

# Effect of age and prior COVID-19 on BBIBP-CorV (Sinopharm) vaccination- induced antibody responses in a region with high seroprevalence

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## Research Article

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# Abstract

## Introduction

Sinopharm (BBIBP-CorV) inactivated virus vaccination for COVID-19 has been administered widely in Pakistan. We investigated the dynamics of BBIBP-CorV -induced antibody responses over a 24 week period in a region with a high seroprevalence.

## Methods

Study subjects (n = 312) were followed up over a 24-week period between May and August 2021. Sera were tested for IgG antibodies to spike and the receptor binding domain (RBD).

## Results

Study subjects were 62% female. Twenty-two percent had a prior history of COVID-19. At 4-, 8- 16- and 24-weeks post-vaccination, the rate of IgG antibodies positive to spike was 57%, 87%, 66% and 90% of individuals, compared with to RBD which was 48%, 62%, 68% and 85% of subjects, respectively. IgG to spike and RBD showed a positive correlation at each interval ( $\rho > 0.6$ ,  $p < 0.0001$ ). Seropositivity to both spike and RBD was reduced in those aged 50 years and over for up until 16 weeks post-vaccination ( $p < 0.05$ ). Individuals with prior COVID-19 infection showed greater antibody responses for up to 16 weeks post-vaccination ( $p < 0.05$ ). SARS-CoV-2 infections were observed with a mean interval of 16 weeks post-vaccination. Antibody responses did not wane for up to 6 months post-vaccination.

## Conclusions

Sinopharm vaccination-induced antibody responses were negatively impacted by age and positively impacted by prior COVID-19 for 16 weeks after vaccination. Importantly, we did not find waning of IgG antibodies to RBD over the study period. Maintenance of antibodies may be the result of continued community exposure and boosting with COVID-19 vaccination.

## Introduction

COVID-19 has resulted in over 6.3 million deaths globally as a consequence of more than 536 million infections (15 June 2022), [1]. COVID-19Vaccination against COVID-19 was first introduced at the start of 2021 and was rolled out in stages based on both access and availability in different populations. The vaccines included formulations based on mRNA expression of spike protein, adenovirus vector-based vaccines, and inactivated vaccine types [2]. The vaccinations have had a major impact on controlling both morbidity and mortality from COVID-19 [3, 4], leading to what in 2022 is thought to be the tail end of the pandemic [5].

Case fatality rates (CFR) from COVID-19 have varied greatly between populations. Pakistan amongst other South Asian countries has so far fared relatively better than other countries in the region and globally well with regard to COVID-19 related morbidity and mortality [6]. In a country of over 200 million, there are 30,000 deaths reported due to COVID-19 (15 June 2022) [7]. Pakistan has a young population, with 64% of individuals aged under 30 years of age [8]. Younger age has been associated with favorable outcomes in COVID-19 [9]. However, the reasons for differential CFRs between countries and regions are unclear but have been related to life expectancy, population pyramids, potential exposure to other coronaviruses, and possible innate immunity [6].

Worldwide about 12 billion doses of COVID-19 vaccinations have been administered with 66% of the global population having received one dose [10]. However, only 18% of individuals in low-income countries have received at least one dose of the COVID-19 vaccine (15 June 2022)[10]. Most of the studies on vaccine efficacy and immunological responses are based on the analysis of populations vaccinated by mRNA type (Pfizer-BNT162b2-, Moderna mRNA-1273) and vector-based vaccines (ChAdOx nCoV-19). There is limited data on inactivated vaccines such as those administered in many low-middle income countries. The vaccine, Sinopharm (inactivated) prepared by Beijing Bio-Institute of Biological Products Co-Ltd. (BBIBP) also called BBIBP-CorV (Vero cells) is administered in two doses, given four weeks apart [11]. Globally, 680 million doses of Sinopharm have been delivered to 79 countries worldwide (15 June 2022) [12].

In Pakistan, 136 million individuals (62%) of the population have received their first dose of vaccination, with 124 million (55%) individuals fully vaccinated (15 June 2022) [7]. Vaccinations were rolled out in February 2021 as emergency use authorization (EUA) was obtained with BBIBP-CorV as one of the primary vaccines administered [13]. Vaccinations were given in a stratified manner first, to health care workers and then to older age groups in the country.

The spike protein of SARS-CoV-2 is highly immunogenic [20] and antibodies to spike protein are associated with protective immunity against the virus [21]. Information regarding immune response and subsequent protection from inactivated vaccines such as BBIBP-CorV is limited [14, 15]. A recent study from Pakistan has shown that full vaccination with BBIBP-CorV was effective in reducing the risk of symptomatic COVID-19 infection (94.3%), hospitalisations (60.5%) and mortality (98.6%), respectively [16]. Studies on immune response induced by BBIBP-CorV have shown differing results. In Sri Lanka, more than 90% seropositivity was observed was seen that 6 weeks post-vaccination [15, 17]. A study from the United Arab Emirates showed seropositivity of 78% in the general population after full vaccination [18], with a similar result shown in a report from Pakistan [19].

It is particularly important to have data from high infectious disease burden regions with limited COVID-19 vaccine access and where primarily, inactivated vaccines were administered as humoral responses against SARS-CoV-2 are driven both by natural infection and COVID-19 vaccinations [20–23]. High seropositivity rates following natural infection have been associated with high population densities and rapid transmission resulting in high rates of infection [17]. In early 2021, we observed COVID-19

antibodies to spike to be present in greater than 50% of unvaccinated healthy blood donors [24]. A study from Karachi showed average seropositivity of 36% between April and July 2020, varying between industrial employees (50%), community (34%), and healthcare workers (13%) [18]. General population based serial serosurveys for COVID-19 in Karachi showed a rise in prevalence rates to 22% by August 2020 [19].

