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RESEARCH ARTICLE

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Omicron infection increases IgG binding to spike protein of predecessor variants

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in India in 2020–2022 was driven predominantly by Wild (Wuhan-Hu-1 and D614G), Delta, and Omicron variants. The aim of this study was to examine the effect of infections on the humoral immune response and cross-reactivity to spike proteins of Wuhan-Hu-1, Delta, C.1.2., and Omicron. Residual archival sera ($N = 81$) received between January 2020 and March 2022 were included. Infection status was inferred by a positive SARS-CoV-2 RT-PCR and/or serology (anti-N and anti-S antibodies) and sequencing of contemporaneous samples ($N = 18$) to infer lineage. We estimated the levels and cross-reactivity of infection-induced sera including Wild, Delta, Omicron as well as vaccine breakthrough infections (Delta and Omicron). We found an approximately two-fold increase in spike-specific IgG antibody binding in post-Omicron infection compared with the pre-Omicron period, whilst the change in pre- and post-Delta infections were similar. Further investigation of Omicron-specific humoral responses revealed primary Omicron infection as an inducer of cross-reactive antibodies against predecessor variants, in spite of the weaker degree of humoral response compared to Wuhan-Hu-1 and Delta infection. Intriguingly, Omicron vaccine-breakthrough infections when compared with primary infections, exhibited increased humoral responses against RBD (7.7-fold) and Trimeric S (Trimeric form of spike protein) (34.6-fold) in addition to increased binding of IgGs towards previously circulating variants (4.2 - 6.5-fold). Despite Delta breakthrough infections showing a higher level of humoral response against RBD (2.9-fold) and Trimeric S (5.7-fold) compared to primary Delta sera, a demonstrably reduced binding (36%–49%) was observed to Omicron spike protein. Omicron vaccine breakthrough infection results in increased intensity of humoral response and wider breadth of IgG binding to spike proteins of antigenically-distinct, predecessor variants.

KEYWORDS

Breakthrough infection, Delta, Humoral immunity, Immune responses, Omicron, SARS coronavirus, SARS-CoV-2 spike variants

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2] has resulted in approximately 6.3 million deaths worldwide. The SARS-CoV-2 genome has acquired multiple mutations resulting in increased transmissibility and/or immune evasion phenotypes, leading to multiple waves of infection of immense global public health impact. The WHO classified Alpha, Beta, Gamma, Delta, and Omicron, as variants of concern (VOCs).[3] Amino acid changes in the spike protein of these VOCs resulted in immune evasion, increased spike receptor-binding domain (RBD) affinity to human angiotensin-converting enzyme 2 (hACE2) receptor, and in turn improved viral cell entry and replication in host cells.[4]

India has reported >40 million confirmed cases and over 525,000 deaths due to COVID-19 between January 2020 and June 2022.[5] The national COVID-19 vaccination program in India, launched in January 2021, was implemented with the adenovirus-vector based COVISHIELD™ and inactivated Wuhan-Hu-1 virus based COVAXIN® vaccines.[6] These vaccines demonstrated a reasonable efficacy (>70%) against symptomatic infections, but uniform high efficacy against severe disease and hospitalization.[7] A total of around 2 billion vaccine doses had been administered till June 2022 in India.[5,8] The introduction of a mass vaccination program in India contained widespread virus transmission to a large extent. Currently, most vaccines are based on spike protein of ancestral Wuhan-Hu-1 strain and the antibody response has effectively neutralized Wuhan-Hu-1 strain and also multiple VOCs through booster doses.[9–13] The continued evolution of SARS-CoV-2 and waning of vaccine- or infection-induced neutralization over time has necessitated vaccine booster doses to maintain efficacy against new VOCs.[14–17] A durable and broad immunity is key to containment of current and future variants of SARS-CoV-2.

To date, India has experienced three waves of COVID-19 between January 2020 and March 2022. The first wave was caused by multiple lineages of SARS-CoV-2 including the ancestral strain, and variants with the D614G mutation in spike protein. Delta variant which originated in India, was predominant during the second wave from March 2021 and replaced its predecessors (Alpha, Beta, and Gamma) in India.[18] SARS-CoV-2 Omicron (B.1.529) variant designated as a VOC by the WHO in November 2021, was the primary driver of the third wave of COVID-19 in India. Of the VOCs, Omicron is the most distinct variant, evolutionarily, with 36 coding mutations in spike protein.[19] The Omicron variant has been shown to cause breakthrough infections owing to enhanced affinity to hACE2 receptor, infection by endosomal host entry route, and immune evasion (either pre-existing infection or vaccine-induced immunity or therapeutic antibodies).[13,20,21] Omicron spread rapidly and has replaced all previous Delta sub-lineages to become the dominant variant circulating globally. Genomic surveillance has reported evolution of omicron into sub-lineages BA.1, BA.2, BA.3, more recently BA.4, BA.5 and others.[21,22] BA.1 spread globally till February/March 2022, and later was gradually replaced by BA.2. Most recent reports suggest the emergence of BA.4 and BA.5 in several countries, including India.[23]

