Research letter

Comparison of neutralizing antibody response in first and second waves of SARS-CoV-2 pandemic in India

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The emergence of multiple variants of concerns like Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and other variants, such as Epsilon (B.1.427/B.1.1429), Zeta (P.2), Eta (B.1.525), Theta (P3), Iota (B.1.526) and Kappa (B.1.617.1) has contributed to the worldwide development of multiple waves of the formidable COVID-19 pandemic.[1] Mid-September 2020 was associated with a maximal spike in the daily cases in India, with its lowest case load in January 2021 during the first wave with the G Clade (with D614G mutation-B.1) being causal. At the end of first wave, COVID-19 seemed to be restrained, the public became complacent and government relaxed its containment approaches allowing elections and religious gatherings, which lead to the resurgence of the second wave.[2] The Delta variant with 10 mutations in the spike protein seen in the second wave in India (60%) was associated with a surge in the number of cases by May 2021 because of higher transmissibility than the B.1 strain.[2,3] Further, the second wave witnessed greater disease severity and therefore more robust antibody responses, earlier seroconversion (<day 16) and higher IgG levels.[4,5] Antibody responses with virus neutralizing capability are one of the key factors for the development of immunity to prevent re-infection. However, it remains unclear if the circulating antibody response changes with time following natural infection. Additionally, the prognostic value of antibody measurements with respect to reinfection has yet to be established.

Here, we evaluated and compared the neutralizing antibody responses among COVID-19-recovered patients from the first and second waves of the pandemic in Maharashtra, India. Serum samples were collected from qRT-PCR-confirmed COVID-19-recovered subjects between March 2020 to January 2021 ($n = 99$) and April 2021 to May 2021 ($n = 99$) from designated hospitals within the Pune Municipal Corporation and Pimpri-Chinchwad Municipal Corporation, Maharashtra. Age-matched and time-matched (Median time was Say 40 from post-onset of disease/first day of PCR positivity) serum samples with no history of re-infection and COVID-19 vaccination were selected for this study.

The median age of the first-wave cohort ($n = 99$) was 35 years 95% + 1 confidence interval (CI), 32–41], of which 53.5% ($n = 53$) were males, 44.44% (44) were females, 9.09% ($n = 09$) individuals were asymptomatic and 91.83% ($n = 90$) were symptomatic with 3.06% ($n = 3$) presenting with severe symptoms. Similarly, the median age of the second-wave cohort ($n = 99$) was 34 years (95% + 1 CI, 31–36), of which 55.5% ($n = 55$) were males, 44.4% ($n = 44$) were females, 28.28% ($n = 28$) were asymptomatic and 71.71% ($n = 71$) were symptomatic with 24.24% ($n = 24$) presenting with severe symptoms during their hospitalization before recovery.

The anti-SARS-CoV-2 spike (S1RBD) IgG antibodies were determined using the Abbott Alinity i Chemiluminescent microparticle immunoassay. Sera from the second-wave cohort (GMT-1139.8, 95% + 1 CI: 796.6–1631) showed a significant increase in binding antibody concentrations compared with the first wave (GMT-758.3, 95% + 1 CI: 511.9–1124; $n = 92$ with $P < 0.01$) (Figure 1A), indicating strong immune responses during the second wave. The plaque reduction neutralization test (PRNT).[6] was used to assess the neutralizing capacity of sera against the B.1 and Delta strains and to check for cross-protection of sera from the first-wave cohort against homologous (B.1 prototype) and heterologous strains (Delta-B.1.617.2), and second-wave sera against Delta (homologous) and B.1 prototype (heterologous strain).
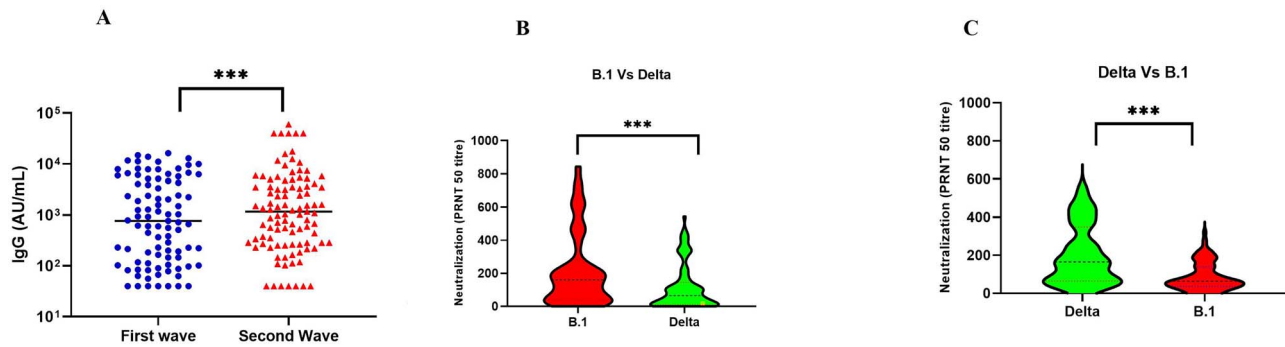
Figure 1. Comparison of first and second wave humoral response and neutralization activity against SARS-CoV-2 homologous and heterologous strains: A. IgG antibody response measured in first and second wave using CMIA. B and C. Sera of B.1 and Delta is tested homogenously (B.1 vs B.1 and Delta vs Delta) and heterogeneously (B.1 vs Delta and *vice versa*). The IgG levels (AU/ml) and neutralizing titers on Y axis are represented in the logarithmic (A) and linear scale (B and C). Data are presented as median, Wilcoxson test was used, and two-tailed *P* values were calculated ***$P < 0.001$. NIBSC code: 20/130 and 20/136 were used as reference standards for Enzyme linked immuno sorbet assay (ELISA) and PRNT, respectively.