Vaccinations were first administered in February 2021 at the Aga Khan University Hospital after it was designated as an Adult Vaccination Center by the Department of Health, Government of Sindh. In this study, we have investigated the dynamics of BBIBP-CorV -induced IgG responses to spike and receptor binding domain (RBD) proteins in a healthcare associated cohort who were followed as part of a seroprevalence study in Karachi, Pakistan [25]. SARS-CoV-2 reactive antibodies were determined in the vaccinees followed up at regular intervals for 24 weeks post-vaccination. We measured IgG to both spike and RBD protein to investigate immunity against SARS-CoV-2 driven by BBIBP-CorV vaccination. The results were analyzed in the context of age, sex and prior history of COVID-19 infection.

## Methods

This study was approved by the Ethical Review Committee of The Aga Khan University (projects #2020-5152-11688).

## Study subjects

This study builds on a previously conducted seroprevalence study conducted at Aga Khan University where we studied antibody responses to spike and RBD in COVID-19 patients and a healthy uninfected healthcare associated cohort [26]. We recruited adult study subjects aged over 18 years with written informed consent. Ninety percent had received their first dose of BBIPP-CorV vaccination between February and March 2021. BBIBP-CorV vaccination was administered as per guidelines of the National Covid Operation and Command (NCOC), Government of Pakistan [7]. The vaccine route was an intramuscular injection in the deltoid area. The time interval between the first and second doses of Sinopharm/BBIBP-CorV was four weeks as per manufacturer's recommendations [11]. Subjects were recruited by a consecutive convenience sampling method. They included healthcare workers, Aga Khan University (AKU) employees, and their family members who volunteered to participate in the study. A verbal history of prior laboratory-confirmed COVID-19 infection based on either a PCR confirmed SARS-CoV-2 test or positive a COVID-19 antibody test result was taken at the time of recruitment. There was no preferential selection of cases based on any prior COVID-19 history.

During the study period, active surveillance of infection was not performed. However, subjects were encouraged to inform the study team if they developed laboratory-confirmed COVID-19 infection post-vaccination.

Participants were requested to give blood samples for up to four time points during the study. Sampling was conducted at monthly intervals which was calculated based on the date of the first dose of BBIBP-

CorV administered (Fig. 1).

## Sample collection

Three millilitres of whole blood was collected in a serum collection tube. Serum was separated from blood and stored at  $-80^{\circ}\text{C}$

## ELISA for IgG to Spike and RBD

Recombinant spike and RBD protein were obtained from iBET/ITQB, NOVA University, Portugal. All serum samples were tested in duplicate using an in-house enzyme-linked immunosorbent assay (ELISA)[26] and as per the protocol described by Figueiredo-Campos *et al.* [21]. Briefly, SARS-CoV-2 Spike and/or RBD protein were used to coat plates with 50 $\mu\text{l}$  of Spike or RBD protein at a concentration of 2  $\mu\text{g}/\text{ml}$  in PBS. Wells were blocked and then incubated with 100  $\mu\text{l}$  serum samples for 2 hours. Wells were washed and stained with secondary antibody conjugated with Horse Radish Peroxidase (HRP). Plates were read for color intensity as optical density units (OD) at 450nm using an Elisa reader. A serum pool containing high titers of IgG antibodies to SARS-CoV-2 proteins was used to develop a calibration curve with end to end titration. This was used to establish a positive cut-off value at 0.5 at 450 nm for both Spike and RBD. Duplicate samples of pooled positive IgG sera at each dilution were assessed to check for intra- and inter-plate variability (Supplementary Figs. 1–2).

## Comparison with commercial assay

Twenty-five randomly selected serum samples were also tested for antibodies using the LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy as per manufacturer's instructions for comparative analysis. Manufacturer recommended interpretation of results: Negative ( $< 12.0$  AU/mL), Equivocal (12.0–15.0 AU/mL) and Positive ( $> 15.0$  AU/mL).

## Statistical analysis

The Statistical Package for the Social Sciences version 24.0 (SPSS Inc. Chicago, IL, USA, 2013) was used to carry out descriptive statistics of 312 participants for demographic variables (gender, age). In addition, health and chronic conditions, history of previous COVID-19 infection, were analysed. The normality of data was checked through Shapiro-Wilk test; mean  $\pm$  SD was used to describe normally distributed and median (IQR) for skewed continuous data. Data are presented in both frequencies and percentages. Chi-square test was used to compare the frequencies of IgG antibodies with respect to age groups (either less than 50 years or those 50 years and above), gender and COVID-19 infection as variables. The threshold of significance was a p-value  $< 0.05$ .

Correlation between the RBD titers and neutralizing potential was determined using the Spearman's rank correlation test using the GraphPad prism. A p value  $< 0.05$  was considered as significant.