Breakthrough infections by Omicron in vaccinated individuals elevated antibody (including neutralization antibodies) levels against ancestral Wuhan-Hu-1 as well as cross-reactive antibodies to spike proteins of other VOCs, attributable to immune imprinting[8,12,24] due to its increased antigenic distance from Wild-type and other VOCs. More importantly, it is still elusive whether pre-Omicron VOCs (i.e., Alpha and Delta) induce a cross-reactive response to other VOCs. Furthermore, the intensity and breadth of cross-reactive responses in natural and breakthrough infections in an Indian context have not yet been reported. To this end, we examined the levels of spike variant-specific humoral responses induced by natural infection with SARS-CoV-2 variants causing the 3 COVID-19 waves in India (either Wuhan-Hu-1 or Delta or Omicron), vaccination and vaccine breakthrough infections (either Delta or Omicron) in an Indian context. We show that cross-reactive antibodies to other variants including those reported not reported/detected in India (C.1.2, detected in South Africa), was observed in Delta and highest in Omicron primary infections with a further boosting of these cross-reactive antibodies in Delta and Omicron vaccine-breakthrough infections.

2 | METHODS

2.1 | Serum and respiratory samples

Residual, deidentified archival sera from blood samples received in the laboratory for routine diagnostic testing between January 2020 and March 2022 were included for testing in this study. The dates of blood and respiratory sample collection for SARS-CoV-2 PCR were obtained from laboratory records. Respiratory samples were tested for SARS-CoV-2 viral RNA using a commercial real-time RT-PCR (Altona Realstar® SARS-CoV-2 RT-PCR). The study was approved by the Institutional Review Board of Christian Medical College, Vellore (IRB Nos. 12917&12691).

2.2 | SARS-CoV-2 genome sequencing and phylogenetic analysis

As corresponding respiratory samples were not available to confirm infection, we inferred the infection type (Wild, Delta or Omicron and Delta and Omicron breakthrough among vaccinated) based on the circulation of variants over the study period by sequencing of respiratory samples in the same week (Figure 1A–C) as the corresponding sample. We inferred the circulating clade and VOCs in Vellore, by sequencing contemporaneous samples from Vellore ($N = 18$) from the same week as the positive PCR specimens. Representative high-quality, whole genome sequence data from India ($N = 172$) along with sample collection date were obtained from GISAID/Virus Pathogen Resource and analysed in a Bayesian framework implemented in BEAST v2.0. MCMC chains were checked

