regards to height, gender, comorbidities and immunosuppressive treatment. Median anti-spike antibody titre for non-responders was 1.9 (IQR 0.33–5.45) AU/mL and that of responders were 2313.0 (IQR 389.1–6518.0) AU/mL ($P < 0.001$) (Table 2 and Figure 2B).
Table 2. Comparison between responder and non-responders in COVID-19-naïve two-dose vaccinated KTRs (no history of COVID-19)

| Parameters                              | Total (n = 103) | Responders (anti-spike antibody titre >50 AU/mL) (n = 50) | Non-responders (anti-spike antibody titre <50 AU/mL) (n = 53) | Mean or median difference/odds ratio (95% CI) | P-value |
|-----------------------------------------|----------------|-----------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------|---------|
| Demographics                            |                |                                                           |                                                               |                                             |         |
| Age (years), mean (SD)                  | 45.91 (12.21)  | 43.40 (11.67)                                             | 48.28 (12.34)                                                 | 4.88 (0.18 to 9.59)                           | 0.042   |
| Height (m), mean (SD)                   | 1.66 (0.09)    | 1.67 (0.10)                                               | 1.65 (0.09)                                                   | −0.017 (−0.05 to 0.019)                       | 0.350   |
| Weight (kg), mean (SD)                  | 65.86 (12.82)  | 68.55 (14.41)                                             | 63.22 (10.63)                                                 | −5.23 (−10.16 to −0.30)                       | 0.038   |
| Gender, n (%)                           |                |                                                           |                                                               |                                             |         |
| Male                                    | 70 (68.0)      | 37 (74.0)                                                 | 33 (62.3)                                                     | 1.73 (0.74–4.0)                              | 0.202   |
| Comorbidities, n (%)                    |                |                                                           |                                                               |                                             |         |
| DM                                      | 45 (43.7)      | 25 (50.0)                                                 | 20 (37.7)                                                     | 0.61 (0.28–1.33)                             | 0.210   |
| HTN                                     | 86 (83.5)      | 42 (84.0)                                                 | 44 (83.0)                                                     | 0.93 (0.33–2.64)                             | 1.00    |
| CLD                                     | 6 (5.8)        | 1 (2.0)                                                   | 5 (9.4)                                                       | 5.10 (0.58–45.32)                            | 0.206   |
| COAD                                    | 1 (1.0)        | 1 (2.0)                                                   | 0 (0.0)                                                       | b                                           | 0.485   |
| Vascular disease (CAD/PVD)              | 2 (1.9)        | 2 (4.0)                                                   | 0 (0.0)                                                       | b                                           | 0.233   |
| Chronic allograft dysfunction           | 35 (34.0)      | 18 (36.0)                                                 | 17 (32.1)                                                     | 0.84 [0.37–1.90]                             | 0.684   |
| Diabetic neuropathy                     | 25 (24.3)      | 14 (28.0)                                                 | 11 (20.8)                                                     | 0.67 [0.27–1.67]                             | 0.391   |
| Baseline immunosuppressant, n (%)       |                |                                                           |                                                               |                                             |         |
| MMF                                     | 88 (85.4)      | 40 (80.0)                                                 | 48 (90.6)                                                     | 2.40 (0.76–7.60)                             | 0.129   |
| CNI                                     | 101 (98.1)     | 49 (98.0)                                                 | 52 (98.1)                                                     | 1.96 (0.07–17.44)                            | 1.00    |
| Vaccination details                     |                |                                                           |                                                               |                                             |         |
| Vaccine type                            |                |                                                           |                                                               |                                             |         |
| Covishield                              | 75 (72.8)      | 36 (72.0)                                                 | 39 (78.0)                                                     | P = 0.488 (responders versus non-responders) |         |
| Covaxin                                 | 25 (24.2)      | 14 (28.0)                                                 | 11 (22.0)                                                     |                                             |         |
| Anti-spike antibody (AU/mL), median (IQR)| 17.1 (1.6–212.5)| 2313.0 (389.1–6518.0)                                    | 1.9 (0.33–5.45)                                               | 2173 (1012.8–3447.6)                         | <0.001  |
| Duration from last vaccine dose (days), median (IQR) | 54.0 (29.0–120.0) | 41.50 (25.0–90.50)                                      | 74.0 (34.0–137.0)                                             | −20.0 (−42.0 to −1.0)                        | 0.036   |
| Duration of transplant to vaccination (weeks), median (IQR) | 281 (132 to 378) | 233.0 (142 to 423)                                      | 295 (123 to 360)                                             | −3.0 (−96 to 63.0)                           | 0.911   |