## Results

### *Description of study*

We recruited 312 individuals who had received the BBIBP-CorV vaccine. Vaccinations were first rolled out on an urgent basis in February 2021 [13] and due to the time taken for regulatory approvals of this study, four weeks post-vaccination was the earliest timed sample had for cases. However, the study population here was similar, with some overlap, to that in our earlier seroprevalence study where we observed earlier whereby unvaccinated, uninfected study subjects in a healthcare associated cohort tested between October 2020 and May 2021 were found to have a 35% seropositivity to spike and 21% seropositivity to RBD antigen [25]. Therefore, we presume that the baseline antibody responses to spike and RBD in our study subjects prior to vaccination with BBIBP-CorV would be similar to that observed earlier for the comparable group.

We recruited a total of 312 participants with a total of 752 blood samples collected for analysis. There was a gradual loss to follow up after 8 weeks. The age range of study subjects was 20 to 101 years with a mean age of  $40.7 \pm 16.5$  years, Table 1. Seventy-four percent of subjects (n=231) were aged 50 years or lower, with 26% (n=81) aged greater than 50 years old. Sixty-three percent of individuals were females. Eighty-nine individuals (29%) had a history of COVID-19. Of these, 68 (22%) had COVID-19 prior to enrollment in the study; median period 23 weeks prior to enrollment (range 3 – 56 weeks). Twenty-one (6.7%) individuals suffered COVID-19 for the first time after full vaccination and during the recruitment period. Of note, the age of individuals who had COVID-19 before the study was significantly lower than those who got COVID-19 post-vaccination ( $p=0.0039$ ). We did not find any difference between sex and the age groups of individuals who had a history of COVID-19 infection (either before enrollment in the study or in those who suffered COVID-19 after vaccination).

The number of serial samples given by each of the 312 individuals varied (Fig. 1). Most participants (71%) submitted samples 8 weeks after the first dose of their vaccination (hence two weeks after full vaccination). Subsequently, for 58% the sample was available after 16 weeks of vaccination. We saw a further loss in follow-up especially by 24 weeks when only 44% of the total cohort submitted a serum sample for testing.

### ***Dynamics of IgG Antibody responses to Spike after BBIBP-CorV vaccination***

To understand the IgG responses further, we compared the levels of IgG antibodies in sera sampled over the 24 week period. Overall, there was an increasing trend in antibody levels with time after vaccination,  $p<0.0001$  (Fig. 2A). Between eight and twenty-four weeks we observed a biphasic response whereby there was a reduction in antibody levels between 8 and 16 weeks ( $p<0.0001$ ). This was followed by an increase in antibody levels by twenty weeks post-vaccination ( $p<0.02$ ). No difference in levels of spike IgG was observed between those measured at 20 and 24 weeks post-vaccination.

A similar trend of IgG antibodies to spike was noted in the context of seropositivity of individuals. The proportion of individuals with seropositivity to spike increased from 57% at 4 weeks up to 87% at 8 weeks, some reduction to 66% at 16 weeks followed by an increase to 82% seropositivity at 20 weeks and 90% by 24 weeks post-vaccination (Fig. 2B).

### ***Dynamics of IgG antibodies to RBD after BBIBP-CorV vaccination***

IgG antibodies to RBD in the vaccinated study cohort were determined as a surrogate marker of neutralizing activity against SARS-CoV-2. IgG antibody levels to RBD were found to progressively increase between 8 and 24 weeks after vaccination,  $p < 0.0001$  (Fig. 3A). IgG antibody levels were higher at 16 weeks ( $p = 0.036$ ), 20 weeks ( $p < 0.0002$ ), and 24 weeks ( $p < 0.0001$ ) as compared with 8 weeks post-vaccination. We did not observe any decay in IgG antibodies to RBD over this period.

There was an increasing trend of IgG seropositivity to RBD; increasing from 48% after 4 weeks, 61% and 62% at 8 and 12 weeks, 68% and 73% at 16 and 20 weeks. By 24 weeks after BBIBP-CorV vaccination, 85% of study subjects having a positive IgG response to RBD (Fig. 3B).

### ***Relationship between IgG antibodies levels to spike and RBD***

The differing trend in early BBIBP-CorV vaccination-induced IgG responses to spike and RBD was examined at different time intervals. IgG antibody levels to spike were significantly greater than those to RBD at 4 and 8 weeks after vaccination (MWU;  $p < 0.024$ ,  $p < 0.001$ , respectively, Fig. 4A-B). At subsequent time intervals, there was no difference found between antibody levels to spike and RBD (Fig. 4C-F). Spearman's rank correlation analysis between IgG to spike and RBD was found to be positive at each of the time intervals post-vaccination (4, 8, 12, 16, 20, and 24 weeks; Supplementary Fig. 3). However, it was evident that the strength of positive correlation between IgG to spike and RBD binding increased over the time post-vaccination, from week 4 (SR  $\rho = 0.88$ ,  $p < 0.0001$ , Sup Fig 3A) until week 24, post-vaccination (SR  $\rho = 0.92$ ,  $p < 0.0001$ , Supplementary Fig. 3F).

### ***Correlating in-house ELISA with commercial IgG antibody testing***

We compared a subset of specimens that has been tested for IgG to RBD in our ELISA assay with a commercial assay that determines IgG to S1 and S2 antigens of SARS-CoV-2. The LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin assay was used for this purpose to test 25 random selected sera, including 5 IgG negative sera and 20 IgG positive sera using in-house ELISA method (Supplementary Table 1). The



results obtained from the DiaSorin showed complete concordance with that obtained by our ELISA assay. These data indicate that the assay had analytical sensitivity and specificity of 100% for identifying neutralizing antibodies to SARS-CoV-2 as compared with the DiaSorin assay.