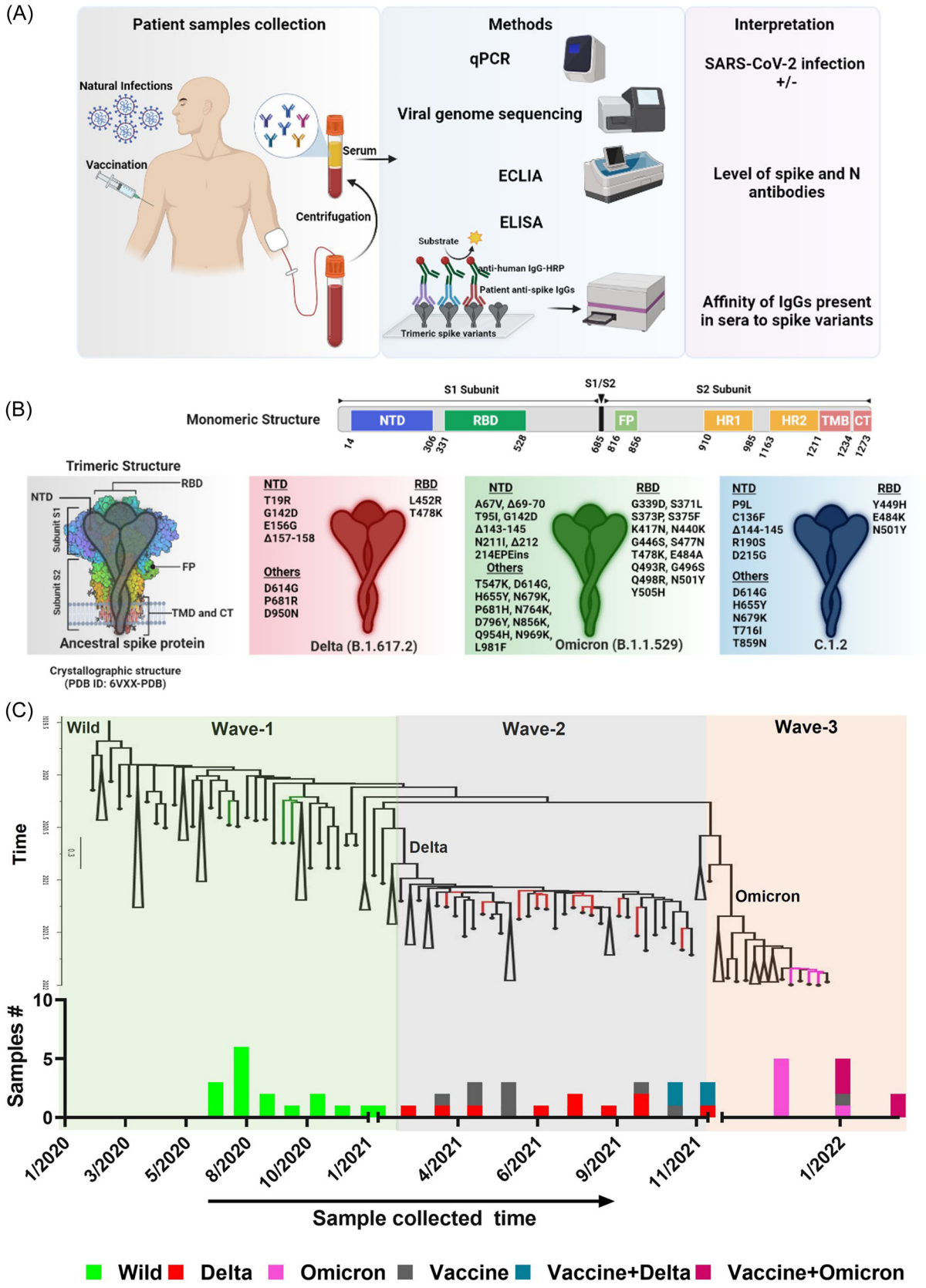

FIGURE 1 Prevalence and waves of SARS-CoV-2 variants in Indian population. (A) Schematic representation of this study, (B) Graphical representation and mutational profile of spike variants, (C) MCC phylogeny of SARS-CoV-2 spike gene from 2020 to 2022 in Vellore (City of Tamil Nadu) and India with the sample collection date.

for convergence using Tracer and Maximum Clade Credibility (MCC) tree was generated using Tree Annotator and annotated in FigTree (v1.4.4). The sequence data generated in the study will be uploaded to GISAID.

2.3 | SARS-CoV-2 N and spike antibodies detection

Serum samples included in this study were tested for antibodies to nucleoprotein (anti-N) and spike receptor binding domain (anti-RBD) (Roche Elecsys ECLIA) platform and antibodies to trimeric full spike protein (anti-Trimeric S) using the Diasorin SARS-CoV-2 Trimeric S kit on the Liaison XL system. The assay and interpretation were as per the manufacturer's recommendations.

2.4 | SARS-CoV-2 variant antigens, and ELISA

Purified trimeric form of spike protein (ACRO Biosystems) expressed in HEK-293 cells was used to detect the level of antibody binding against Wuhan-Hu-1, Delta (B.1.617.2) and Omicron (B.1.1.529) and C.1.2 by ELISA. The C.1.2 variant was identified and monitored in South Africa in mid-2021. C.1.2 by virtue of its heavily mutated spike, showed high immune evasion compared to Delta variant in convalescent and vaccine-elicited sera.[25] We evaluated C.1.2 for a better understanding on immune evasion.

2.5 | Sampling and study design

Serum samples collected between January 2020 and March 2022 were included in this study spanning the three waves of SARS-CoV-2 in India. Serum samples from seven SARS-CoV-2 noninfected (called pre-pandemic) and 74 infected individuals were used. First, we broadly classified serum samples based on the date of blood collection. Samples from January to March 2022 ($N = 20$) were classified as "Omicron period" and from June 2020 to December 2021 ($N = 54$) as the 'pre-Omicron period' samples. Similarly, samples from March to December 2021 ($N = 36$) were categorized as the "Delta period" and those from June 2020 to February 2021 ($N = 18$) as the "Pre-Delta period."

To examine the effects of natural and breakthrough infection on antibody levels and binding, we further categorized sera into groups such as a) pre-pandemic, b) Wild (Wuhan-Hu-1 and D614G mutant), c) Delta, d) Omicron, e) vaccine, f) vaccinated with Delta breakthrough (Vaccine+Delta), g) vaccinated with Omicron breakthrough (Vaccine +Omicron), based on level of anti-N, anti-S and/or PCR positivity, and period (sample collected date) as described Table 1. The overall study overview, variant spike mutational profile and phylogenetic confirmation of virus circulation is shown in Figure 1A–C, respectively. Phylogeny confirmed circulation of the D614G (green branches) between June 2020 to March 2021 (Wave-1 green shading), Delta (B.1.617.2 and descendants, red branches, Wave-2, grey shading) between March and December 2021 and Omicron (BA.2 pink, Wave-3 peach shading) after mid-December 2021 in Vellore.

2.6 | Percentage change in geometric mean binding

For each group of serum samples (e.g. "Wild sera" etc.), the geometric mean of Spike IgG ELISA OD (IgG binding) of the heterologous antigen (i.e., Delta, Omicron and C.1.2) was normalized to the homologous antigen (Wuhan-Hu-1) and the increase/decrease towards the heterologous antigen was expressed as a percentage.

