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Letter to the Editor

## Clinical, immunological and genomic analysis of the post vaccinated SARS-CoV-2 infected cases with Delta derivatives from Maharashtra, India, 2021

*Dear editor,*

Recently, Paula *et al.*, demonstrated the impact of time elapsed on the magnitude and decay kinetics of SARS-CoV-2 RNA loads in upper respiratory tract of breakthrough cases with SARS-CoV-2 Delta variant.[1] Globally, the breakthrough infection mainly found to occur due to waning immune response post natural infection/vaccination or the immune evasion of the VOCs.[2] India has experienced the devastating second wave of the pandemic with emergence of Delta variant from April 2021.[3] The variant has accounted for major breakthrough and re-infection cases in various countries irrespective of the vaccine platforms.[2,4–7] The Maharashtra state found to be the highly affected state with major number of COVID-19 cases recorded during March-June 2021 particularly from Mumbai and Pune cities with the high community transmission.[3] The nationwide study on breakthrough infections post-vaccination in India demonstrated 87% of breakthroughs occurred due to the Delta variant.[8] The higher frequency of breakthrough infections after complete vaccination has raised the question about the effectiveness of vaccine in controlling the SARS-CoV-2 infection specifically against the VOCs. Here, we report the clinical, immunological, cytokine and genomic analysis of SARS-CoV-2 infected cases post vaccination with Covaxin/Covishield reported from seven hospital sites of Mumbai and Pune, Maharashtra state, India.

The study was conducted in the hospitals at Pune ($n = 5$) and Mumbai ($n = 2$) of Maharashtra State, India during and post the second wave of the pandemic. The oro/nasopharyngeal [OP/NP] samples, blood samples were collected from the Covaxin or Covishield vaccinated individuals at the time of SARS-CoV-2 infection ($n = 448$) categorized into four groups: Covaxin (single dose, $n = 9$; CVSD), Covaxin (double dose, $n = 40$, CVDD), Covishield (single dose, $n = 149$, CSSD), and Covishield (double dose, $n = 240$, CSDD) and recovery ($n = 256$) during April to August 2021. The samples were screened for the SARS-CoV-2 variant, viral load, IgG and neutralizing antibody response and the cytokine analysis (Supplementary Fig. 1).

Of the 448 infected cases, male were predominant (44–65%) in all the four groups. Diabetes Mellitus type-II was the most common co-existing illness/co-morbidity (22–35%) ranging from, followed by hypertension (20–35%) asthma (2–11%) and obesity (2–7.5%) in all groups. Of all the cases, 77 to 88% cases were hospitalized, while 2.5–22% was at home isolation. Of all the cases, 338 (75.44%) cases were mild while 90 (20.04%) and 20 (4.46%) cases were moderate and severe respectively. Most of the cases were mild (55- 82%), while 11% in CVSD and 6.71% in CSSD were severe as compared to the CVDD (5%) and CSDD (2.8%) (Supplementary Fig. 2). The severity was significantly less with two doses of the vaccination as compared to first dose. On follow-up at the time of recovery between 14 and 21 days, 20–35% of all infected cases amongst all the four groups had residual complain of dry cough and generalized weakness with mild improvement of taste/smell.

SARS-CoV-2 RNA was detected in 362 samples (80.8%) of the 448 participants during the first collection and 66 (25.7%) out of 256 in follow-up samples (Viral RNA copy numbers ranged from $1 \times 10^2$ to $1.9 \times 10^9$ for the E gene, $2.8 \times 10^4$ to $2.8 \times 10^9$ for the E subgenomic RNA and $9.1 \times 10^3$ to $9.7 \times 10^{10}$ for the N subgenomic RNA) (Supplementary Fig. 3). Complete SARS-CoV-2 genome (>98%) could be retrieved from 224 samples [CVDD ($n = 5$), CVSD ($n = 22$), and CSDD ($n = 74$), CSSD ($n = 123$)] using next generation sequencing. Sequences with genome retrieval less than 98% were not used for further analysis . Majority of the cases characterized with variant Delta & Delta derivatives (AY) & all delta variants belongs to clade GK. Delta AY.50 ($n = 72$) was the major SARS-CoV-2 lineage in infected cases followed by Delta B.1.617.2 ($n = 58$), Delta AY.43 ($n = 14$), Delta AY.16 ($n = 12$), Delta AY.122 ($n = 07$), Delta AY.127 ($n = 06$), B.1.617.1 (Kappa) ($n = 5$), Delta AY.76 ($n = 05$), Delta AY.70 ($n = 04$), Delta AY.61 ($n = 04$), Delta AY.44 ($n = 04$), Delta AY.126 ($n = 04$), Delta AY.125 ($n = 04$), Delta AY.121 ($n = 03$), Delta AY.120 ($n = 03$), AY.47 ($n = 02$), Delta AY.16.1 ($n = 02$), Delta AY.14 ($n = 02$), B.1.351 ($n = 01$), B.1.1.306 ($n = 01$), Delta AY.99 ($n = 01$), Delta AY.98.1 ($n = 01$), Delta AY.87 ($n = 01$), Delta AY.86 ($n = 01$), Delta AY.58 ($n = 01$), Delta AY.37 ($n = 02$), Delta AY.10 ($n = 01$) (Fig. 1 & Supplementary Fig. 4). The AY.50 variant was detected in all the months with dominance in June and July. CSSD and CSDD groups had higher number of infections in the month of June and July with AY.50 and B.1.617.2 variants. The mild cases were infected with the lineages i.e., Delta ($n = 88$), Kappa ($n = 5$), Beta ($n = 1$), Delta derivatives ($n = 80$) and B.1.1306 ($n = 1$). Similarly, the variants in the moderate cases were predominantly Delta ($n = 27$) and Delta derivatives ($n = 16$) with a single case of B.1. The severe SARS-CoV-2 cases, found to be affected with Delta ($n = 9$) and Delta derivatives ($n = 7$). Delta variant predominated in moderate and severe SARS-CoV-2 cases as compared to the mild cases which also had other variants including B.1, Kappa and Beta.

Anti-SARS-CoV-2 IgG antibody titres were compared at the time of infection and recovery amongst 256 cases of 448 breakthrough cases. The significant reduction in Geometric mean titer (GMT) of IgG antibody were observed with the whole virion antigen, S1-RBD and N protein ELISA for individuals administered with CSDD and CSSD, while non-significant reduction in CVDD and CVSD group. It is observed that at the time of recovery, the antibody response for all the four groups had shown better immune response compared to its infective stage for both the individuals groups administered with either Covishield or Covaxin Fig. 1 (Fig. 2 A-D). Similarly, the neutralizing antibody response was compared among 53

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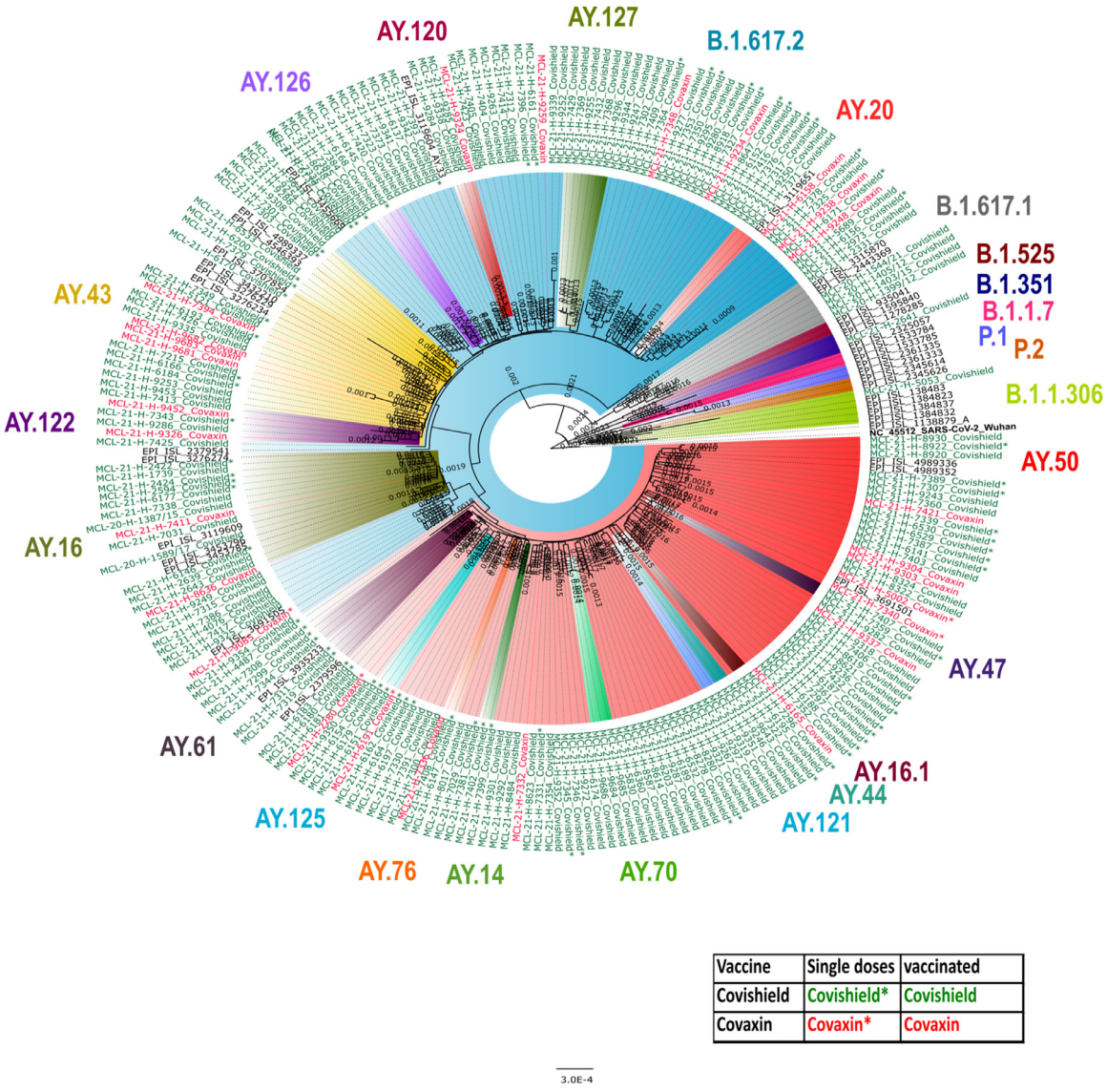


**Fig. 1. Phylogenetic tree of the SARS-CoV-2 genomes retrieved from COVID-19 post immunized cases**: A Neighbour-joining tree of the 224 SARS-CoV-2 sequences retrieved in this study, along with the representative SARS-Cov-2 sequences from different clades with a bootstrap replication of 1000 cycles. SARS-CoV-2 sequences retrieved from different vaccinated conditions are marked on branched in different colours. Covishield with one dose green asterisk; Covishield with two doses green; Covaxin with one dose red asterisk and Covaxin with two doses red color. The representative pangolin lineages are also marked on branches in different colours. FigTree v1.4.4 and Inkscape were used to visualize and edit the generated tree.

paired sera CVDD (16), CVSD (4), CSSD (18), CSDD (15) at the time of infection and recovery. All the groups demonstrated the higher NAb response at the time of recovery than at the time of infection. The GMT against Omicron was found to be lesser than B.1 and Delta variant suggesting the partial immune escape against both the Covishield and Covaxin Fig. 2 (Fig. 2 E-H). The cytokines IL-4, IL-5, IL-6, IL-13 and IL-15 indicated cell mediated and enhanced humoral immune responses in Covishield two doses vaccinees providing better protective immunity (Supplementary Fig. 5).

Despite the fact that vaccines are effective in preventing the SARS-CoV-2 infection, many breakthrough and reinfection cases were observed in the second wave when this data was collected. The shifting paradigm would be mainly due to the reducing/waning immune responses post either natural infection or vaccinations, emergence of the new SARS-CoV-2 variant, its immune escape potential. In summary, we report SARS-CoV-2 breakthrough infections predominated by the Delta variant from the western part of India. Most of the cases being mild and all have recovered uneventfully with a very low fatality of 0.4%. The infections were associated with prolonged PCR positivity and low level of the IgG antibody titres at the time of the infection post vaccination, emphasizing the need for the booster dose.

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## Declaration of Competing Interest

Authors do not have a conflict of interest among themselves.

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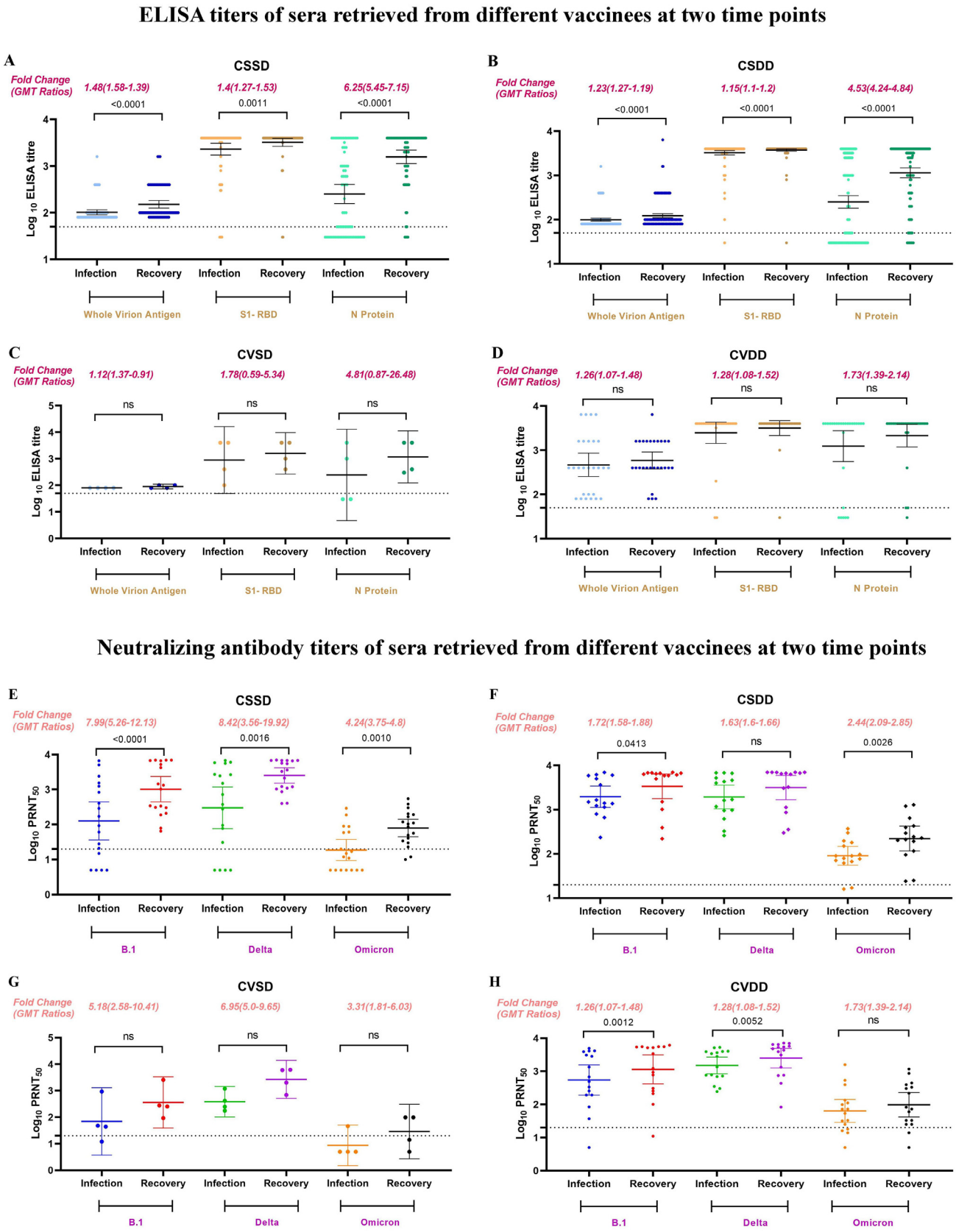


**Fig. 2. ELISA titers of sera retrieved from different vaccine at two time points**: Scatter plot depicting the ELISA titer of the individual sera vaccinated with A) one dose of Covishield vaccine and B) two doses of Covishield along with C) one dose of Covaxin vaccine and D) two doses of Covaxin against the Whole virion (blue), S1-RBD (D614G) (brown), and N protein (green). A two-tailed pair-wise comparison was performed using the Wilcoxon matched-pairs signed-rank test with a p-value of 0.05. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD). **Neutralizing antibody titers of sera retrieved from different vaccine at two time points**: Scatter plot depicting the neutralizing antibody titer of the individual sera vaccinated with E) one dose of Covishield vaccine and F) two doses of Covishield along with G) one dose of Covaxin vaccine and H) two doses of Covaxin against B.1 (blue, red), Delta (green, violet), and Omicron (orange, black). A two-tailed pair-wise comparison was performed using the Wilcoxon matched-pairs signed-rank test with a p-value of 0.05. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as mean values +/− standard deviation (SD).

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.014.

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