### ***Impact of age and sex of COVID-19 on antibody responses***

We investigated the impact of age and sex on IgG antibody responses after BBIBP-CorV vaccination. The proportion of study subjects with positive IgG responses was compared into age groups divided into those aged 50 years and under with those aged 50 years and over.

The frequency of individuals with IgG seropositivity to spike was greater in those under the age of 50 years as compared with study subjects 50 years and older, as compared to the group at 8 ( $p<0.001$ ), 12 ( $p<0.001$ ), and 16 ( $p=0.014$ ) weeks after BBIBP-CorV vaccination (Fig. 5A). Similarly, the frequency of those seropositive to RBD at 8 ( $p<0.010$ ), 12 ( $p<0.001$ ), and 16 ( $p=0.002$ ) weeks was greater in those under the age of 50 years as compared with the older age group (Fig. 5B).

We examined whether there was any sex related difference in the seropositivity of IgG to SARS-CoV-2 antigens. We found greater seropositivity of IgG in females than in males measured to both to spike ( $p<0.0001$ ) or RBD ( $p<0.0001$ ) when measured at 12 weeks post-vaccination (Fig. 5 C-D). Notably, at this time interval of 12 weeks post-vaccination, there were relatively more females ( $n=66$ ) as compared with males ( $n=42$ ).

### ***IgG seropositivity in the context of COVID-19***

In total, eighty-nine (29%) of study subjects had confirmed COVID-19 either, either prior to ( $n=68$ ) or after vaccination ( $n=21$ ). The frequency of positive responses to spike after 4 ( $p=0.025$ ), 12 ( $p=0.021$ ), and 16 ( $p=0.002$ ) weeks of vaccination, was greater in those who were vaccinated and also had a history of SARS-CoV-2 infection as compared with those who had received BBIBP-CorV vaccination but did not have a known history of natural infection (Fig. 6A). Similarly, seropositivity to RBD after 4 ( $p=0.004$ ), 12 ( $p=0.02$ ), and 16 ( $p=0.009$ ) weeks of vaccination was greater in those with a history of COVID-19 than in those who did not (Fig. 6B).

We separately examined the IgG levels of vaccinees who had COVID-19 either prior to enrollment or, developed disease during the study period. Sixty-eight study subjects had COVID-19 before enrollment in the study, Table 1. Of these, 63 (92.6%) individuals were aged below 50 years and five (7.4%) were 50 years and above. All vaccinees with prior COVID-19 had a positive IgG response to spike and RBD at the

time they were enrolled in this study. None of them developed COVID-19 a second time after vaccination and during the follow-up period of the study.

Further, 21 study subjects developed COVID-19 after vaccination and enrollment in the study.

The average time interval of developing COVID-19 post-vaccination was 16 (4.2) weeks after the first dose of BBIBP-CorV vaccine. Sixty-seven percent of COVID-19 post-vaccination cases developed it after 16 weeks of first vaccination (Supplementary Fig. 4).

Thirteen (62%) participants were below 50 years, with eight (18%) aged 50 years and above. Assessing earlier data for IgG to RBD, at enrollment, of those who suffered COVID-19 after vaccination we found that, 11 (52%) were negative for IgG to RBD; measured at 4 weeks (n=2) and 8 weeks (n= 9) after vaccination. All of these twenty-one individuals displayed a positive IgG response to RBD after developing COVID-19.

## Discussion

We report BBIBP-CorV -induced dynamics of IgG antibody responses to spike and RBD over a 24 week period in a population with a high baseline seroprevalence. Our study subject were adults otherwise healthy without any significant co-morbid conditions. At that time, SARS-CoV-2 alpha variants had been introduced in Pakistan [27]. The exposure period during the six month follow-up of this study was through September 2021, co-coincident with a fourth wave of the pandemic which was mainly caused by delta variants [28].

We observed a significant trend towards increased IgG antibodies to spike ( $p < 0.0001$ ) and RBD ( $p < 0.0001$ ) between four and twenty-four weeks after vaccination with BBIBP-CorV. After four weeks of receiving the first dose of BBIBP-CorV, 57% of individuals had a positive IgG response to spike and this rose to 87% at 8 weeks, followed by a reduction by 16 weeks, increase to 82% and finally a 90% seropositivity to spike was observed by 24 weeks after vaccination. The dynamics of IgG antibodies to RBD differed and a slower by continual increase in seropositivity was observed such that at 4 weeks, 48% of individuals were seropositive rising to 84.5% by twenty-four weeks.

The differences in the dynamics of IgG responses to spike and RBD may lie in differences of recognition of epitopes in the two antigens. IgG antibodies to spike may be to a broader spectrum of cross-reactive epitopes which would explain higher levels as well to Spike compared to RBD, and the dip may indicate a shift of antibody responses towards a more defined set of epitopes.

We found that the relative levels of IgG antibodies were higher to spike as compared to RBD at both four- and eight- weeks post-vaccination, likely reflective of the increased number of reactive antibody epitopes present in the larger protein as compared with its RBD sub-unit.

Also, differences in spike and RBD responses may depend on epitopes being recognized by the individuals, that is, T independent or (via Pathogen Associated Molecular Patterns) or T dependent epitopes with finer specificity.