| aThree patients received vaccine other than Covishied and Covaxin.  
| bCannot be computed due to zero count.  

Out of 103 COVID-19-naive two-dose vaccinated KTRs, 75 subjects received Covishield™ and 25 were vaccinated by Covaxin™ (Table 1). The seroconversion rate in KTRs receiving Covishield™ (36 out of 75, 48%) versus Covaxin™ (14 out of 25, 56%) was comparable (P = 0.488) (Table 2). The distribution of subjects receiving Covishield™ and Covaxin™ between the responders (Covishield™: 36/50 and Covaxin™: 14/50) and non-responders (Covishield™: 39/50 and Covaxin™: 11/50) was also comparable (P = 0.488). However, amongst the responders, subjects receiving Covishield™ had a significantly higher anti-spike antibody titre [median (IQR): 378.05 (161.5–4272); P = 0.042]. Interestingly, the median time interval for anti-spike antibody assessment past vaccination was higher for non-responders [74 days (IQR 34–137)] as compared with responders [41.5 days (IQR 25–90.5); P = 0.036] (Table 2).

Association and correlation of anti-spike antibody levels with demographics, comorbidities and other baseline characteristics of the KTRs

No significant association of anti-spike antibody levels assessed in KTRs from all cohorts with demographics, comorbidities, vaccination and anti-spike antibody assessment details was observed (Table 3). Similar results were obtained when we studied the association of anti-spike antibody titres of only seropositive KTRs (anti-spike antibody levels >50 AU/mL) with the baseline characteristics (Supplementary data, Table S1). Further, the correlation between anti-spike antibody levels in post-COVID-19 (non-vaccinated, single-dose and two-dose vaccinated) and COVID-19-naive two-dose vaccinated KTRs with clinical variables such as age, transplant duration up to vaccination, duration from onset of COVID-19 to vaccination and days from last dose of vaccine to serological assessment was studied (Table 4). No significant correlation was observed except in cohort 3, where anti-spike antibody levels from previously infected KTRs who received both doses of vaccine showed a negative correlation between age and anti-spike antibody levels (Spearman’s correlation coefficient: −0.380; P = 0.038).

DISCUSSION

The current study, to the best of our knowledge, is the first to investigate the longitudinal serological response to SARS-CoV-2...
natural infection and subsequent vaccination in KTRs in India. We have assessed the anti-spike antibody levels in 105 COVID-19-infected KTRs who have not been vaccinated (cohort 1) or vaccinated with either a single (cohort 2) or two doses (cohort 3) with 103 COVID-19-naïve two-dose vaccinated KTRs (cohort 4).

Recent studies have demonstrated that KTRs elicit an impaired immune response to the SARS-CoV-2 vaccine, with only 4–48% of KTRs showing detectable anti-spike IgG after complete vaccination [19–24, 30]. However, all these investigations were done in response to mRNA vaccines, which are currently unavailable in India. In our study cohorts, the majority of the subjects were vaccinated with Covishield™, an adenovirus-vectored vaccine expressing the SARS-CoV-2 spike protein (ChAdOx1-nCoV or AZD1222, acquired from Oxford University and AstraZeneca, manufactured by Serum Institute of India, Pune, India) and rest with inactivated whole virus–based vaccine Covaxin™ (BBV-152, manufactured by Bharat Biotech, Hyderabad, in collaboration with the Indian Council of Medical Research (ICMR), India). The data available on the serological response to Covishield™ and Covaxin™ is based on immune responses from immune-competent individuals [35–37], and similar information from KTRs is scarce. Recently, Prendecki et al. [38] reported immunological responses to two-dose vaccination with ChAdOx1 (Oxford University–AstraZeneca) in KTRs; only

