Research Letter

Immune responses against different variants of SARS-CoV-2 including Omicron following six months of administration of heterologous prime-boost COVID-19 vaccine

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Running title: Reduced response against Omicron in COVID-19 vaccinees implies for booster

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Highlights

Comparative analysis at one- and six-months post-vaccination showed modest reduction in S1-RBD IgG antibody and NAb titers against B.1, Alpha, Beta and Delta variants in heterologous and homologous vaccine recipients groups. However, significant reduction in NAb titers against Omicron in vaccinees' sera post-six months underlines need for cautious prospective follow-up.

Text

Within a short span of time SARS-CoV-2 vaccines developed through usage of different platforms have been approved across the globe. Such vaccines have acted as disease modifying tools by reducing the severity of the clinical course of infection and mortality due to COVID-19. However, the recent emergence of SARS-CoV-2 variant of concern (VOC) Omicron with its high transmissibility, has led to rapid surge in the number of COVID-19 cases including the breakthroughs and re-infections.[1] Against this background, marked immune escape potential of the Omicron variant has been at the center stage of discussion. There has also been growing interest in mix and match booster vaccination against COVID-19 across the globe in the light of newly emerging VOCs and many countries have started following the heterologous booster vaccination approach.[2] Preliminary studies have reported the safety, and improved humoral and cellular immune responses with a heterologous boost approach than a homologous one.[2]

Since December 2021, India has been witnessing a rapid surge in cases caused by Omicron in some major urban settings and Delta variants of SARS-CoV-2 infection in some other places.[3] Even with the aggressive vaccination campaign with Covaxin (whole virion inactivated vaccine/BBV152, BBIL, India) and Covishield (viral vector vaccine/ChAdOx1 nCoV- 19, SII, India), breakthrough infections and re-infections have been reported in the country. Under the national COVID-19 vaccination program, the inadvertent vaccine interchangeability with 1st dose of Covishield and 2nd of Covaxin was reported in twenty individuals in Audai Kalan village of Siddharthnagar district of eastern Uttar Pradesh, India.[4] Earlier we reported the safety and immunogenicity of the heterologous vaccination regimen of Covishield (CS) followed by Covaxin (CV) and compared it with two other

cohorts, receiving either two doses of homologous Covishield or Covaxin.[4] Out of the eighteen individuals in the heterologous group reported earlier,[4] we could follow-up 17 individuals in the second round at 6 months following administration of the second dose. Here, we report the findings of the IgG antibody responses against S1-RBD and the neutralizing antibody (NAb) titres of the heterologous and homologous vaccination regimes against the SARS-CoV-2 VOCs (Alpha, Beta, Delta) and its comparison with prototype B.1 at 1 and 6 months and with Omicron post-six months.

Venous blood (5 ml) was collected in serum separator gel tube vacutainers from each of the participants at 6 months after the second dose. The samples were tested for anti-SARS-CoV-2 IgG antibodies against S1-RBD ELISA as described earlier.[5]

Further, the plaque reduction neutralization test (PRNT50) was also performed against the B.1 (NIV2020-770, GISAID accession number: EPI_ISL_420545), Alpha [B.1.1.7, hCoV-19/India/20203522 SARS-CoV-2 (VOC) 202 012/01], Beta (B.1.351, NIV2021-893, GISAID accession number: EPI_ISL_2036294), Delta (B.1.617.2, NIV2021-1916, GISAID accession number: EPI_ISL_2400521) and Omicron (B.1.1.519, NIV2021-11828, GISAID accession number: EPI_ISL_8542931) variants, which were isolated from clinical specimens collected from SARS-CoV-2 infected individuals as described earlier.[4, 6, 7]

Of the 98 vaccine recipients, 88 individuals could be followed up at 6 months following administration of the second dose. The heterologous group CS/CV (n = 17, first dose Covishield, second dose Covaxin administered at an interval of six weeks) comprises of 10 males (M/F: 10/7) with a median age of 62 years (IQR 53-68.5). Among the homologous CS/CS group (n = 36, two doses given six weeks apart), 21 were males and 15 were females (M/F: 21/15) with a median age of 65.5 years (IQR 62–69.5). The

homologous CV/CV group (n = 35, two doses administered four weeks apart) had 14 males (M/F: 14/21) with a median age of 56 year (IQR 46–62) (Figure 1 A).