Importantly, IgG to spike and RBD showed a significant positive correlation at each of the time intervals studied from 4 until 24 weeks, with an increasing significance and rho value. The increasing strength of correlation is probably induced with the increasing level of antibodies driven by vaccination over the period of time. IgG to RBD is associated with neutralizing activity to SARS-CoV-2 as a measure of successful COVID-19 vaccination [29]. In this respect our ELISA assay was in complete concordance with a commercial assay which measures response to specific chains of RBD and that with IgG RBD we were detecting protective antibodies.

We found seropositivity to spike was 87.3% and that to RBD was 61.5% at 8 weeks post-vaccination, a time associated with full vaccination. Therefore, IgG to RBD was absent in the 39.5% of all study subjects after 8 weeks of with BBIBP-CorV. Our data differ from those reported in a study from Sri Lanka by Jeewandara *et al.* of BBIBP-CorV vaccinated group at 0, 4 and 6 weeks, which reported 95% seroconversion at 6 weeks and also found these associated with neutralizing activity to SARS-CoV-2 [30]. However, it correlates with the lower [29] antibody responses to BBIBP-CorV measured (50%) in dialysis patients in the UAE measured at 6 weeks of vaccination [18]. It also correlates with data from Pakistan that showed 78% of antibody responses to RBD in individuals vaccinated with BBIBP-CorV [19].

The differential dynamics of IgG to spike and RBD may have significance in the context of quality of antibody responses produced by the vaccination. IgG to RBD are associated with neutralizing activity [29] [31]. BNT162b2 mRNA and ChAdOx vaccinations have been shown to effectively induce IgG antibodies to spike and neutralizing antibodies, within 14 days of the second dose, or full vaccination [29, 32]. The slower rise in BBIBP-CorV -induced IgG to RBD we observed correlates with earlier reports [15].

BBIBP-CorV vaccination-induced IgG seropositivity to spike followed the same trend in those aged below 50 years as compared with those aged 50 years and above. However, the levels of seropositivity were significantly higher in the younger as compared with the other age group between 8 and 16 weeks after BBIBP-CorV vaccination. Analysis of induced IgG antibodies to RBD further showed that study subjects aged less than 50 years of age had a more dynamic response than those who were older. These data correlate with those reported by Ferenci *et al.* from Hungary who showed that RBD-specific antibody responses after 2 doses of BBIBP-CorV were present in 90% of cases below 50 years but were reduced in those who were older [33]. BBIBP-CorV vaccination data from Sri Lanka also shows reduced immune responses in individuals aged 60 years and above [30].

A likely explanation could be that during the early weeks post-vaccination, there may be a T independent B cell expansion (TI) and therefore similar IgG responses are observed in both age groups. Subsequently, in the younger age group (< 50 years) T cells activation occurs earlier, leading to a peak antibody response between 12 and 16 weeks, followed by a plateau after this time point. T follicular helper cell independent expansion has been shown to occur in response to SARS-CoV-2 infection in mice, resulting

in high affinity antibodies [34]. Therefore, resident T cells may drive antigen specific B cell expansion. In the older age group, T cell activation is compromised [35] and therefore, the antibody response dramatically drops with removal of antigen antibody complexes. However, the T independent responses continue to produce IgG antibodies as likely indicated by slow rise of IgG antibodies to RBD [31]. Further identification of epitope specificity and IgG subclass antibody determination is required.

We found that seropositivity of IgG to spike and RBD in females was greater than males at 12 weeks after vaccination. A comparison of antibody responses to ChAdOx1 has been shown to induce higher levels in females than males [32]. Sex-specific differences related to COVID-19 have been observed between males and females, with increased COVID-19 morbidity in males. Inflammatory cytokine levels shown to be raised in females than males [36–38].

Those with a history of COVID-19 displayed significantly greater higher antibody seropositivity than those who did not for up to 16 weeks after vaccination. This correlates with reports by Aijaz *et al* who observed that seropositivity rates from natural infection were greater than those induced by Sinopharm vaccination [19]. Antibody positivity protects against SARS-CoV-2 reinfection for at least 7 months [39]. The IgG response to natural infection by SARS-CoV-2 is thought to last up to 10 months or more [21, 40]. Difference in dynamics of IgG responses with and without prior COVID-19 may be due to the re-activation of T cell responses present due to natural infection [41, 42], with cellular immunity activating B cell class switching leading to increased IgG production.

Our study has some limitations. The vaccination rollout was rapid and on an emergency basis in our study population, healthcare worker and older age group populations. Due to the time taken for regulatory and ethical approvals for the study at the time of the urgent roll-out of BBIBP-CorV vaccination in Pakistan, we could not unfortunately determine baseline data exactly for the subjects in the BBIBP-CorV vaccine study. However, in our earlier seroprevalence study (conducted March to October 2020), we had observed that seropositivity to spike to be 35% and 21.3% to RBD in our healthy uninfected controls [25]. In this study, many vaccinees belonged to the larger study therefore we can estimate that this could be similar to their baseline antibody levels.

Neutralizing assays against SARS-CoV-2 were not conducted in this cohort. However, we have shown previously that sera samples positive for IgG to RBD had neutralizing activity against SARS-CoV-2 [43]. Therefore, IgG to RBD would be associated with greater protection against disease. We were unable to sample each study subject multiple times during the study period and for some we only have one or two serial samples available. This was due to the reluctance of study subjects to give follow up blood samples. Probably due to the high level of anxiety on the university hospital campus during the period covered by this study, the delta variant surge of 2021.