2.7 | Statistical analysis

Statistical significance of serum IgG binding groups (see above) across variants was analysed using nonparametric one-way analysis of variance-Friedman test with Dunn's correction. The nonparametric Mann–Whitney test was used to analyse significance between groups. Level of statistical significance was denoted by * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, ****for $p < 0.0001$, ns for = nonsignificant values. The statistical significance and visualization of data were performed in GraphPad Prism version 8 (GraphPad Software).

TABLE 1 Grouping of serum samples

Groups	Sample size	Anti-N antibodies	Anti-S antibodies	PCR	Period
Pre-pandemic	7	-	-	-	Jan–May 2020
Wild	17	+/++	+/++	+	Jun 2020–Mar 2021
Delta	8	+/++	+/++	+	Apr 2021–Dec 2021
Omicron	5	+/++	+/++	+	Jan–Mar 2022
Vaccine	22	-	+/++	-	Jan 2021–Mar 2022
Vaccine+Delta	14	+/++	+++	+	Apr–Dec 2021
Vaccine+Omicron	8	+/++	+++	+	Jan–Mar 2022

Note: Wild- Wuhan-Hu-1 (Prototype) and G614 strains; + (low); ++ (medium); +++ (high).

3 | RESULTS

3.1 | Omicron infection-induced IgG antibodies to have higher binding towards heterologous spike variants

We assessed the level of antibodies (anti-N, anti-RBD, and anti-Trimeric S) and binding to four antigens (Wuhan-Hu-1, Delta, Omicron, and C.1.2 spike trimer) between the Delta (pre- vs. post) and Omicron period (pre- vs. post) sera. The levels of anti-N, anti-RBD, and anti-Trimeric S were found to be similar (Mann–Whitney test, $p > 0.05$) between Delta and pre-Delta (Figure 2A–C) as well as Omicron and pre-Omicron periods (Figure 2E–G). However, the antibody binding (measured OD value) to each of the antigens were similar between Delta and pre-Delta periods (Figure 2D), but showed a 2–3 times increased binding between the Omicron and pre-Omicron period (Figure 2D,H). This indicated that Omicron infections contributed to an increased antibody binding to heterologous spike variants in the study population.

3.2 | Primary Omicron infection induces poor anti-spike humoral response, but cross-reacts with heterologous spike variants

To evaluate whether immune cross-reactivity against spike variants is a characteristic signature of Omicron, first, we compared antibody levels in sera from three primary infection groups—Wild, Delta and Omicron. The levels of anti-N (Figure 3A) and anti-RBD antibodies (Figure 3B) were similar among Wild, Delta and Omicron sera. However, levels of anti-Trimeric S were found to be lower in Delta and Omicron sera compared with Wild sera ($p < 0.05$) (Figure 3B). We further compared IgG antibody binding of these 3 sera groups to Wuhan-Hu-1, Delta, C.1.2, Omicron trimeric spike proteins using indirect ELISA (Figures 3C–E and S1). To compare the antibody binding of Wild sera between variants, we computed the ratio of geometric mean (GM) OD of binding of Wild sera to spike of heterologous variants, normalized to Wuhan-Hu-1 spike and expressed in percentage. We found a 30%, 66%, and 40% reduction in Wild sera binding to Delta ($p > 0.05$), Omicron ($p < 0.0001$), and C.1.2 ($p < 0.0001$) spike proteins, respectively (Figure 3C,F). Similarly, Delta sera demonstrated reduced binding to Omicron (49%, $p < 0.0001$) and C.1.2 (15%, $p > 0.05$) and increased (17%) to Wuhan-Hu-1 spike variants (Figure 3D,F). Conversely, in Omicron sera, the percent GM ratio of binding to spike variants showed an increased relative antibody binding to all heterologous spike variants (Figure 3E,F). Collectively, these data suggest that sera from individuals infected with Omicron have a poorer humoral immunity (low level of spike antibodies), but it exhibits increased antibody cross-reactivity to predecessor heterologous variants.

3.3 | Omicron breakthrough infection after vaccination boosts the magnitude of humoral response as well as cross-reactivity towards spike protein of all variants

Further, we examined the impact of vaccine breakthrough infections with Delta and Omicron on spike antibody levels and cross-reactivity