Of the first wave samples 8.42% ($n = 08$) and 25.26% ($n = 24$) of the first-wave samples did not show neutralizing antibody titers (NAb) against the B.1 and Delta strains, respectively, whereas 16 sera (16.84%) ($n = 16$) showed NAb titers only for the B.1 strain. The GMT of first-wave sera against B.1, and Delta was 84.26 (95% + 1 CI, 53.76–132.1, $n = 95$) and 20.02 (95% + 1 CI, 10.79–37.16, $n = 95$), respectively. The GMT ratio of B.1 to Delta was 4.2, indicating a significant reduction in neutralization titers for Delta as compared with B.1 (Figure 1B). Similarly, 2.1% ($n = 02$) and 10.52% ($n = 10$) of the second-wave sera did not show NAb titers against the Delta and B.1 strains, respectively, whereas a total of 85 (89.47%) samples showed NAb titers against both B.1 and Delta. The GMT of the second wave sera against Delta and B.1 was 127.24 (95% + 1 CI, 96.25–168.2, $n = 95$) and 37.58 (95% + 1 CI, 24.07–58.71, $n = 95$), respectively, and the GMT ratio of Delta to B.1 was 3.4—emphasizing a significant reduction in cross-neutralization titers for B.1 compared with Delta (Figure 1C). Thus, a 1.51-fold increase was observed in neutralizing antibody GMT ratio for the second wave, compared with the homologous NAb titers in both first and second waves (i.e. GMT ratio of B.1 against first wave sera and GMT ratio of Delta against second wave sera), indicating higher antibody responses ($P < 0.001$) during the second wave.

In general, our observations are in line with previous reports, which state that humoral responses are higher in the second wave.[7] Surprisingly, a 3.4-fold reduction in the GMT ratio of Delta to B.1 with second wave sera was observed compared with the 4.2-fold reduction of the first wave sera of B.1 to Delta, indicating the immune response against Delta variant imparts a 1.2-fold higher cross-neutralization of Delta variant heterologous strains like B.1. Even though the government of Indian initiated a vaccine drive in January 2021, due to vaccine production challenges, it was insufficient to cover the enormous population by the time Delta variant was spread in the country. However, 80% of hospital admissions are all severe cases requiring intensive care units, and COVID-19-related mortality was observed only in unvaccinated populations.[8] Further, among the breakthrough cases during second wave infected with the Delta, majority of them did not progress to sever disease.[9] Hence, though the low cross-neutralizing antibody titers to heterologous strains (of first and second wave) were observed in this study, the data indicate that the serum of patients infected previously with one SARS-CoV-2 strain might provide protection against the rapidly emerging variants.

However, our study had few limitations including small sample size and unavailability of followed samples for monitoring the level of cross-protection during reinfection.

In conclusion, our observation represents that the second wave dominated by the Delta strain, elicited a robust immune response than the first wave and would be valuable to the efforts for developing vaccines. Additional studies with larger cohort with systematic follow-up will help in better understanding of antibody dynamics against emerging SARS-CoV-2 variants in the country.

Conflict of interest

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References

1. SARS-CoV-2 variant classifications and definitions. *Centers for Disease Control and Prevention*. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html (22 July 2021, date last accessed).
2. Choudhary OP, Priyanka SI, Rodriguez-Morales AJ. Second wave of COVID-19 in India: dissection of the causes and

lessons learnt. *Travel Med Infect Dis* 2021; **43**:102126. 10.1016/j.tmaid.2021.102126.

3. Kumar A, Dwivedi P, Kumar G *et al*. Second wave of COVID-19 in India could be predicted with genomic surveillance of SARS-CoV-2 variants coupled with epidemiological data: a tool for future. *Med Rxiv* 2021. 10.1101/2021.06.09.21258612.

4. Zhang B, Zhou X, Zhu C *et al*. Immune phenotyping based on the neutrophil-to-lymphocyte ratio and IgG level predicts disease severity and outcome for patients with COVID-19. *Front Mol Biosci* 2020; **7**:157. 10.3389/fmolb.2020.00157.

5. Huang AT, Garcia-Carreras B, Hitchings MDT *et al*. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* 2020; **11**:4704. doi.org/10.1038/s41467-020-18450-4.

6. Deshpande GR, Sapkal GN, Tilekar BN *et al*. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. *Indian J Med Res* 2020; **152**:82–7. 10.4103/ijmr.IJMR_2382_20.

7. Ezzikouri S, Majidi H, Redwane S *et al*. SARS-CoV-2 infection elicits a greater humoral immune response in the second wave than the first wave. *Research Square* 2021. https://doi.org/10.21203/rs.3.rs-419367/v1.

8. Choudhary OP, Choudhary P, Singh I *et al*. India's COVID-19 vaccination drive: key challenges and resolutions. *Lancet Infect Dis* 2021; **21**:1483–4. 10.1016/S1473-3099(21)00567-3.

9. Gupta N, Kaur H, Yadav PD *et al*. Clinical characterization and genomic analysis of samples from COVID-19 breakthrough infections during the second wave among the various states of India. *Viruses* 2021 7; **13**:1782. 10.3390/v13091782.