Vaccine efficacy studies of inactivated types such as, BBIBP-CorV and CoronaVac conducted in China, India and Chile [14, 44, 45], shown that these are effective against preventing hospitalisations and severe COVID-19. In Pakistan, BBIBP-CorV vaccination was shown to reduce hospitalization, mortality and symptomatic COVID-19 in the older age group [16]. We were not able to calculate a vaccine efficacy from

this data set as the accrual time prior to vaccination was greater than the post vaccine. Further, there was the changing impact of the differing rates of exposure through the study period and the absence of a control group.

Importantly, we did not observe any waning of antibodies over the six month study period. This differs from reports of waning humoral immune responses to BNT162b2 over 6 months in Israel demonstrated by decreased IgG levels and neutralizing antibodies over 3 months, accompanied by breakthrough infections [46]. A case control study of vaccine efficacy against SARS-CoV-2 variants using BNT162b2 in Qatar also showed protective antibody levels to decrease after vaccination [47]. A study in Sri Lanka showed a waning of BBIBP-CorV induced antibodies by 12 weeks after vaccination [30]. Here, the absence of antibody waning may be a consequence of continued SARS-CoV-2 exposure in the community, boosting cross-reactive responses. The period studied was coincident with the second and third waves of the COVID-19 pandemic in Pakistan, when alpha and then delta variants were predominant leading to a peak in morbidity and mortality by June 2021 [28]. Incidentally, we observed that the most common interval at which study subjects were infected with SARS-CoV-2 was 16 weeks after vaccination. As most of our vaccinated study subjects were recruited between February and March 2021, the 16 week period afterwards is coincident with the peak of the delta wave of the pandemic in June/July 2021.

## **Conclusions**

Our study provides the important immunological insights as to the effect of inactivated COVID-19 vaccine type, BBIBP-CorV from South Asia. We observed an age-dependent effect with reduced IgG responses in those aged 50 years and over, supporting a role for booster vaccinations in this group. The effect of COVID-19 infection enhancing antibody responses to vaccination were evident. These are likely linked to antibody maintenance observed in our study group post-vaccination, thereby suggesting that vaccination-induced immunity in a high SARS-CoV-2 transmission region may differ and this can impact longer term immunity against disease. Hence, recommendations for COVID-19 vaccinations should be made in the context of local seroprevalence and ongoing transmission in the population.

## **Declarations**

### **Conflicts of interest**

The authors have no potential conflicts of interest to declare.

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## Tables

Table 1. Characteristics of study subjects

Characteristic	n (%)	Females	Males	P value
<b>Age</b>				
All	312 (100)	196 (62.8%)	116 (37.2%)	
≤ 50 years (n, %)	231 (74)	154 (66.7)	77 (33.3)	
>50 years (n, %)	81 (26)	42 (52)	39 (48)	
<b>History of COVID-19</b>				
Yes (n, %)	89 (28.8%)	60	29	
<b>Age (years Mean, SD)</b>				
All	44 (14) y			
COVID-19 pre vaccination	34.4 (10.9) y			P=0.0039
COVID-19 post vaccination	44.2 (13.8) y			
All prior COVID (n, %)	68 (21.8%)	47	21	
<b>Age-wise</b>				
≤ 50 years		40	19	NS
>50 years		7	2	
All PV-CoV	21 (7.1%)	13	8	
<b>Age-wise</b>				
≤ 50 years		4	9	NS
>50 years		4	4	