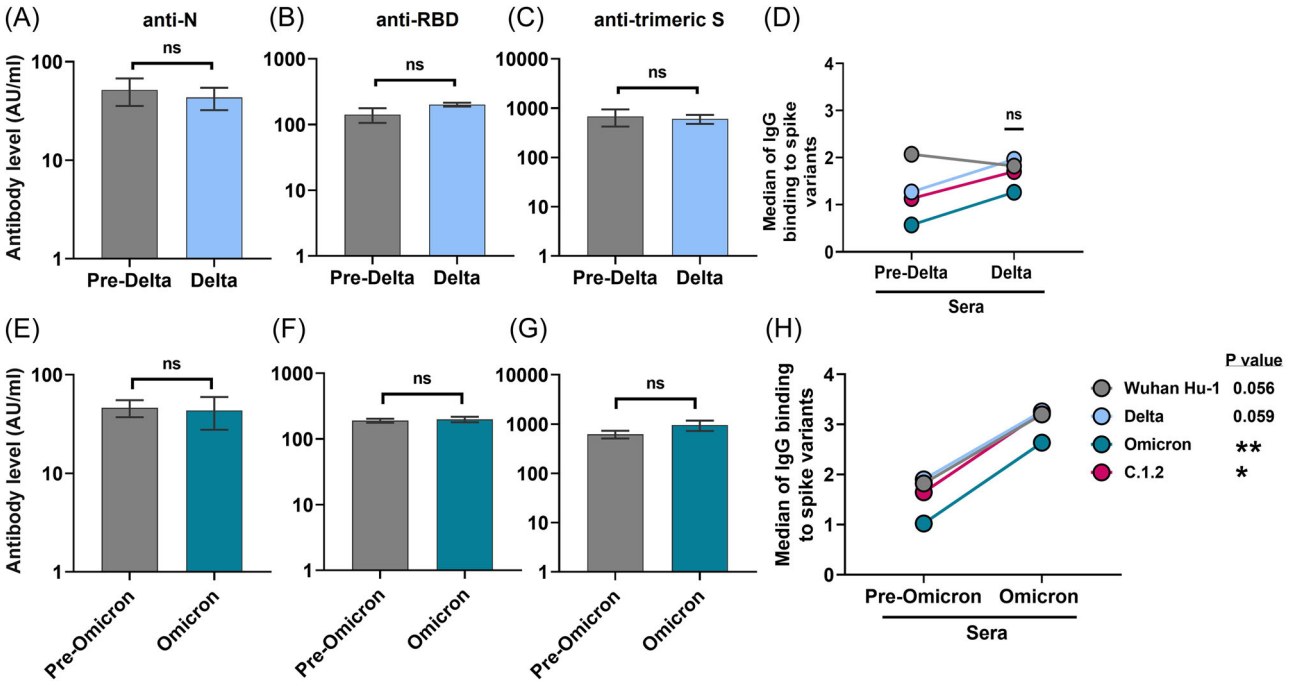

FIGURE 2 The levels and cross-reactivity of humoral response induced by pre-Delta and Delta, pre-Omicron, and Omicron infections towards heterologous spike proteins. The levels of (A&E) anti-N, (B&F) anti-RBD, (C&G) anti-Trimeric S antibodies, and (D&H) Median OD value (of antibody binding) against different spike variants in respective serum groups.

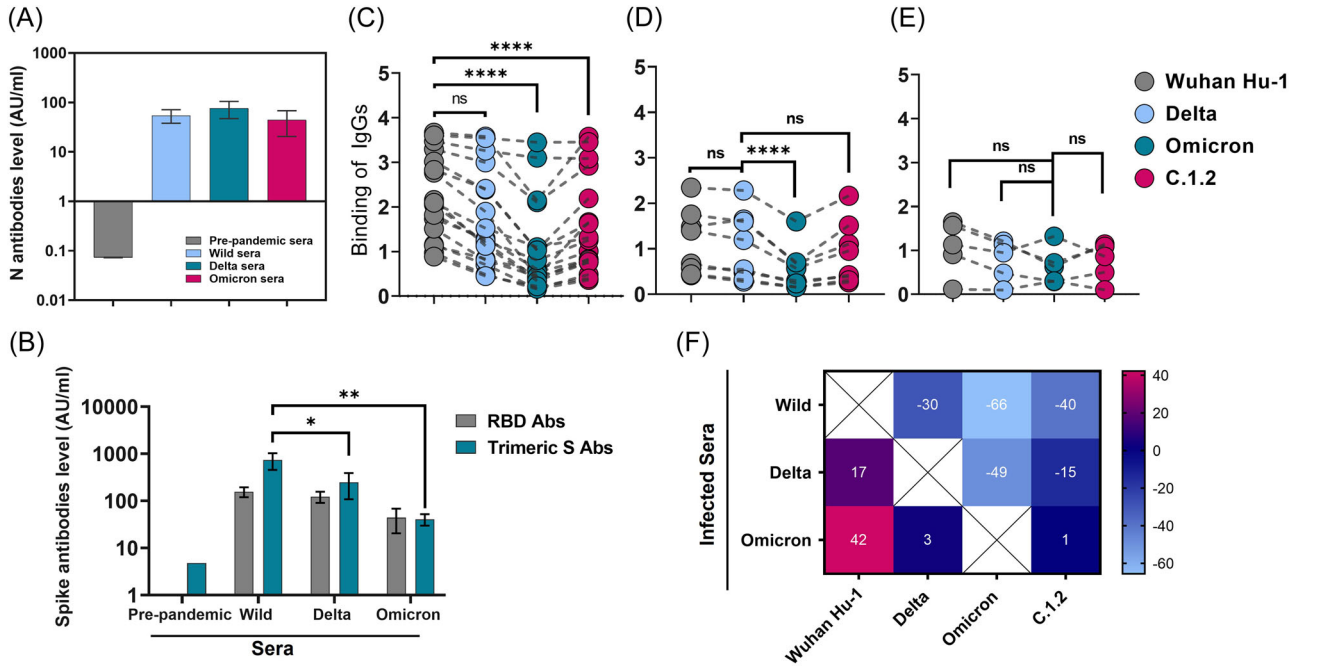

FIGURE 3 Humoral responses induced by Delta and Omicron infection is cross-reactive towards heterologous spike proteins. The level of antibodies against (A) N protein, (B) RBD & Trimeric S protein. Levels of IgG binding towards spike variants in (C) Wild, (D) Delta and (E) Omicron Infected sera. (F) The GM ratio of IgGs binding to the variants (%).

