

Research letter

Title: Neutralization of Delta variant with sera of Covishield™ vaccinees and COVID-19 recovered vaccinated individuals

Running title: Neutralization of Delta variant

Authors: ¹#Gajanan N. Sapkal, Ph.D, *¹Pragya D. Yadav, Ph.D, ¹Rima R. Sahay, M.D., ¹Gururaj Deshpande, Ph.D, ²Nivedita Gupta, M.D, Ph.D., ¹Dimpal A Nyayanit, Ph.D., ¹Deepak Y. Patil, Ph.D, ¹Anita M. Shete, Ph.D, ³Sanjay Kumar, M.Ch, ¹Priya Abraham, M.D, Ph.D, ²Samiran Panda, M.D, ²Balram Bhargava, D.M.

#Equal first author

Affiliations:

¹Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India Pin-411021

²Indian Council of Medical Research, V. Ramalingaswami Bhawan, P.O. Box No. 4911, Ansari Nagar, New Delhi, India Pin-110029

³Department of Neurosurgery, Command Hospital (Southern Command), Armed Forces Medical College, Pune, Maharashtra, India Pin-411040

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***Corresponding author**

Dr. Pragya D. Yadav,
Scientist 'E' and Group Leader,
Maximum Containment Facility,
Indian Council of Medical Research-National Institute of Virology,
Sus Road, Pashan, Pune, Maharashtra, India Pin-411021.
Phone: +9120-26006111, Fax No. 91-20-26122669
Email: hellopragya22@gmail.com

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Keywords: Adenoviral-vector vaccines, ChAdOx-1, AstraZeneca, AZD1222, variants of concern, vaccine effectiveness

Highlights

The study demonstrated that the breakthrough cases and the COVID-19 recovered individuals with one or two doses of Covishield™ vaccine had relatively higher neutralizing responses against the Delta variant in comparison to the participants who were administered either one or two doses of Covishield™.

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Text

The SARS-CoV-2 lineage B.1.617 was initially detected from India during October 2020 and since then further mutated as sub lineages Kappa (B.1.617.1), Delta (B.1.617.2) and B.1.617.3 variant. Delta Variant has now mutated as Delta AY.1 and Delta AY.2, detected from India and many other countries. Delta variant has been reported to be 60% more transmissible than the Alpha variant (B.1.1.7) and the WHO has designated Delta variant as a Variant of Concern (VOC). The second wave of the COVID-19 pandemic in India was dominated by Delta variant, affecting millions of people causing a serious public health crisis. Similarly, it spread rampantly and dominated over the Alpha variant in the UK and gained foothold in over 92 countries.¹

The worldwide endeavor of scientists to create a safe and effective COVID-19 vaccine has resulted in the availability of 18 vaccines, which have received Emergency Use Authorization.² Currently, available vaccines appear to induce robust humoral and cellular immune responses against the SARS-CoV-2 spike protein.^{3,4} However, the newly emerged SARS-CoV-2 variants have led to breakthrough infections after completion of vaccination regimen.⁵ Hence, it is crucial to evaluate natural, vaccine-induced humoral immunity to SARS-CoV-2, and the phenomenon of breakthrough infection to understand the immune escape due to emerging VOCs.

Covishield™ is a replication-deficient viral vector based-SARS-CoV-2 recombinant vaccine, which has been rolled out under the national COVID-19 vaccination program in India. Lacobucci *et al.*, demonstrated significant immune responses following first dose and complete seroconversion in the subjects after second dose of Covishield™ in a study conducted in England and Wales.⁶ As the Delta variant has important mutations in spike

region; it could pose a real challenge to the vaccines specifically developed targeting spike gene.

Our earlier investigations demonstrated a reduction in the neutralizing antibody (NAb) titer in the sera of Covishield™ vaccinees against Kappa variant.⁷ Here, we have assessed the NAb response of individuals immunized with Covishield™ (first dose and second dose), COVID-19 recovered individuals who were vaccinated (first dose and second dose) and breakthrough infections (due to Kappa and the Delta variant).

A comparative assessment of Covishield™ vaccinated individuals' (n=116) sera in different categories was performed against prototype strain B.1 (D614G) and Delta variant. Sera under this study were grouped into five categories: I. one dose (n=31), II. Two doses (n=31), III. COVID-19 recovered plus one dose (n=15), IV. COVID-19 recovered plus two doses (n=19) and V. breakthrough COVID-19 cases (n=20). All the sera were collected four weeks post-vaccination for category I-IV participants. For category V patients, sera were collected two weeks post two-dose vaccination which were SARS-CoV-2 positive using rRT-PCR. COVID-19 recovered cases (category III & IV) were of B.1 lineage whereas breakthrough cases (category V) belonged to lineages Kappa and Delta variants as confirmed by next-generation sequencing. The samples were also screened for S1-RBD and N protein ELISA. Except for category I and II samples were not screened for N protein ELISA.