## Figures

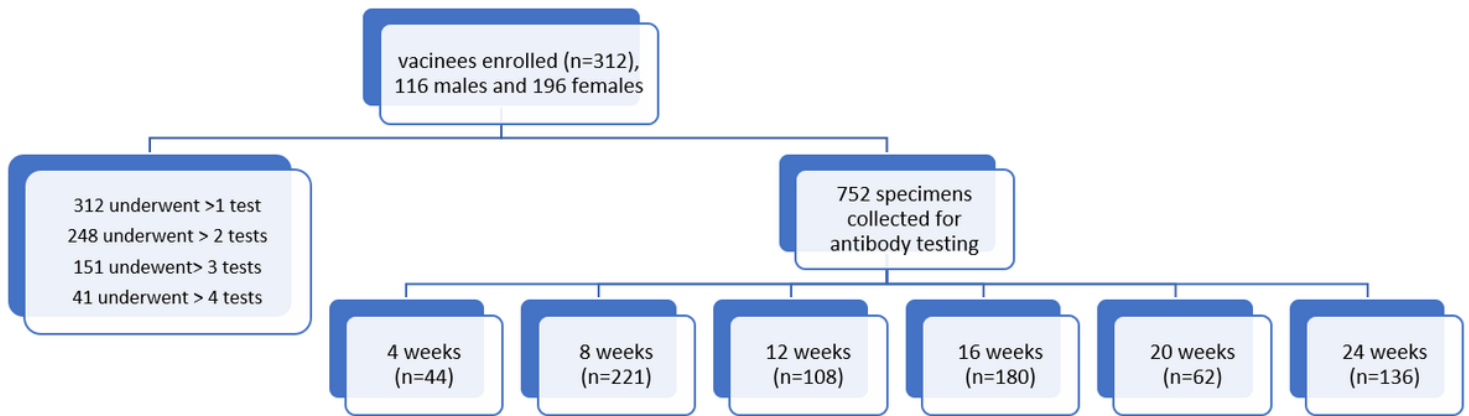


Figure 1

Study timeline and description of BBIBP-CorV/ Sinopharm study

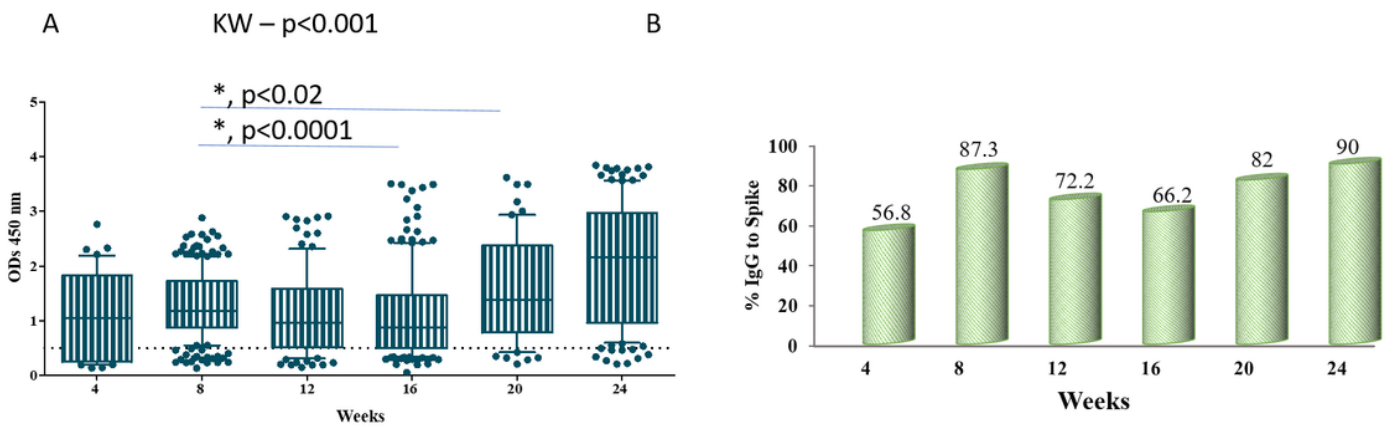
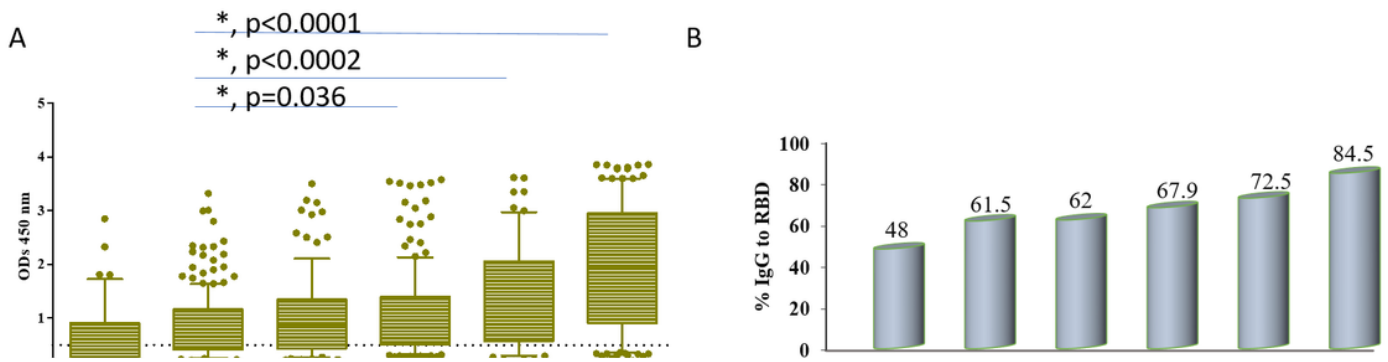


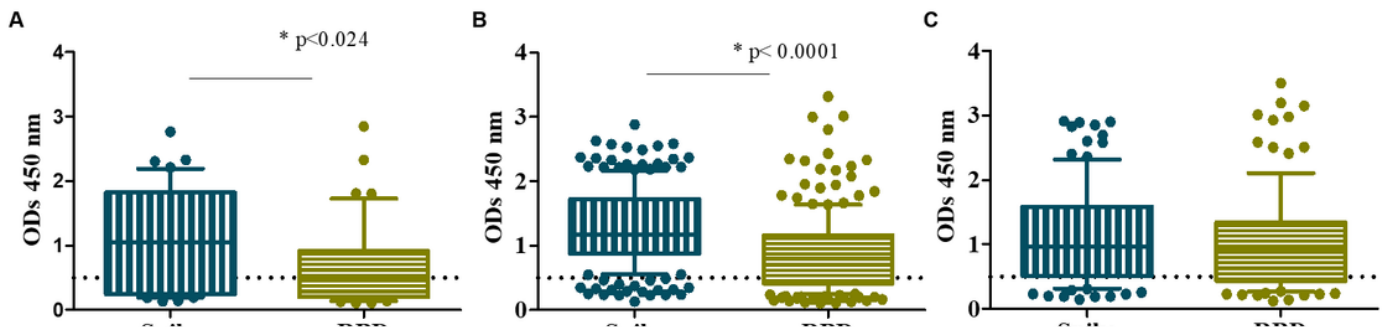
Figure 2

**Dynamics of post-vaccination IgG responses to spike protein.** Sera was collected post vaccination between 4 and 24 weeks. IgG antibodies were tested in each donor. A) shows levels of antibodies, B) shows frequency of positive IgG responses at each time period tested.