to spike variants. Vaccine+Delta sera and Vaccine+Omicron sera showed similar levels of anti-N antibodies (Figure 4A) but showed higher levels of anti-RBD and anti-Trimeric S when compared with Vaccine sera (Figure 4B). In Vaccine sera, we observed that the percentage GM ratios normalized to Wuhan-Hu-1 spike showed reduced relative binding to Delta (13%), Omicron (54%, $p < 0.0001$), and C.1.2 (34%, $p = 0.0065$) (Figure 4C,F). Among Vaccine+Delta sera, the percent GM ratios normalized to Delta spike showed a reduction towards spike protein of Omicron (36%, $p < 0.0001$) and C.1.2 (9%) and marginal increased binding to Wuhan-Hu-1 spike (8%) (Figure 4D,F). Surprisingly, we observed that the percent GM ratio of IgG binding in Vaccine+Omicron sera showed an increased binding towards heterologous spike proteins of Wuhan-Hu-1 (1%, $p = 0.0221$), Delta (11%, $p = 0.0402$) and C.1.2 (10%) compared to homologous spike (Omicron) (Figure 4C,F). These findings suggest that Omicron breakthrough infections after vaccination boosts spike humoral immunity, with an increasing breadth of cross-reactivity and higher levels of binding antibodies to spike proteins of heterologous predecessor variants.

3.4 | Omicron vaccine breakthrough infection induces robust IgG responses against heterologous variants compared with primary infection

To summarize the impact of breakthrough infection, we compared levels of spike (anti-RBD and anti-Trimeric S) antibodies and GM ratio of antibody binding to spike variants between the following groups: Wild vs Vaccine and breakthrough infection groups (Vaccine+Delta vs Delta, Vaccine+Omicron vs Omicron) (Figures 5A,B and S2). Compared with Wild sera, Vaccine sera showed higher anti-RBD (1.5x), but lower anti-Trimeric S (2.5x-fold) antibodies level with similar antibody binding against spike variants ($p > 0.05$). Compared with Delta sera, breakthrough Delta infection (Vaccine+Delta) showed an increase in antibody level and higher binding to all spike proteins (range 2.5x–3.3x). However, Omicron breakthrough (Vaccine+Omicron) caused elevated spike antibody levels (35x) and increased level of antibody interaction to all heterologous variant proteins (range from 4.2x–6.5x) indicating that Omicron breakthrough infections increase absolute antibody levels (depth) and binding towards all spike variants (breadth) of antibody response.

4 | DISCUSSION

In this study, we probed postinfection (including vaccine breakthrough) and postvaccination archival sera to decipher the impact of the SARS-CoV-2 Wild (Wuhan-Hu-1 and D614G mutant), VOCs-Delta (B.1.617.2) and Omicron (B.1.1.529) on the levels of anti-spike antibodies and variant-specific antibody interactions. In our study, we found that both primary and breakthrough infection with Omicron resulted in an increased IgG antibody binding towards heterologous predecessor variants. Breakthrough infections with Omicron increased the depth and breadth of antibody response to previous tested variants of SARS-CoV-2.