Table 3. Association of anti-spike antibody levels with baseline characteristics and comorbidities in post-COVID-19 non-vaccinated, vaccinated (both single and two-dose vaccinated) and COVID-19-naïve two-dose vaccinated KTRs. The data shown corresponds to anti-spike antibody titres (AU/mL)

| Characteristics | Post-COVID-19 KTRs |
|-----------------|-------------------|
|                 | Non-vaccinated n = 57 | P-value | Vaccinated (single-dose and two-dose vaccinated) n = 48 | P-value | COVID-19-naïve two-dose vaccinated n = 103 | P-value |
| Demographics    |                   |         |                                           |         |                                         |         |
| Age             |                   |         |                                           |         |                                         |         |
| <60 years       | 768 (250–2228) n = 47 | 0.973   | 3697 (716–10 450) n = 37 | 0.873   | 57.90 (2.23–2551) n = 92 | 0.184   |
| >60 years       | 499 (153–6348) n = 10 |         | 3540 (371–10 626) n = 11 |         | 3.90 (0.80–220.0) n = 11 |         |
| Gender          |                   |         |                                           |         |                                         |         |
| Male            | 768 (250–1941) n = 39 | 0.993   | 3697 (974–10 360) n = 35 | 0.719   | 87.2 (1.35–2756) n = 70 | 0.522   |
| Female          | 694 (184.8–4294) n = 17 |         | 2298 (426–12 220) n = 13 |         | 6.80 (2.0–1339) n = 33 |         |
| Transplant duration |       |         |                                           |         |                                         |         |
| ≤1 year         | 1710 (523–10 760) n = 5 | 0.284   | Three cases | 0.841   | NA                                         | NA       |
| >1 year         | 745 (249–2228) n = 51 |         | 3540 (716–10 450) n = 45 |         | NA                                         | NA       |
| Vaccination details |              |         |                                           |         |                                         |         |
| Anti-spike antibody levels (AU/mL) from onset of COVID (weeks) |                   |         |                                           |         |                                         |         |
| <12 weeks       | 768 (205–3168) n = 31 | 0.921   | 3114 (633–6188) n = 10 | 0.339   | NA                                         | NA       |
| >12–24 weeks    | 1132 (249–1941) n = 11 |         | 750 (519–4835) n = 7 |         | NA                                         | NA       |
| >24 weeks       | 750 (382–9774) n = 15 |         | 5883 (1233–10 780) n = 31 |         | NA                                         | NA       |
| Anti-spike antibody levels from last vaccine dose (days) |                   |         |                                           |         |                                         |         |
| <21 days        | NA | 0.910 | 3619 (510–7960) n = 10 | | 31.1 (0.08–1191) n = 10 | 0.280   |
| >21 days        | NA |         | 3741 (733–10 560) n = 38 |         | 17.1 (1.9–2534) n = 93 |         |
| Comorbidities   |                   |         |                                           |         |                                         |         |
| Any             |                   |         |                                           |         |                                         |         |
| Present         | 76 (250–2390) n = 50 | 0.969   | 3697 (682–10 550) n = 47 | | NA | 0.930 | 17.10 (1.90–1801) n = 9 | 0.390 | 390.9 (0.70–3416) | 0.12 |
| Absent          | 1228 (66.8–4508) n = 6 |         | One-case only | | NA | 0.930 | 0.930 | 17.10 (1.90–1801) n = 9 | 0.390 | 390.9 (0.70–3416) | 0.12 |
| DM              |                   |         |                                           |         |                                         |         |
| Present         | 7680 (238–1772) n = 29 | 0.825   | 3481 (532–5403) n = 25 | 0.197   | 119 (4.0–4710) n = 45 | 0.022   |
| Absent          | 745 (250–4150) n = 29 |         | 7022 (1233–12 120) n = 23 |         | 8.0 (0.40–839.5) n = 58 |         |
| HTN             |                   |         |                                           |         |                                         |         |
| Present         | 754 (249–2228) n = 47 | 0.713   | 3697 (614–10 580) n = 45 | 0.873   | 23.5 (1.80–2219) n = 86 | 0.657   |
| Absent          | 1710 (181–4294) n = 9 |         | 3436 n = 3 |         | 4.40 (1.0–2214) n = 17 |         |
| CLD             |                   |         |                                           |         |                                         |         |
| Present         | 1196 (645–4680) n = 5 | 0.339   | Only two cases | 0.255   | 5.0 (0.8–52.10) n = 6 | 0.084   |
| Absent          | 745 (228–2228) n = 51 |         | 3706 (733–10 560) n = 46 |         | 56.10 (2.05–2534) n = 97 |         |
| COAD            |                   |         |                                           |         |                                         |         |
| Present         | 2 cases | 0.026 | 4234 (371–10 620) n = 7 | 0.090 | One case | 0.719 | 0.719 | 17.0 (1.6–2219) | 0.102 |
| Absent          | 877 (273–2950) n = 54 |         | 3540 (862–10 450) n = 41 |         | 17.0 (1.6–2219) | 0.102 |
| Vascular disease (CAD/PVD) |       |         |                                           |         |                                         |         |
| Present         | Two cases | 0.449 | 2997 (480–6472) n = 34 | 0.297 | Two cases | 0.038 | 0.038 | 16.90 (1.6–1759) | 0.101 |
| Absent          | 678 (280–2877) n = 51 |         | 3628 (918–11 110) n = 34 |         | 16.90 (1.6–1759) | 0.101 |
| Chronic allograft dysfunction |       |         |                                           |         |                                         |         |
| Present         | 1196 (515–6791) n = 11 | 0.348   | 4000 (2700–10 780) n = 11 | 0.244 | 100.5 (0.40–978.9) n = 35 | 0.473   |
| Absent          | 754 (216–2552) n = 45 |         | 3481 (583–9172) n = 37 |         | 16.45 (2.0–2595) n = 68 |         |