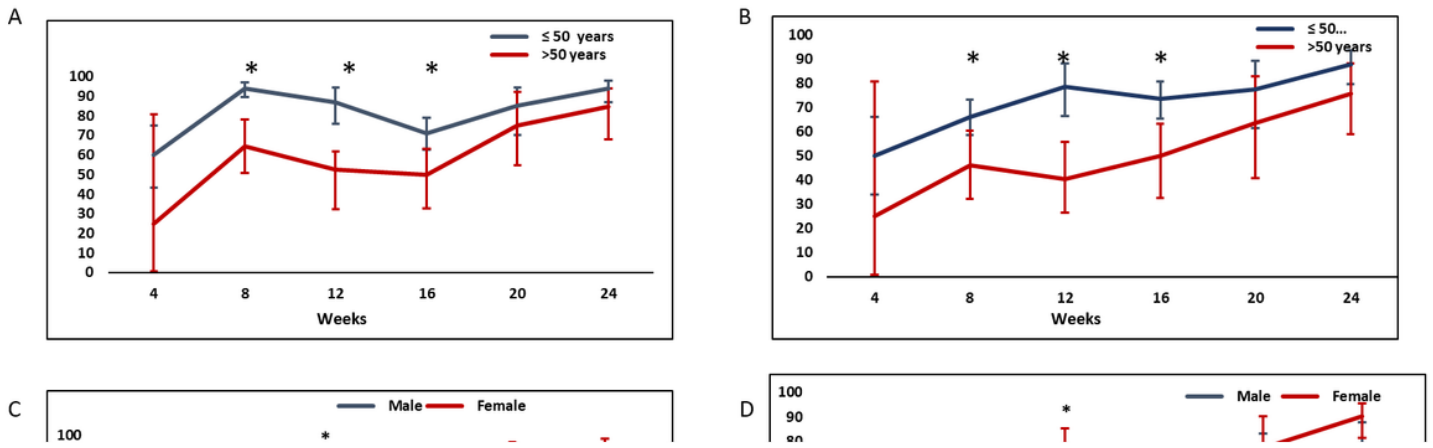
**Figure 3**

**Dynamics of post-vaccination IgG responses to spike protein.** Sera was collected post vaccination between 4 and 24 weeks. IgG antibodies were tested in each case. A) shows levels of antibodies in each case, B) shows frequency of positive IgG responses in each time period tested.



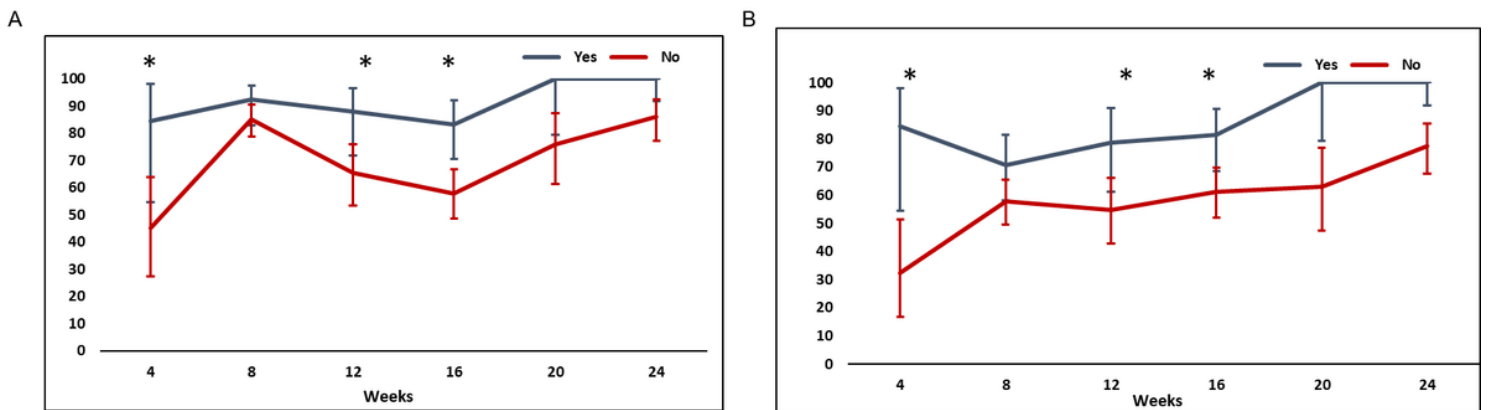
**Figure 4**

**Comparison of spike and RBD IgG recognition after vaccination.** Sera was collected post vaccination between 4 and 24 weeks. IgG antibodies were tested in each donor and graphs depict IgG antibody levels to spike and RBD after A, 4 weeks. B, 8 weeks. C, 12 weeks. D, 16 weeks. E, 20 weeks and F, 24 weeks of first-dose of vaccination. '\*' indicate p values  $< 0.05$  using MWU.



**Figure 5**

**Effect of age and gender on IgG responses to Spike and RBD after vaccination.** Graphs depict the percentage of positive cases with IgG antibodies in each case. Positivity of IgG to based on age cut off 50 years to A) spike and B) RBD. Positivity of IgG based gender in response to C) spike and D) RBD. '\*' indicate p values < 0.05 at each particular time point between groups



**Figure 6**

**Effect of COVID-19 history on IgG responses to Spike and RBD after vaccination.** Graphs depict the percentage of positive cases with IgG antibodies in each case. Positivity of IgG to based on a prior history of COVID-19 for A) spike and B) RBD. '\*' indicate p values < 0.05 at each particular time point between groups

## Supplementary Files

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