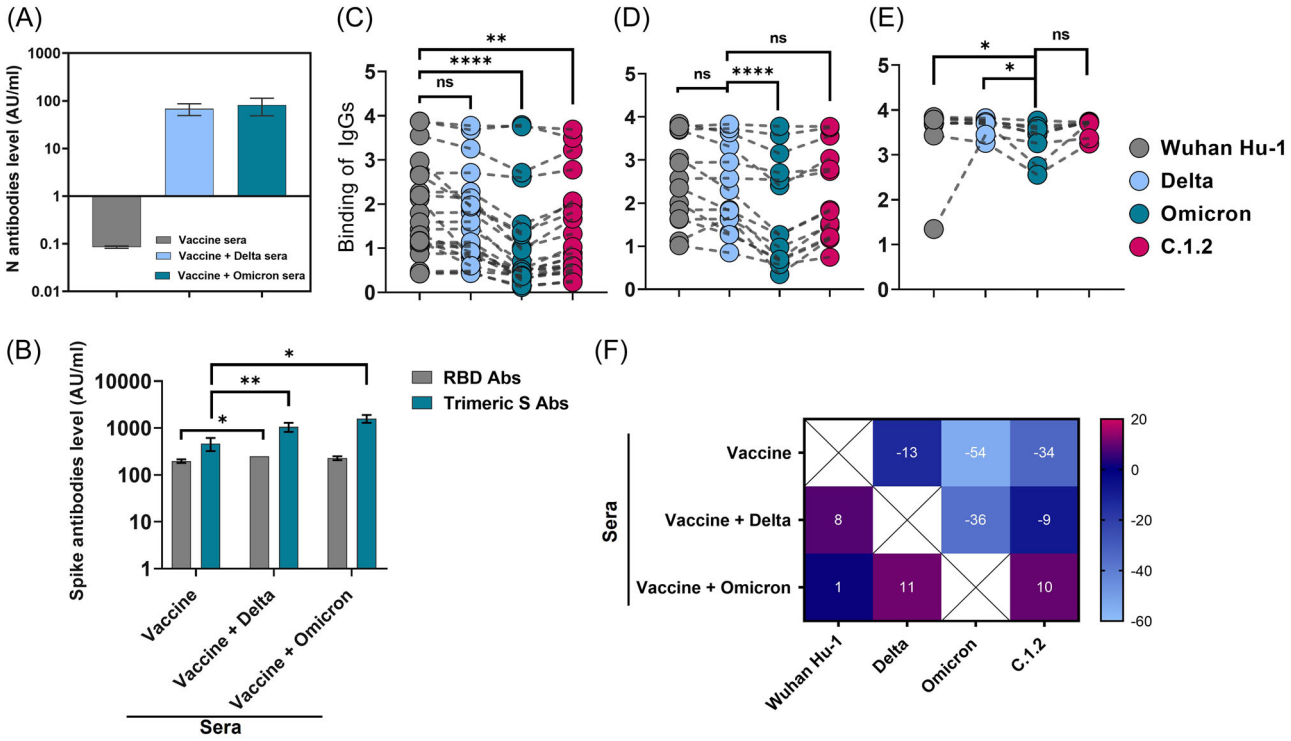

FIGURE 4 Delta and Omicron infections elicited differential immune imprinting in vaccinated individuals. Level of (A) anti-N, (B) anti-RBD & anti-Trimeric S antibodies in serum groups. The IgG antibody binding towards spike variants in (C) vaccinated, breakthrough infections including (D) Vaccine+Delta and (E) Vaccine+Omicron infected sera, (F) the GM ratio between the spike variants to the type of infection (%).

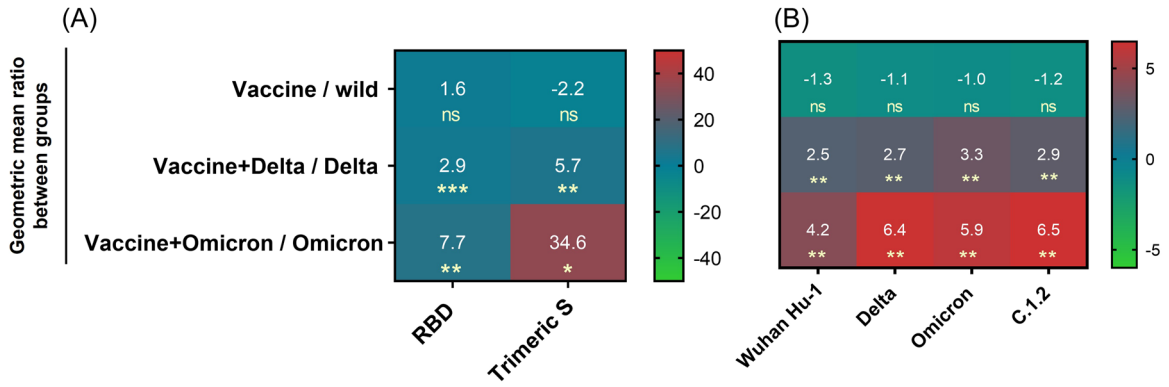

FIGURE 5 Differences in the levels and cross-reactivity of humoral responses in primary and breakthrough infections. Geometric mean ratio of (A) level of antibodies against RBD & Trimeric S and (B) Binding of IgGs against spike variants, between indicated groups.

4.1 | Primary infection with Omicron induces a lower level but cross-reactive antibody response

The antibody to spike for Wild sera demonstrated decreasing binding towards heterologous spike proteins of Delta, Omicron, and C.1.2. However, it was noted that postinfection sera of 'succession' variants (Delta or Omicron) uniformly displayed high binding towards the ancestral spike (Wuhan-Hu-1). Spike protein of Omicron (BA.1 and BA.2) are antigenically dissimilar compared to spike protein of Wild and other reported VOCs due to high antigenic distance.[26] We also found similar results in concurrence with the existing findings (Figure 3F). Molecular studies have shown demonstrable immune escape of SARS-CoV-2 variants due to unique mutations in N-terminal domain (NTD) and RBD of spike,[13,20,27] causing alteration in epitopes or shield the antibody binding site by glycosylation.[4] Omicron with the highest number of spike mutations (23 mutations) compared with C.1.2 (8 mutations) and Delta (6 mutations) in NTD and RBD, leads to diminished binding of "ancestral" Wuhan-Hu-1 strain-induced antibodies (immune escape). We found that the levels of

antibodies induced by Omicron were lower than the Wuhan-Hu-1 strain (Figure 2B). A lower antibody level can be attributed to Omicron, as such, being less "antigenic," resulting in a poorer homotypic immune response.[28]

4.2 | Breakthrough infection among vaccinated

An effect similar to "Wild sera" (Figure 3C), was observed in "Vaccine sera" (Figure 4C), as all vaccines used globally are based on the Wuhan-Hu-1 spike. Delta and Omicron strains have been shown to evade vaccine-induced immunity and cause breakthrough infections.[13,29–32] In Delta breakthrough, a preferential boosting of ancestral (Wuhan-Hu-1) and homologous (Delta) immunity was seen but not to successor (Omicron) variants. With Omicron breakthrough sera, increased binding was noted to the Wuhan-Hu-1 and Delta than Omicron possible due to activation of memory B cells producing antibodies against epitopes common to vaccine strain and other previous VOCs.[8,12,24] A reduced interaction compared to ancestral strains is a further indication of a reduced "antigenicity" of Omicron, as noted above. The spike IgGs elicited by Omicron primary infection and vaccine breakthrough are able to cross-react with the spike of all ancestral variants tested in this study, consistent with previous reports.[12,24,33] This study confirms a significant increase in specific IgGs binding heterologous and predecessor spike proteins of Wuhan-Hu-1 and Delta relative to Omicron spike during Omicron breakthrough.

In concurrence with previous reports,[33] our findings also demonstrated that the level of antibodies (anti-RBD and anti-Trimeric S) induced by Omicron breakthrough infection were raised for Delta (3-fold for RBD and 6-fold for Trimeric S) and maximum for Omicron (8-fold for RBD and 35-fold for Trimeric S), compared to primary Omicron infection. This observed effect is characteristic of hybrid immunity, where the presence of immune memory acquired by either infection or vaccination, results in a robust response on secondary exposure. Delta or Omicron breakthrough infections following vaccination produced a heightened humoral response against RBD and Trimeric S compared to vaccination alone, consistent with several studies.[12,22,33] Hybrid immunity due to Omicron breakthrough infection also results in cross-variant neutralizing immunity covering VOCs.[24,34] Recent studies have identified the generation of broad cross-reactive antibodies as either immune imprinting due to conserved epitope or change in the glycosylation patterns across variants of spike protein.[8,12,35] Our study provides evidence of the quantum and heterologous reactivity towards spike variants of Omicron vaccine breakthrough sera in an Indian context. This may have implication for emergence of newer/novel lineages in the future. The emergence of Omicron and boosting of population-level immunity to previous circulating variants, effectively minimizes the chance of emergence of a new variant from unsampled virus diversity, particularly in areas with continued virus circulation and poor vaccine coverage/uptake. This data supports the continued use of vaccination as hybrid immunity would dampen the circulation of multiple VOCs. A large-scale epidemic with newer sub-lineages has not emerged in India (BA.4/BA.5. or BA.2.12.1/BA.2.75) after BA.1/BA.2 emergence in January 2022. This is likely the effect of high vaccine coverage and hybrid immunity. The impact of hybrid immunity on novel recombinant forms has not yet been studied.

4.3 | LIMITATIONS OF THE STUDY

Our sampling timeframe covers over 27 months of the pandemic. As this was a cross-sectional study, it is very likely undocumented infection as well as waning immunity to either infection or vaccination would have resulted in a few misclassifications between groups. However, this has been minimized by the use of multiple parameters (PCR, sequencing as well as antibody estimation to both N and S) to classify groups. A follow-up cohort with temporal sampling would have provided a clearer interpretation of infection. Previous studies describing the phenomenon of immune escape and imprinting have been conducted in postvaccinal sera[13,22] but without documented infection history. To our knowledge, this is the first study to be comprehensively probe naturally-infected and breakthrough infections to decipher variant-specific immune profiles.

AUTHOR CONTRIBUTIONS

Gokulnath Mahalingam, Mahesh Moorthy, and Srujan Marepally contributed to the conceptualization of the article. Gokulnath Mahalingam, Ramya K. T. Devi, Yogapriya Periyasami, Porkizhi Arjunan, Rajesh Kumar, Roshlin Susan Mathew, and Tamil Venthan Mathivanan contributed to the methodology of the article. Gokulnath Mahalingam, contributed to interpretation, and visualization of data. Mahesh Moorthy, and Srujan Marepally supervised the investigation. Gokulnath Mahalingam, Mahesh Moorthy, and Srujan Marepally contributed to manuscript preparation. Gokulnath Mahalingam, Ramya K. T. Devi, Prasanna Samuel Premkumar and Mahesh Moorthy contributed to statistical analysis of the data. Gokulnath Mahalingam, Alok Srivastava, Jayaprakash Muliyil, Mahesh Moorthy, and Srujan Marepally contributed to reviewing and editing the manuscript. All authors had access to the data. Gokulnath Mahalingam, Mahesh Moorthy, and Srujan Marepally accessed the original data and vouch for its authenticity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Original data can be accessed upon request.

ETHICAL APPROVAL

The Institutional Review Board of Christian Medical College, Vellore (IRB Nos. 12917&12691).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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