*IQR could not be calculated due to insufficient n values.*

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In agreement with the available literature, we also observed that only 48.5% KTRs (50/103) with no history of COVID-19 (COVID-19-naïve two-dose vaccinated cohort) responded positively (anti-spike antibody titre of ≥50 AU/mL) despite receiving a complete two doses of the vaccination regimen. This is lower in comparison with a recent report from India by Kute et al. [39], where 19 out of 31 uninfected KTRs (61.2%) seroconverted, probably because they considered a lower cut-off of ≥15 AU/mL (as opposed to 50 AU/mL in our study) as an indication of an antibody response.

In our study, proportions of responders and non-responders receiving Covishield™ versus Covaxin™ were comparable. No significant difference was observed in the seroconversion rate of Covishield™ and Covaxin™. However, the serological response to vaccination was significantly lower in responders who received Covaxin™ as compared with those who were administered Covishield™. Similar observations have been reported by two independent studies, including one on rheumatology patients [36, 40]. Interestingly, the non-responders were significantly older than the responders, with older age being associated with poorer vaccination responses [20].

Notably, amongst KTRs who were previously infected with COVID-19, only four subjects reported anti-spike antibody titre of <50 AU/mL, out of which three were non-vaccinated and one subject had received both vaccine doses. This is in agreement with a recent study, where only 5% of previously infected KTRs were seronegative after vaccination [38]. Similarly, in a study in an immunocompetent population, investigators reported that 100% of healthcare workers with a history of COVID-19 became seropositive after vaccination [36].