A significant reduction was observed in the S1-RBD IgG antibody titers in all the three groups i.e., CS/CV group (4.13-fold; p-value <0.0001), CS/CS group (6.80; p-value <0.0001)) and CV/CV group (4.87-fold; p-value <0.0001)) (Figure-1 B). The groups were compared using the Wilcoxon matched-pairs signed rank test to assess the statistical significance. Even with the waning antibody response at 6 months, the heterologous group had shown better immune response compared to the homologous groups administered with either Covishield or Covaxin. The comparison of the S1-RBD GMT ratios for heterologous group (1 month/6 month) to the S1-RBD GMT ratios for homologous Covishield and Covaxin group (1 month/6 month) demonstrated an increase in the IgG titer by 1.65 and 1.18 fold respectively (Figure-1 B).

In the comparative analysis at 1 and 6 months, significant reduction in ratio of the NAb geometrical mean titer (GMTs) was observed in the heterologous group as well as the homologous groups against B.1, Alpha, Beta and Delta (Figure 1 C, D, E, F). Despite significant fold-reductions in GMT of heterologous group, their NAbs were higher compared to that of the homologous groups.

The comparison of the B.1, Alpha, Beta and Delta GMT ratios for homologous Covishield (1 month/6 month) to the respective B.1, Alpha, Beta and Delta GMT ratios for heterologous group (1 month/6 month) demonstrated 0.4 fold, 0.5, 0.39 and 0.34 fold decrease in the neutralization titers respectively. Similarly, the comparison to GMT ratios of Covaxin group demonstrated 0.44, 0.53, 0.36 and 0.58 fold decrease to heterologous group (Figure 1 C, D, E, F).

Further, vaccinees' sera collected at 6 months were analyzed for the NAb titers against the Omicron and compared with NAb titers against other VOCs. The heterologous group demonstrated significant fold-reduction in the NAb titers compared to B.1 [Alpha: 1.28, Beta: 3.45, Delta: 1.75, Omicron: 19.16]. Similarly, the homologous groups had demonstrated significant fold-reduction in NAb titers compared to B.1; CS/CS group (Alpha: 1.63, Beta: 3.43, Delta: 2.27, Omicron: 23.15) and the CV/CV group (Alpha: 1.67, Beta: 2.56, Delta: 2.83, Omicron: 24.21) [Figure-1 G, H, I]. It is very clear from the geometric mean titre of the heterologous group at 1, 6 months and their ratio compared to homologous vaccination groups that the heterologous Covishield and Covaxin vaccination is immunogenically superior to homologous vaccination.

The NAb titers against Omicron were almost negligible in all the three groups (cut off value of the assay: 20). Only 23.5%, 30.5% and 22.8% of the sera from the CS/CV, CS/CS and CV/CV groups respectively showed NAb titers against Omicron [range:20-50]. In few samples which showed neutralization titer of that heterologous group showed 19-fold reductions, which was slightly less than the reductions observed with homologous CS/CS (23-fold) and CV/CV (24-fold) groups against Omicron. The proportion of vaccinees that showed >20 PRNT titers against Omicron strain for all other three groups were found to be statistically significant using the Chi-Square analysis.

Stuart *et al.* have reported robust immune responses using the heterologous prime boost approach with the vector/mRNA platform of the vaccines compared to the homologous vaccination strategy along with comparable safety and reactogenicity.[8] However in the light of emergence of Omicron variant, further studies with different vaccine platforms are needed.

Keeping in view the ongoing third wave of the pandemic with the emergence of Omicron, the National Technical Advisory Group on Immunization (NTAGI), Government of India has recommended the precautionary doses for those individuals who have completed 9 months after the second dose of Covishield or Covaxin.[9] Immunization with the additional dose has been initiated for all the health care and front-line workers as well as for the elderly above 60 years (with comorbidities) from 10th January 2022.

The gradual shift of VOCs from Delta to Delta-sub-lineage to Omicron along with the observed waning of immunity post-six months of vaccination, has prompted discourses around devising innovative vaccination strategy. The present investigation findings contribute meaningfully to such discussions. Regardless of the findings of this study, longitudinal monitoring for breakthrough severe disease should remain a part of any surveillance system.

Ethical statement

The study was approved by the Institutional Ethics Committee of the ICMR-Regional Medical Research Centre (ICMR-RMRC), Gorakhpur (IHEC Number-RMRCGKP/EC/2021/2.1). Written and informed consent was obtained from all the participants enrolled in the study before the collection of clinical data and samples at both the time points.

Author contributions

PDY and RKS and contributed to study design, data analysis, interpretation and writing and critical review. GS, GD, RRS, GRD, RS, DAN, DYP, AMS, KZ and AKC contributed to data collection, interpretation, analysis, writing and critical review. PDY, RRS, DYP, NG, RKS, SP, PA and BB contributed to the critical review and finalization of the paper.

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Competing interests:

No competing interest exists among the authors.