The NAb titers against B.1 and Delta variants were compared for sera of each category. NAb against B.1 were not observed in 11/31 (35.5%) participants in category I. Similarly, NAb against the Delta were not observed in 18/31 (58.1%) and 5/31 (16.1%) participants of categories I and II respectively. The GMT ratio of the Delta vs B.1 for

categories I- V were 0.22 (95% confidence interval (CI): 0.19-0.25; p-value <0.0001); 0.31 (95% CI: 0.22-0.43; p-value <0.0001); 0.34 (95% CI: 0.32-0.37; p-value <0.0001); 0.38 (95% CI: 0.38-0.39; p-value <0.0001), and 0.53 (95% CI: 0.49-0.56; p-value <0.0001) respectively (Figure 1 A-E). NAb titers for Delta relative to B.1 were reduced in the sera of the participants belonging to categories I (78%), II (69%), III (66%), IV (38%) and V (47%). This finding suggests increased susceptibility of two dose vaccinees to Delta variant.

The GMT for B.1 strain in categories I-V was 16.12 (95%CI: 6.507-39.94; p-value <0.0001), 73.47 (95% CI: 50.7-106.5; p-value <0.0001), 868.9 (95% CI: 533.3-1416; p-value >0.999), 1312 (95%CI: 949.3-1813; p-value >0.999) and 1344 (95%CI: 700.2-2580) respectively. This indicates that sera of COVID-19 recovered participants who were vaccinated with either one (category III) or two doses (category IV) and breakthrough had higher NAb titers relative to COVID-19 negative participants of category I and II (Figure 1F). Higher NAb titers in sera of COVID-19 recovered cases (categories III and IV) as compared to COVID-19 negative cases (categories I and II) highlights that even one dose of vaccine is enough to protect against re-infection of SARS-CoV-2. Our results corroborated with study conducted with mRNA vaccine by Goel *et al.*⁸ It also supports the role of cross-reactive SARS-CoV-2-specific T-cell-mediated immune response as described by Geers *et al.*⁹

Similarly, GMT for Delta variant in categories I-V was 3.553 (95%CI: 1.252-10.08), 22.43 (95%CI: 10.96-45.9), 298.8 (95%CI: 196-455.5), 501.3 (95%CI: 368.6-681.7) and 706.2 (95%CI: 342.8-1455) respectively (Figure 1G). Participants in category I (4.5 fold) and II (3.2 fold) showed reductions in NAb titers against Delta variants as compared to B.1 lineage. Reduction in GMT was evident in categories III-V, however NAb titers remained

significantly higher to provide enough protection. An increase in NAb titers was observed for both B.1 and Delta variants in the sera of the participants who had completed two vaccine doses relative to one dose. NAb titers against B.1 and Delta variants were highest among breakthrough participants which may be due to spike-specific T-cell responses.¹⁰

IgG specific to RBD protein showed higher antibody response (1:3200) in category III-V group (Figure 1 H). N protein-based ELISA indicated a similar pattern of IgG titer in participants in category III-V (Figure 1 I).

We observed significantly lower NAb titers (3.2-4.5-fold) for the Delta relative to B.1 variant. However, NABs in breakthrough participants and the COVID-19 recovered individuals with one or two-dose vaccination had relatively higher protection against Delta in comparison to the vaccinees with one or two dose vaccination.

Long-term follow-up of participants could help to understand the impact of natural infection and vaccination on long-term protection from SARS-CoV-2 offered by Covishield™. It is important to track the breakthrough infections for immune escape mutants.

Ethical approval

The study was approved by the Institutional Human Ethics Committee of ICMR-NIV, Pune, India under project 'Assessment of immunological responses in breakthrough cases of SARS-CoV-2 in post COVID-19 vaccinated group'.

Author Contributions

GNS, PDY and PA contributed to study design, data analysis, interpretation and writing and critical review. RRS, GRD, DAN, DYP, AMS and SK contributed to data collection, data analysis, interpretation, writing and critical review. NG, SP, and BB contributed to the critical review and finalization of the paper.

Conflicts of Interest

Authors do not have a conflict of interest among themselves.

Financial support & sponsorship

The study was conducted with intramural funding 'COVID-19 of Indian Council of Medical Research (ICMR), New Delhi provided to ICMR-National Institute of Virology, Pune.

Acknowledgement

We Sincere acknowledge the excellent support of Dr. Rajlaxmi Jain, Mr. Prasad Sarkale, Mr. Shreekant Baradkar, Ms. Aasha Salunkhe, Mr. Chetan Patil, Mrs. Triparna Majumdar and Mrs. Savita Patil for extending excellent technical support.

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Figure 1. Neutralization antibody titer of individual sera from different category against SARS-CoV-2 B.1 (D614G) and Delta (B.1.617.2) strains and RBD-S1 and N

protein titer: NAb titer against the B.1 (pink) and Delta (green) was compared between participants administered with one dose Covishield™ vaccine (A), two doses vaccine (B) COVID-19 recovered individuals administered with one dose Covishield™ vaccine (C) COVID-19 recovered individuals administered with two doses (D) and breakthrough participant (E). A two-tailed pair-wise comparison was performed using the Wilcoxon matched-pairs signed-rank test to analyze the statistical significance.

Comparison of NAb titer between participants administered with one dose Covishield™ vaccine (red color), two doses Covishield™ vaccine (green color) Covishield™ vaccine along COVID-19 recovered individuals administered with one dose (purple color) and two doses (indigo color) and breakthrough (black color) participants against B.1 strain (F) and Delta strain (G).

IgG titers of participant's sera from different categories for SARS-CoV-2 RBD protein (H) and N protein ELISA (I). The statistical significance was assessed using two-tailed Kruskal Wallis test with Dunn's test of multiple comparisons to analyze the statistical significance and a p-value less than 0.05 was 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).