Interestingly, the SARS-CoV-2 anti-spike IgG antibody response [with a median antibody titre of 768 (249.0–3022)] in post-COVID-19 non-vaccinated KTRs was significantly higher than the COVID-19-naïve two-dose vaccinated KTRs. This observation is supported by studies that have investigated the development of SARS-CoV-2 antibodies in transplant recipients, including KTRs following natural COVID-19 infection [31–33]. It is to be noted that the low antibody levels in vaccinated uninfected KTRs are likely attributable to non-responders in the cohort. Further analysis of the median anti-spike antibody titres of the responders from the COVID-19-naïve two-dose vaccinated cohort revealed that the immune response elicited by vaccination in uninfected KTRs was comparable to KTRs with COVID-19 history (P = 0.221).

The antibody responses to Covishield™ and Covaxin™ vaccines in KTRs who have been previously infected with SARS-CoV-2 are largely unknown. In our study, we observed that previously infected KTRs showed a significant 4-fold increase in anti-spike antibody response to a single dose of vaccine. However, the median antibody titres between KTRs who received only one dose as compared with those who received both doses were comparable. This is in agreement to multiple studies conducted in non-transplant individuals [35–37, 41, 42] and a study by Benotmane et al. [43] in KTRs, where one dose of vaccine yielded significantly higher anti-spike antibody titre in COVID-19 recovered subjects. The robust post-vaccination immune response in individuals with past COVID-19 infection could be explained by immune memory that may persist for months [44–46], possibly resulting in a quicker and sustained response to COVID-19 vaccines.

We also analysed association of anti-spike antibody levels with demographics, vaccination details and comorbidities, and no significant association was observed. Similar results were reported by Singh et al. [36], with no significant difference in seropositivity rate with regard to age, sex, BMI, blood group, and any comorbidities, including its duration and treatment. However, when we calculated the correlation of anti-spike antibody with clinical variables, we observed a negative correlation of antibody levels with age in post-COVID-19 two-dose vaccinated KTRs; older age has been associated with a poorer immune response to vaccination.

There are several limitations to this study, including the relatively small sample size, especially in KTRs vaccinated with Covaxin. We also acknowledge the variation in anti-spike antibody levels of individuals, which could be due to variation in the interval between transplantation, COVID-19 diagnosis and vaccination days. It has been reported that patients who contracted COVID-19 within the first year of transplant may have a poorer immune response due to immunosuppression therapy [20]. Another important consideration is the variation in timing of antibody testing past infection or vaccination within our study cohorts. It is possible that anti-SARS-CoV-2 antibody levels have started to decline over time in some individuals, especially in KTRs who experience mild COVID-19 symptoms or with compromised immune response [46]. This could be applicable
in our study, where we observed that the median time interval for assessment of anti-spike antibody after the last vaccine dose in non-responders of the COVID-19-naive two-dose vaccinated cohort was longer [74 days (IQR 34–137)] as compared with responders [41.5 days (IQR 25–90.5)]. This could be one of the reasons for low antibody levels in some of the non-responders.

Our findings provide evidence that KTRs infected with COVID-19 develop anti-spike antibody following natural infection, albeit lower than the KTRs that received vaccination subsequent to their recovery from COVID-19. The antibody response significantly increases after the administration of a single dose of vaccine (Covishield™), suggesting that a previous infection with SARS-CoV-2 primed the immune system of the KTRs against COVID-19. Although no increase in the antibody level was observed following the second dose of vaccination, it is possible that the second dose could improve the longevity and durability of the response. However, due to inadequate number, the inference could not be extrapolated to patients receiving Covaxin™. Further studies with larger sample sizes, sequential anti-spike antibody monitoring over time and comparing COVID-19-infected KTRs with COVID-19-naive KTRs are warranted.

SUPPLEMENTARY DATA
Supplementary data are available at cij online.

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AUTHORS’ CONTRIBUTIONS
All authors contributed equally in research design and execution, data collection, analysis and interpretation of data and writing of the manuscript. All authors provided intellectual content of critical importance to the work described, approved the version for publication and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT
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