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References

1. Imperial College of London. Omicron largely evades immunity from past infection or two vaccine doses. https://www.imperial.ac.uk/news/232698/omicron-largely-evades-immunity-from-past/ [Accessed on January 12, 2022]
2. European Medicines Agency. Heterologous primary and booster COVID-19 vaccination Evidence based regulatory considerations. https://www.ema.europa.eu/en/documents/report/heterologous-primary-booster-covid-19-vaccination-evidence-based-regulatory-considerations_en.pdf. Accessed on 4 January 4, 2022
3. Indian COVID-19 Genomic Surveillance. https://clingen.igib.res.in/covid19genomes/ [Accessed on January 12, 2022]
4. Kant R, Dwivedi G, Zaman K, et al. Immunogenicity and safety of a heterologous prime-boost COVID-19 vaccine schedule: ChAdOx1 vaccine Covishield followed by BBV152 Covaxin. J Travel Med. 2021 Dec 29;28(8):taab166. doi: 10.1093/jtm/taab166. PMID: 34652440; PMCID: PMC8524638.
5. Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. The Lancet Infectious Diseases. 2021 Mar 8.
6. Yadav PD, Gupta N, Potdar V, et al. An in vitro and in vivo approach for the isolation of Omicron variant from human clinical specimens. bioRxiv. 2022 Jan 1.

7. Deshpande GR, Sapkal GN, Tilekar BN, et al. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. The Indian journal of medical research. 2020 Jul;152(1-2):82.
8. Stuart ASV, Shaw RH, Liu X, et.al. Immunogenicity, safety, and reactogenicity of heterologous COVID-19 primary vaccination incorporating mRNA, viral-vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-blind, randomised, phase 2, non-inferiority trial. Lancet. 2022 Jan 1;399(10319):36-49. doi: 10.1016/S0140-6736(21)02718-5. Epub 2021 Dec 6. PMID: 34883053; PMCID: PMC8648333.
9. Guidelines for COVID-19 vaccination of children between 15-18 years and precaution dose to HCWs, FLWs & 60+ population with comorbidities. https://www.mohfw.gov.in/pdf/GuidelinesforCOVID19VaccinationofChildrenbetween15to18yearsandPrecautionDosetoHCWsFLWs&60populationwithcomorbidities.pdf [Accessed on January 12, 2022].

Figure legends

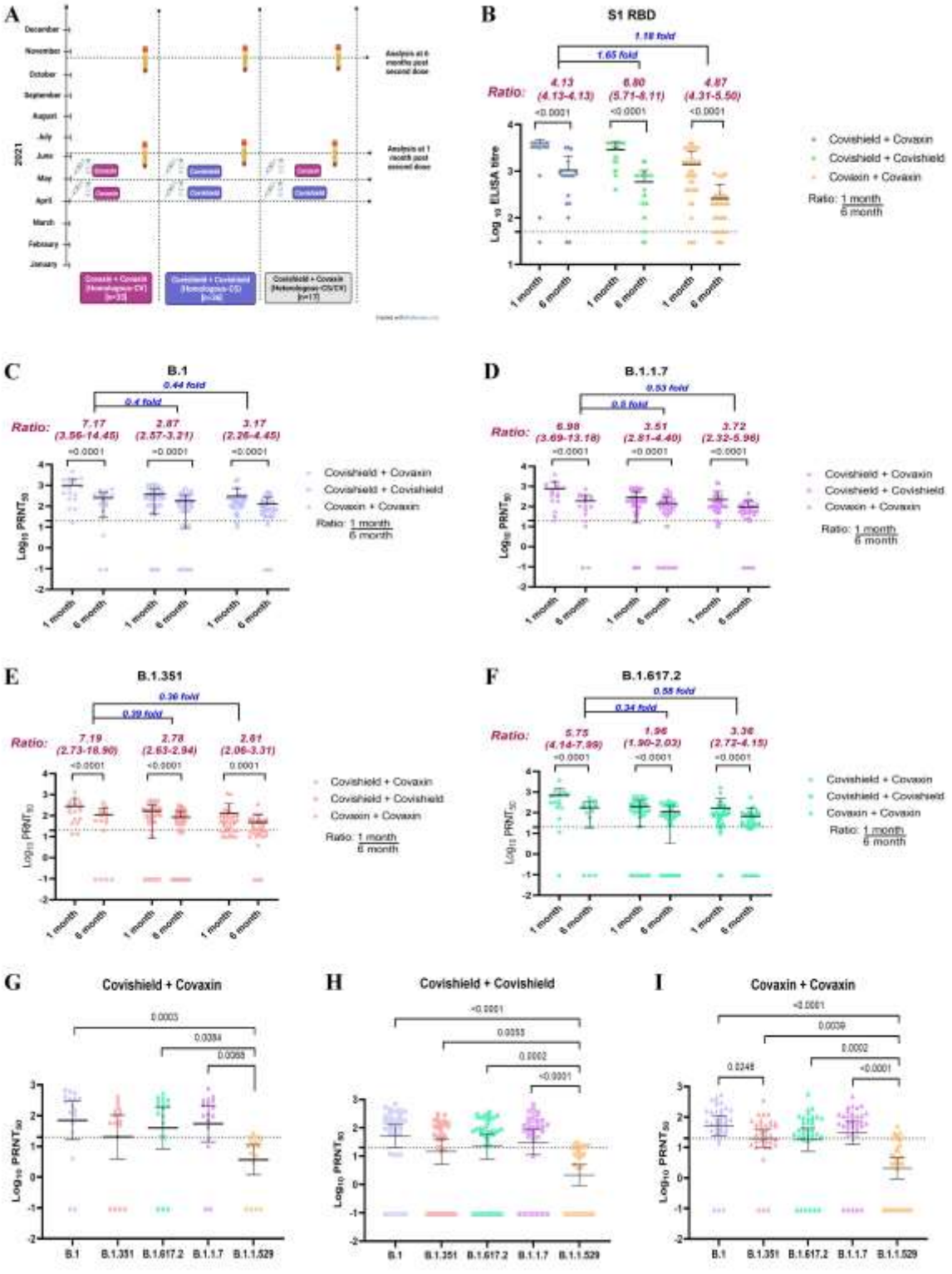
A
December
November
October
September
August
July
June
May
April
March
February
January
2021
Analysis at 6 months post second dose
Analysis at 1 month post second dose
Covaxin + Covaxin (Homologous-CX) [n=33]
Covishield + Covishield (Homologous-CO) [n=34]
Covishield + Covaxin (Heterologous-CO/CX) [n=17]
B
S1 RBD
1.18 fold
1.65 fold
Ratio:
4.13 (4.13-4.13)
6.80 (5.71-8.11)
4.87 (4.31-5.50)
<0.0001
Log 10 ELISA titre
1 month
6 month
Covishield + Covaxin
Covishield + Covishield
Covaxin + Covaxin
Ratio: 1 month / 6 month
C
B.1
0.44 fold
0.4 fold
Ratio:
7.17 (3.56-14.45)
2.87 (2.57-3.21)
3.17 (2.26-4.45)
<0.0001
Log10 PRNT50
D
B.1.1.7
0.53 fold
0.8 fold
Ratio:
6.98 (3.69-13.18)
3.51 (2.81-4.40)
3.72 (2.32-5.96)
E
B.1.351
0.36 fold
0.39 fold
Ratio:
7.19 (2.73-18.90)
2.78 (2.63-2.94)
2.61 (2.06-3.31)
0.0001
F
B.1.617.2
0.58 fold
0.34 fold
Ratio:
5.75 (4.14-7.99)
1.96 (1.90-2.03)
3.36 (2.72-4.15)
G
Covishield + Covaxin
0.0003
0.0084
0.0068
B.1
B.1.351
B.1.617.2
B.1.1.7
B.1.1.529
H
Covishield + Covishield
<0.0001
0.0033
0.0002
<0.0001
I
Covaxin + Covaxin
<0.0001
0.0039
0.0002
0.0248
<0.0001

Figure 1: The flow chart of the study design and ELISA titer of individual sera vaccinated with different vaccine regime: A) Study design of the experiment performed delineating the detailed steps of the sample collection from participants B) Anti-SARS-CoV-2 IgG antibody against S1-RBD for the different vaccine combination (Covishield followed by Covaxin marked as circle; Covishield followed by Covishield as square; Covaxin followed by Covaxin as triangle). The fold reduction provided in magenta color is the geometric mean titer ratio of one and six month. The statistical significance was assessed using a two-tailed Wilcoxon matched-pairs signed rank test; p-value less than 0.05 were considered to be statistically significant. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD). Comparative ratios of the neutralizing antibody geometric mean titres in CS/CV, CS/CS and CV/CV group against B.1, Alpha, Beta and Delta at 1 and 6 months post second dose [C-F]. The neutralizing antibody geometric mean titres in CS/CV, CS/CS and CV/CV group against Alpha, Beta Delta and Omicron compared to B.1 at 6 months post second dose [G-I]. The fold reduction provided in magenta color is the geometric mean titer ratio of one and six month. The statistical significance was assessed using a two-tailed Kruskal-Wallis with Dunn's multiple comparison test; p-value less than 0.05 were considered to be statistically significant. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD).