Vaccine 59 (2025) 127270

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Comparative study of humoral and cellular immunity against SARS-CoV-2 induced by different COVID-19 vaccine types: Insights into protection against wildtype, Delta and JN.1 omicron strains

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ARTICLE INFO

Keywords:
SARS-CoV-2 variants
Humoral immunity
Mixed-vaccine study
Neutralizing activity
Delta
JN.1
Psuedotyped-viral particles
High infectious disease burden population
T cell immunity
QuantiFERON assay

ABSTRACT

We investigated the effectiveness of different COVID-19 vaccinations administered in Pakistan by studying the effect of inactivated virus, mRNA and vector formulations.

This study in 916 participants was conducted between October 2021 and July 2022. Subjects receiving inactivated (A), mRNA (B), one-dose vector (C), and two-dose vector (D) vaccines were sampled at baseline, 6, 12, and 24 weeks. Serum IgG antibodies to wildtype Spike and its receptor binding domain (RBD) were measured. Pseudovirus particle-based neutralizing assays against wildtype, Delta, and JN.1 variants were performed. T cell IFN-γ responses to SARS-CoV-2 antigens were measured.

Participants were aged 37.05 ± 14.44 years and comprised 48.6 % females. Baseline Spike seropositivity rose from 90 % to 96 % by 24 weeks; and 40 % to 90 % against RBD. Group B participants had the highest anti-RBD levels which peaked by 6 weeks. IgG RBD in group A and C increased up until 24 weeks. Anti-RBD levels were reduced in those over 50 years.

At baseline neutralizing titers were present at 38.5 % against wildtype and in 34.2 % against Delta variants. Titers doubled in vaccine groups A-C by 12 weeks, with highest titers in B and lowest in group C participants. At baseline, neutralizing titers against the JN.1 variant were absent but low titers were evident in 10 % of participants after 12 weeks. T cell reactivity to SARS-CoV-2 increased from 31 % at baseline to 50 % in group A and 73 % in group B participants by 6 weeks after vaccination.

Presence of immunity against wildtype and Delta variants in one-third of participants at baseline could be due to sub-clinical infections. Increase in humoral and cellular immunity was greater after mRNA as compared with inactivated vaccinations. As COVID-19 morbidity in the population remained low, our data supports effectiveness of multiple vaccine formulations in protecting against severe COVID-19 in this high transmission population.

1. Introduction

The COVID-19 pandemic caused 700 million cases and 7 million deaths worldwide from December 2019 until January 2025 [1]. Infections caused by the 2019 novel coronavirus SARS-CoV-2 were driven by mutations conferring distance from early Wuhan S, L/V strains with

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https://doi.org/10.1016/j.vaccine.2025.127270
Received 19 February 2025; Received in revised form 19 April 2025; Accepted 12 May 2025
Available online 22 May 2025

the emergence of D614G variants, followed by variants Alpha, Beta, Gamma, and Delta in 2021 then, Omicron and its sub-variants BA.1, BA.2, BA.5 in 2022, XBB in 2023 and JN.1 variant in 2024.

Robust and long-lasting humoral and cellular immunity is crucial for preventing severe illness, hospitalization, and death. Antibodies elicited by SARS-CoV-2 infection rise within 2 to 5 weeks but wane within 5–8 months [2], resulting in low levels until subsequently stimulated by additional infections or vaccinations. T cell activation such as IFN-γ responses to viral antigens limit SARS-CoV-2 [3]. Exhaustion of CD4+ and CD8+ T cells is associated with severe disease [4]. Immunity against SARS-CoV-2 is shown to be hybrid, driven by factors such as prior infections and vaccinations [5].

COVID-19 vaccinations contributed greatly to the resolution of the pandemic through reduction of disease severity and hospitalizations, improving outcomes. Vaccines based on mRNA, whole virus inactivated and viral vector technologies were predominantly used. With COVID-19 now endemic, it is important to assess population-wide immunity against SARS-CoV-2 keeping in mind the need for updated vaccinations particularly, in low-resource settings.

Pakistan experienced approximately 31,000 deaths through the COVID-19 pandemic (up to 23 March 2023) with lesser morbidity reported than many other countries of its relative size. COVID-19 vaccines were rolled out in February 2021 as emergency use authorization (EUA) through a national committee established under the authority of the Drug Regulatory Authority of Pakistan (DRAP) [6]. Inactivated virus vaccines BBIBP-CorV (SinoPharm) and Sinovac-CoronaVac were administered first after which, CanSino Biological Inc.'s adenovirus type (Ad5-nCoV) recombinant vector vaccine and its regionally packaged formulation PacVac was introduced [7,8]. In December 2021, booster doses (Pfizer-BioNTech BNT162b2, Moderna mRNA-1273, ChAdOx nCoV-19, Oxford AstraZeneca) were made available through the COVAX facility in limited supply.

Given its unique demographic (240 million individuals with 50 % under 40 years, median age 20.6 years) and high infectious disease burden, it is necessary to ensure vaccine effectiveness in the context of the local setting. Genetic divergence between the ancestral spike protein and emerging SARS-CoV-2 variants were evident by the breakthrough infections when mutations D614G, L452R, P618R, and T478 emerged [9]. Omicron variants have continued to emerge. JN.1 was classified as a Variant of Interest (VOI) by the World Health Organization (WHO) on December 20, 2023. The JN.1 variant was identified at the Aga Khan University Hospital on 2 Jan 2024 https://www.brecorder.com/news/40284141.Vaccines given were as per availability at vaccination centers as per directives of the Department of Health, Government of Sindh. Available types were inactivated vaccines (type A; CoronaVac, SinoVac and BBIBP-CorV, Sinopharm), mRNA vaccinations (type B; BNT162b2, Pfizer) and single-dose vector vaccines (type C; CanSino BIO, Inc. and PakVac) and two-dose vector vaccines (type D; ChAdOx, Astrazeneca and Sputnik, Gamelya).

This study was carried out between October 2021 and June 2022, a period during which SARS-CoV-2 Delta and Omicron variants were predominant [10,11]. We had a mixed-vaccine setting within a rural population of Matiari, Sindh. Although limited numbers of COVID-19 cases were reported, the population seroprevalence had reached 81 % by November 2021 [12]. The cohort we studied had received the first generation of inactivated, mRNA and vector based COVID-19 vaccines. Here we investigated the dynamics of antibody responses after COVID-19 vaccination through the measurement of antibodies IgG against Spike and RBD proteins prior to, and then 6, 12 and 24 weeks after vaccinations. We used pseudotyped viral particle-based neutralizing assays to compare the inhibitory effect of sera against SARS-CoV-2 spike ancestral, Delta and JN.1 Omicron variants in study subjects. We also investigated SARS-CoV-2 induced T cell activity comparing responses to inactivated and mRNA vaccinations using an IFN-γ release assay (IGRA) against Spike protein and other genome components.

2. Methods

2.1. Study subjects

This study was approved by the Ethical Review Committee (ERC) of Aga Khan University (AKU), ERC #2020–5152-11,688 and by the Department of Health, Government of Sindh, Pakistan. Participants were recruited at government run adult vaccine centers (AVCs) of district Matiari (Saeedabad, Hala and Matiari councils), Sindh. All vaccines were administered in the deltoid muscle as per protocols of the Department of Health, Sindh and National Institute of Health, Pakistan. Two-dose vaccines were given 4 weeks apart.

2.2. Study plan

This was a longitudinal observational study with convenience sampling. We included adults >18 years old, both males and female, and only those who were residents of the district with no current plan to move away for at least 6 months (from the time of enrollment). We excluded those already vaccinated, those who had suffered COVID-19 or, had current infections (viral or bacterial) at the time of enrollment in the study. COVID-19 were self-reported by the study subjects. Age and gender-segregated groups were selected for representation of the four vaccine groups (A-D). Baseline ($n = 916$, prior to first dose) and 6 ($n = 792$), 12 ($n = 734$) and 24 ($n = 820$) follow up samples were taken for each consenting individual resulting in 3262 individual specimens.

Data was collected using a structured questionnaire, through a computer-assisted personal interview (CAPI) approach. The questionnaire included sections on socio-economic status, personal and household demographic information and underlying comorbidities. Samples were collected following international standard operating procedures (SOPs) including the use of personal protective equipment, safe handling of laboratory samples and transportation at appropriate temperatures. The first blood samples were taken at the AVC with follow-ups carried out at the participants' households. Samples were first transported to the Matiari based AKU research laboratory and then to the research laboratory, AKU campus, Karachi.

2.3. ELISA for IgG to Spike and RBD

Recombinant Spike and RBD proteins were obtained from iBET, Lisbon, Portugal, courtesy of Prof. Paula M. Alves. All serum samples were tested at a 1/100 dilution in duplicate using an in-house enzyme-linked absorbent assay (ELISA) as per the protocol described by Figueiredo-Campos et al. which was validated in our laboratory [13,14] and is described in Sup Fig. 1. The cut-off for positive responses of IgG to Spike and RBD was 0.5 OD 450 nm in each case.

2.4. Pseudotyped viral particle-based neutralizing assay for SARS-CoV-2

The pseudotyped viral particles were generated by co-transfection of HEK cells provided by Dr. Colin Adrain, Queen's University Belfast, UK with pLEX-GFP reporter, psPAX2 and pCAGGS Spike and neutralizing assay conducted as described previously [15]. The assay quantifies GFP expression in cells which indicate entry of pseudotyped viral particles. The wildtype Spike protein expressed in the lentivirus vector belonged to the D614G wild-type strain from 2020. The delta Spike gene encoding plasmid used was described previously [15]. The Spike omicron JN.1 containing all the defining mutations of this lineage (ins16MPLF, T19I, R21T, L24del, P25del, P26del, A27S, S50L, H69del,V70del, V127F, G142D, Y144del, F157S, R158G, N211del, L212I, V213G, L216F, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445H, G446S, N450D, L452W, L455S, N460K,S477N,T478K, N481K, V483del, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, S939F, Q954H, N969K, P1143L) was synthesized by

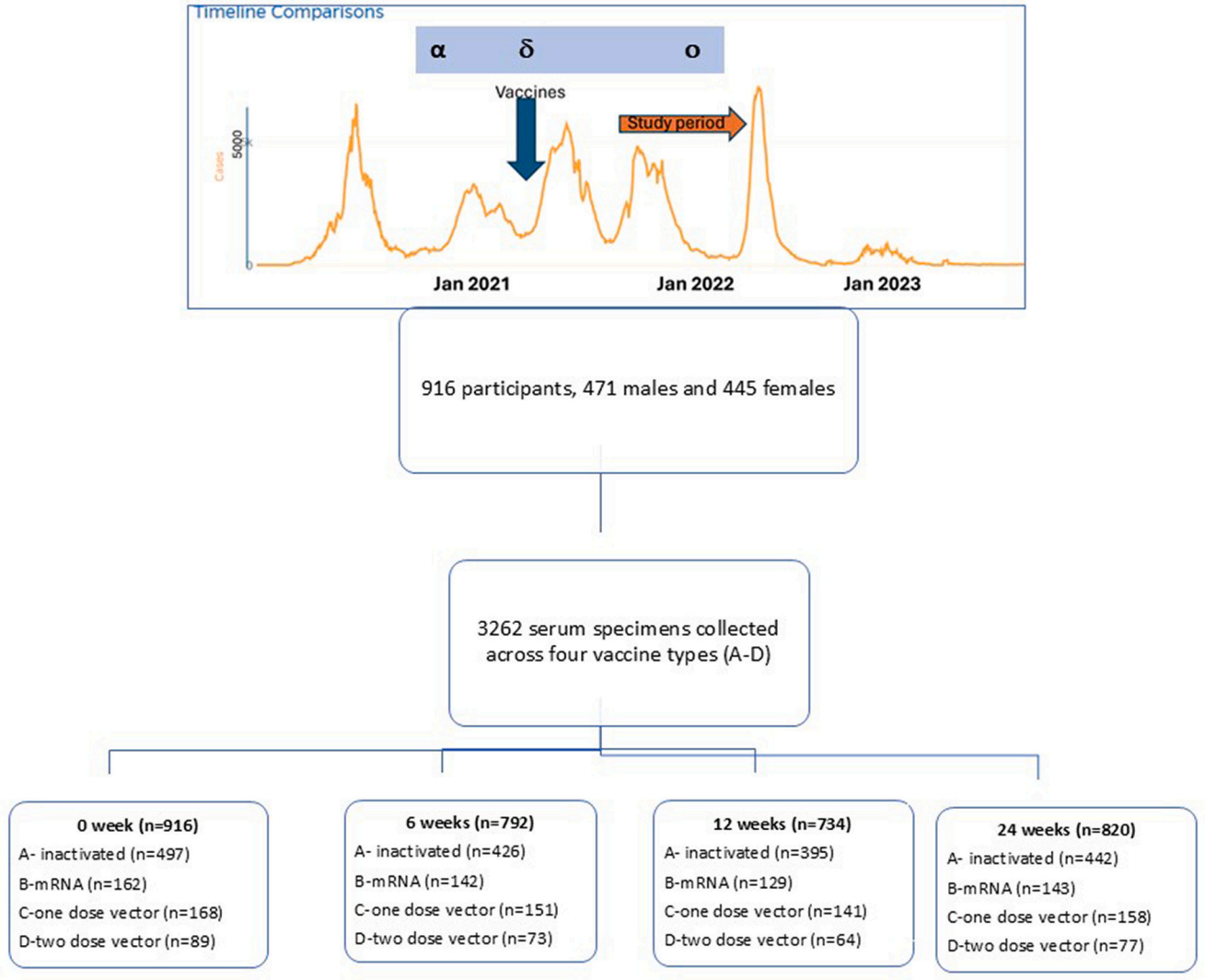


Fig. 1. Description of longitudinal study to compare humoral immunity to different COVID-19 vaccines. Samples were collected between October 2021 and July 2022. The graphic depicts the COVID-19 timeline and the emergence of '**α**' Alpha, '**d**' Delta and '**o**' Omicron variants. Individuals were groups as per the vaccines they received vaccine types; A, inactivated (BBIBP-CorV and SinoVac, CoronaVac), mRNA (BNT162b2), C one-dose vector (CanSinobio and PacVac) and D, two-dose vector (Sputnik and AstraZeneca) respectively. The number of individuals for whom follow up samples were available at each time interval are listed as per the vaccine group.

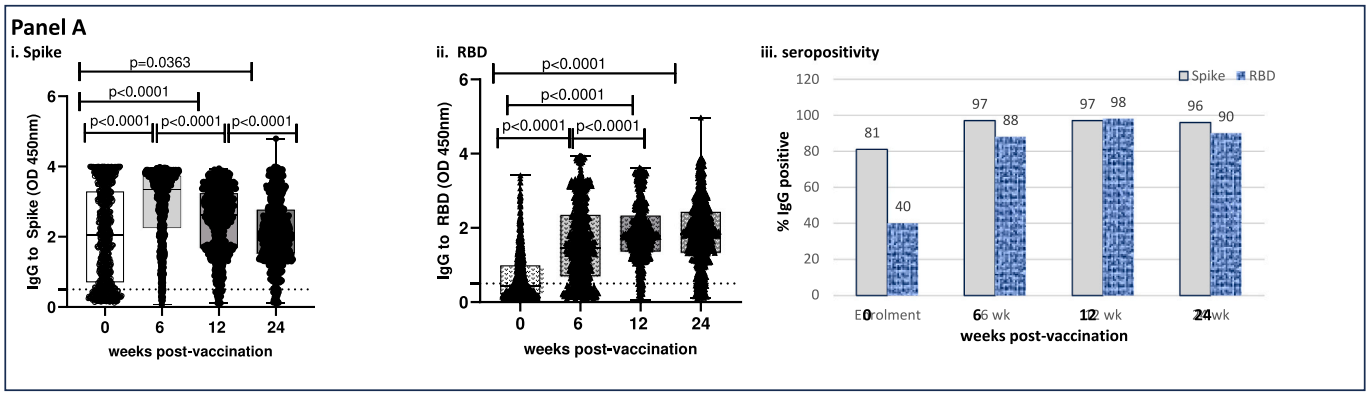
Eurofins Genomics (Germany) and cloned into pCAGGS. The plasmid vectors used for the production of pseudotyped viral particles are shown in Sup Fig. 2. For the neutralization assay, serum samples were three-fold serially diluted over 6 dilutions, beginning with a 1:30 initial dilution. Dilutions were then incubated with Spike pseudotyped lentiviral particles for 1 h at 37 °C. The mix was added to a pre-seeded 96 well plate of 293 T-ACE2 cells, with a final MOI of 0.2. At 48 h post-transduction, the fluorescent signal was measured using the Varioscan Lux multimode microplate reader (Thermofisher scientific, USA). The relative fluorescence units obtained were normalized to those derived from the virus control wells (cells infected in the absence of plasma or serum) after subtraction of the background in the control groups with cells only. The half-maximal neutralization titer (NT_{50}), defined as the reciprocal of the dilution at which infection was decreased by 50 %, was determined using four-parameter nonlinear regression (least squares regression without weighting; constraints: bottom = 0) (GraphPad Prism 10.4).

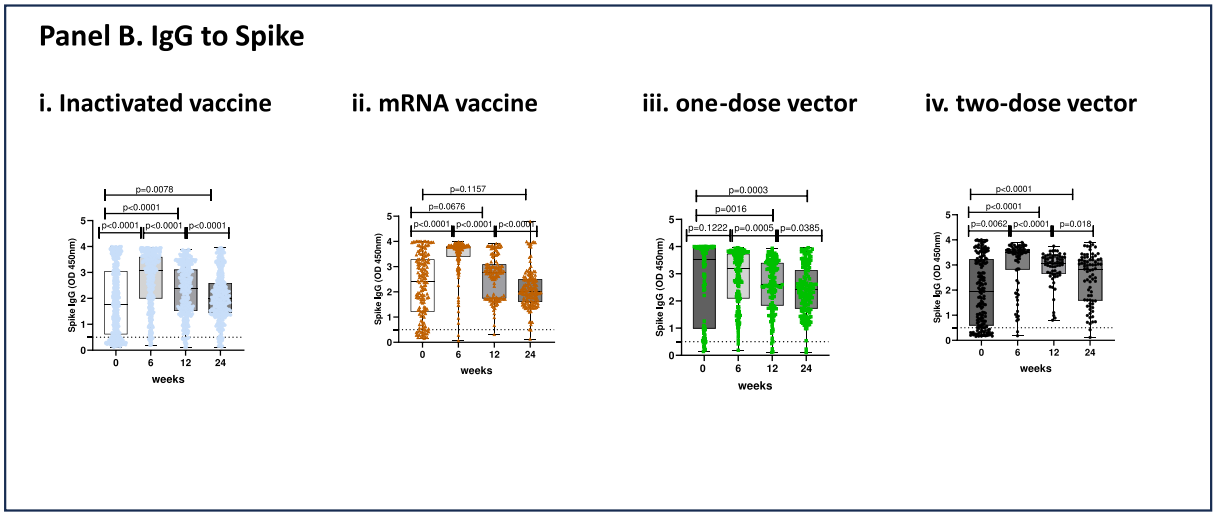
2.5. T cell activation against SARS-CoV-2 antigens

T-cell responses were evaluated via the QuantiFERON (QFN) SARS-CoV2 RUO Starter+Extended Pack (Cat. No. 626915) and QuantiFERON ELISA (Cat. No. 626410) assay as per the manufacturer's instructions (Qiagen, GmBH) and described previously [15]. The QFN SARS-CoV-2 Ag1 tube contains CD4 + epitopes derived from the S1 subunit (RBD) of the Spike protein, the Ag2 tube contains CD4 + CD8 + epitopes from the S1 and S2 subunits of the Spike protein and the Ag3 tube consists of CD4 + CD8 + epitopes from S1 and S2, and epitopes from M and the rest of the genome. Briefly, whole blood was incubated with SARS-CoV-2 antigen Ag1, Ag2 and Ag3, as well as the mitogen tubes. Supernatants were centrifuged and then tested for IFN-γ (IU/ml) using QFN ELISA kit. Samples with IFN-γ $\geq$ 0.15 IU/mL were considered Positive as were the manufacturer's guidelines. Results were calculated for T cell positivity separately against Ag1, Ag2 and Ag3 as well as 'any Ag' (one or more).

2.6. Sample Size Estimation and Statistical Methods

We estimated that 916 individuals (at least three individuals per household) were needed to estimate an expected seroprevalence of 36 % with 5 % precision at 95 % level of confidence, with 80 % power and a non-response rate of 20 %. Categorical variables such as sampling frame





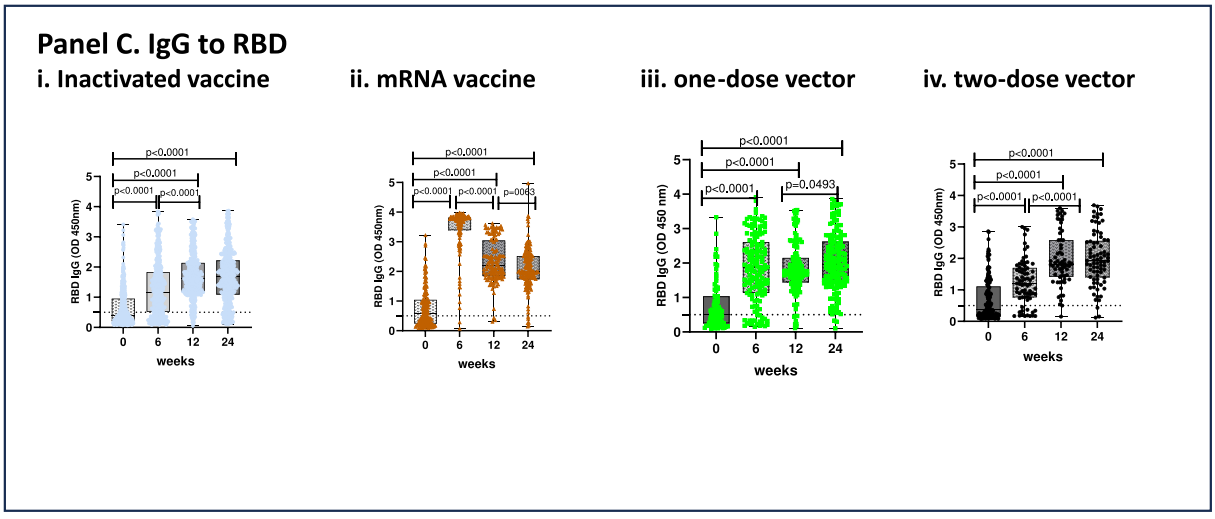


Fig. 2. Increase in IgG antibodies to SARS-CoV-2 Spike after COVID-19 vaccination. The graphs show IgG antibodies measured in 916 individuals who received vaccinations, testing at enrolment (prior to vaccination, 0 weeks), after 6, 12 and 24 weeks after first dose of vaccine. ELISA assays were carried on sera at a dilution 1:100 with each of the antigens, to determine the intensity of antibody binding (OD 450 nm). **Panel A. Baseline seropositivity. i,** IgG to Spike protein. ii, IgG to RBD measured in 916 vaccinated individuals at each time interval respectively. IgG antibody levels are depicted as box and whiskers plots (95 %CI) with horizontal lines depicting median values. The dotted line denotes the cut off at 0.5 OD 450 nm. Statistical analysis was conducted using the Mann-Whitney *U* test; significant values identified as '*', $p \leq 0.05$. iii, the frequency of seropositivity to Spike and RBD respectively in the vaccinees tested at 0, 6, 12 and 24 weeks is shown. **Panel B. IgG to Spike protein. Panel C. IgG to RBD.** The graphs depict IgG to Spike compared separately between different vaccine (i) types A (inactivated, $n = 497$), (ii) B (mRNA, $n = 162$), (iii) C (one-dose vector, $n = 168$) and (iv) D (two-dose vector, $n = 89$) respectively. Antibody levels are depicted prior to vaccination (0 weeks), after 6, 12 and 24 weeks after first dose of vaccine. IgG antibody levels are shown as box and whiskers plots with 95 % CI and median values as horizontal lines. Statistical analysis between groups was run using the Mann-Whitney U (MWU) test, '***', $p < 0.0001$, '*', $p \leq 0.05$.

and differing samples per vaccine were reported as frequencies and proportions. Seroprevalence was reported by age, gender, COVID-19 exposure and clinical symptoms. Pearson Chi-square test was used to establish an association between categorical variables and *p*-value for trend to compare the seroprevalence over time by baseline characteristics. Adjusted odds ratios (OR) with 95 % confidence intervals were reported.

Antibody levels (IgG to Spike and RBD) are presented as median values and compared using Wilcoxon-Sign rank and Mann-Whitney *U* tests. *P* value ≤0.05 was considered significant. Change in seropositivity after vaccination from baseline compared with results at 6, 12 and 24 weeks was determined with 95 % CI and compared using Chi-squared test.

A *p*-value of ≤0.05 was considered statistically significant. Analysis was carried out using STATA Version 17 (Stata Corp, Stata Statistical Software). Two-tailed, nonparametric Mann–Whitney *U* test and ANOVA test were performed on numerical data. Spearman's rank correlation analysis compared the antibody response and neutralizing titers. GraphPad Prism version 10.4 was also for graphical and statistical analysis.

3. Results

3.1. Demographics of the Study cohort and vaccines administered

We investigated the longitudinal effect of COVID-19 vaccinations conducted between October 2021 and July 2022 (Fig. 1) in age and gender-matched groups who received the four different vaccine types administered in the population; A, inactivated vaccines; B, mRNA vaccines; C, one-dose vector vaccines and D, two-dose vector vaccines. Our total cohort comprised 916 individuals. The largest vaccine group was A ($n = 497$, 2.2 % Sinopharm, 52.1 % Sinovac), followed by group B ($n = 162$, BNT162b2), group C ($n = 168$, CanSino BIO) and group D ($n = 89$, AstraZeneca and Sputnik received in limited supply) vaccines. Although we intended to study the dynamics of IgG antibodies in the complete cohort, it was not possible to recruit all individuals at each follow up. Thus, we sampled 792 participants at 6 weeks, 734 at 12 weeks and 820 at 24 weeks after vaccination respectively.

We measured IgG antibodies to Spike and RBD in all collected sera. Individuals were aged 37.1 (± 14.4) years and included 48.8 % females (**Sup** Table 1). The most common comorbid conditions found in

Table 1
Seroprevalence according to sociodemographic characteristics.

	All		Enrolment		After 6 weeks		After 12 weeks		After 24 weeks	
	N	%	Spike positive	p-value	Spike positive		Spike positive	p-value	Spike positive	p-value
All	916	100	744 (81.2 %)		770 (97.2 %)		712 (97 %)		792 (96.6 %)	
Age groups										
18–30 Years	387	42.2	318 (82.2 %)	NS	334 (98.2 %)	NS	315 (99.1 %)	<0.001	336 (98 %)	NS
31–49 Years	363	39.6	294 (81.0 %)		296 (95.8 %)		276 (96.8 %)		313 (96 %)	
50 Years and Above	166	18.1	132 (79.5 %)		140 (97.9 %)		121 (92.4 %)		143 (94.7 %)	
Gender										
Male	471	51.4	384 (81.5 %)	NS	379 (96.9 %)	NS	358 (97.3 %)	NS	405 (96.2 %)	NS
Female	445	48.6	360 (80.9 %)		391 (97.5 %)		354 (96.7 %)		387 (97 %)	
Vaccines by type										
A: Inactivated (Sinovac/Sinopharm)	497	54.3	390 (78.5 %)	0.010	410 (96.2 %)	NS	378 (95.7 %)	NS	421 (95.2 %)	NS
B: mRNA(Pfizer)	162	17.7	140 (86.4 %)		140 (98.6 %)		129 (99.2 %)		140 (97.9 %)	
C: one-dose vector (CanSino/Pak Vac)	168	18.3	133 (79.2 %)		149 (98.7 %)		141 (97.2 %)		156 (98.7 %)	
D: two-dose vector(AstraZeneca/ Sputnik)	89	9.7	81 (91.0 %)		71 (97.3 %)		64 (100 %)		75 (97.4 %)	

	All		Enrolment		After 6 weeks		After 12 weeks		After 24 weeks	
	N	%	RBD positive	p-value	RBD positive	p-value	RBD positive	p-value	RBD positive	p-value
All	916	100	410 (44.8 %)		643 (81.2 %)		702 (95.6 %)		753 (91.8 %)	
Age groups										
18–30 Years	387	42.2	165 (42.6 %)	NS	284 (83.5 %)	NS	309 (97.2 %)	NS	320 (93.3 %)	NS
31–49 Years	363	39.6	172 (47.4 %)		250 (80.9 %)		272 (95.4 %)		294 (90.2 %)	
50 Years and Above	166	18.1	73 (44.0 %)		109 (76.2 %)		121 (92.4 %)		139 (92.1 %)	
Gender										
Male	471	51.4	216 (45.9 %)	NS	316 (80.8 %)	NS	350 (95.1 %)	NS	391 (92.9 %)	NS
Female	445	48.6	194 (43.6 %)		327 (81.5 %)		352 (96.2 %)		362 (90.7 %)	
Vaccines by type										
A: Inactivated (Sinovac/Sinopharm)	497	54.3	205 (41.2 %)	0.057	321 (75.4 %)	<0.001	375 (94.9 %)	NS	387 (87.6 %)	<0.001
B: mRNA(Pfizer)	162	17.7	84 (51.9 %)		132 (93 %)		127 (97.7 %)		137 (95.8 %)	
C: one-dose vector (CanSino/Pak Vac)	168	18.3	75 (44.6 %)		130 (86.1 %)		137 (94.5 %)		155 (98.1 %)	
D: two-dose vector(AstraZeneca/ Sputnik)	89	9.7	46 (51.7 %)		60 (82.2 %)		63 (98.4 %)		74 (96.1 %)	

Chi-Squared test was used to determined statistical significance between seropositive and seronegative individuals between each category, $p < 0.05$ considered significant.

participants were hypertension in 5.2 %, diabetes in 1.8 %, whilst two individuals had a history of hepatitis (C virus), and two had a history of tuberculosis. There were no differences between the demographics or medical history of vaccine groups (A-D) **Sup Table 2**. No individual reported to have developed COVID-19 or tested positive during the study period.

3.2. *All vaccines induced increase in IgG antibodies to RBD but not Spike, differential response in younger and older aged individuals*

We investigated the effect of COVID-19 vaccinations by comparing IgG to Spike and RBD with baseline levels. IgG antibodies to Spike increased by 6 weeks ($p < 0.0001$), showing waning by 12 and 24 weeks but still remaining consistently raised (Fig. 2 **panel Ai**). IgG to RBD increased rapidly within 6 weeks after vaccination ($p < 0.0001$), and further by 12 weeks ($p < 0.0001$). No anti-RBD waning was observed in levels at 24 weeks after vaccination, Fig. 2 **Aii**.

Baseline Spike seropositivity was 81 % prior to vaccination rising to 96 % by 24 weeks whilst seropositivity to RBD rose from 40 % at baseline to 90 % after 24 weeks**,** Fig. 2 **Aiii**. No gender associated differences were found between IgG Spike or RBD seropositivity. However, Spike seropositivity significantly differed between older and younger age groups at 12 weeks after vaccination ($p < 0.001$), Table 1. Further evaluation of age demonstrated that those aged 50 years and over showed lower Spike levels as compared with younger individuals ($p < 0.001$, **Sup Table 3**), as shown previously [15]. IgG seropositivity to RBD was reduced in individuals older than 50 years at −6 ($p = 0.018$), −12 ($p = 0.043$) and 24 ($p = 0.038$) weeks of receiving the first dose of vaccine (Sup Table 3). Of note, RBD seropositivity differed significantly between vaccine groups at −6 ($p < 0.001$) and − 24 weeks ($p < 0.001$), with those who received inactivated vaccinees (group A) with the lowest seropositivity, Table 1.

3.3. *Distinct kinetics of RBD IgG responses after different COVID-19 vaccinations*

Compared of longitudinal dynamics of IgG to Spike induced by different vaccines revealed that participants of group A (inactivated), B (mRNA) and D (two-dose vector) showed an increase in antibodies by 6 weeks ($p < 0.001$) with antibody waning after 12 weeks (Fig. 2 **panel B, i, ii and iv**). However, in group C (one-dose vector), antibodies to Spike did not increase above baseline levels (Fig. 2 **Biii**).

The levels of IgG to RBD differed between groups, with rising levels observed up to 24 weeks in groups A, C and D whilst, group B participants showed a rapid increase by 6 weeks followed by waning by 24 weeks (Fig. 2 **panel C**). Compared between vaccine groups, participants in group B had the highest antibody levels at weeks 6 and 12, whilst at 24 weeks these were higher in group C (**Sup Table 4**). At 24 weeks, 67 (8.2 %) individuals remained RBD seronegative; these comprised 12.4 % of group A, 4.2 % of group B, 1.9 % of group C and 3.9 % of group D participants.

3.4. *Neutralizing antibodies are higher and longer lasting against wild-type SARS-CoV-2 after mRNA vaccination*

We investigated neutralizing capacity in a random subset of sera from group A, B and C participants, to focus on the predominant vaccines employed in the population. We first tested 80 sera collected from participants prior vaccination using a wildtype pseudovirus assay developed by Alenquer et al. [16,17]. At baseline, 38 % of individuals had a modest neutralizing potential against the wildtype Spike psuedotyped viral particles. In group A participants showed a 6.9× fold change, a significant increase in neutralizing antibodies by 6 weeks ($p < 0.0001$, Fig. 3 **Ai**) followed by a 0.32× decrease by 12 weeks ($p = 0.0655$). In group B participants, we also observed a 7.2× increase in

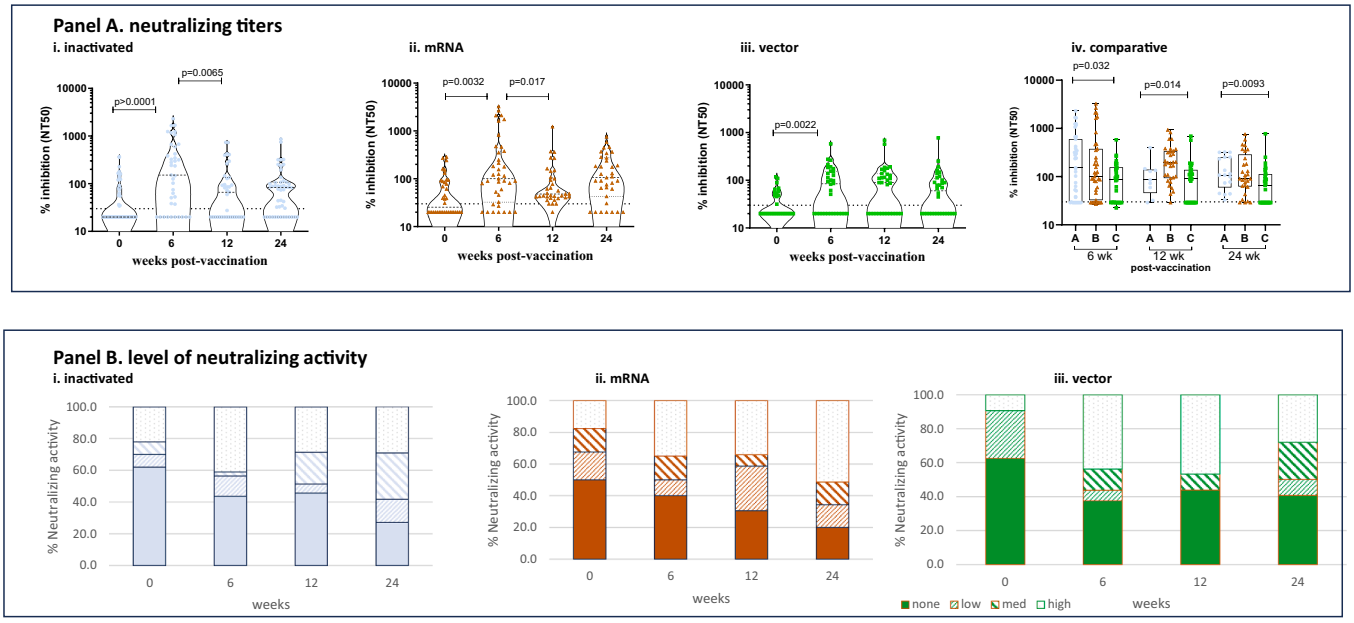


Fig. 3. Assessment of neutralizing titers to SARS-CoV-2 wild type pseudovirus after vaccinations. The graphs depict SARS-CoV-2 neutralizing titers in sera of individuals who received different vaccine types A (inactivated, $n = 54$), B (mRNA, $n = 33$) and C (one-dose vector, $n = 32$) respectively. Sera from groups.
Panel A shows the effect of vaccination on psuedovirus titers after enrolment (0 weeks), 6, 12 and 24 weeks. The violin plot depicts in a scatter the for neutralizing titer (NT_{50}) for individuals in vaccine groups, **i**, inactivated (group A), **ii,** mRNA (group B) and **iii,** one-dose vector types (group C). The horizontal dotted line marks the cut-off $NT_{50} = 30$, negative titers are plotted with an arbitrary value $NT_{50} = 20$. Statistical analysis between groups was run using the Mann-Whitney *U* (MWU) test, '*', $p < 0.05$. **iv**, compares the neutralizing titers in vaccine groups A, B and C at 6, 12 and 24 weeks respectively. Statistical analysis was conducted using the ANOVA test.
Panel B depicts the quality of neutralizing titers present in the vaccinees tested after 6, 12 and 24 weeks after received different vaccine types with classification as none (<30), low 30–75, medium 76–100 and high (>100) NT_{50} in sera tested in response to vaccine groups i, ii and iii. The data labels represent the % of individuals in each category of the bar plot.

virus-neutralizing capacity by 6 weeks ($p = 0.0032$, Fig. 3 **Aii**), followed by a decrease by 12 weeks (0.26×, $p = 0.017$). In group C, neutralizing titers increased 2.8× by 6 weeks ($p = 0.0022$, Fig. 3 **Aiii)** with limited waning by 12 weeks (1.1×). Comparison of titers between vaccination groups differed at 6 ($p = 0.032$), 12 ($p = 0.014$), and 24 ($p = 0.0093$) weeks (Fig. 3 **Aiv**). At 12 weeks, titers measured in group B were greater than those in groups A ($p = 0.04$) or C ($p = 0.007$) participants. Titers in group C were lower than group B vaccinees at 6 weeks ($p = 0.02$) and 24 weeks (p = 0.01).

We ranked virus neutralizing capacity into low, medium and high levels as per NT_{50} values; none (<30), low (30–75), medium (76–100) and high (> 100) and compared between vaccine types. We found differences between neutralizing activity between vaccine groups at 12 weeks, p = 0.007 but not at 24 weeks, $p > 0.05$, Fischer's exact test (Fig. 3 **panel B)**. In group A, 41 % of individuals displayed high titers at 6, 28.6 % at 12 weeks, and 29.2 % at 24 weeks. By 24 weeks, 27.1 % of groups A participants had no serum neutralizing antibodies (Fig. 3 **Bi**). In group B, 35 % of participants displayed high titers at 6 weeks, 34.1 % at 12 weeks, and 51.4 % at 24 weeks, whilst 20 % had none by 24 weeks (Fig. 3 **Bii**). In group C, high titers were seen in 43.8 % of participants at 6 weeks, 46.9 % at 12 weeks, and 28.1 % at 24 weeks, but none in 40.6 % of participants by 24 weeks (Fig. 3 **Biii**).

3.5. COVID-19 vaccines induce differential neutralizing titers against delta and JN.1 omicron variants

Next, we investigated neutralizing capacity Delta variants as these were prevalent at the time of this study. We tested sera in a subset of participants which was from baseline, then 12 and 24-weeks after vaccination. Notably, prior to vaccination 34.2 % of participants had neutralizing capacity against delta variants. Within group A, titers increased x1.8 by 12 weeks ($p < 0.0001$), reducing by 24 weeks (Fig. 4, **panel Ai**). Group B vaccinees also showed x2.78 increase in titers by 12 weeks (p < 0.0001), with waning by 24 weeks (Fig. 4 **Aii**). Group C participants displayed a x2 increase in neutralizing activity after 12 weeks ($p < 0.01$), Fig. 4 **Aiii**. Titers differed between vaccine types at 12 ($p = 0.001$) and 24 weeks ($p = 0.018$), Fig. 4 **Aiv**.

The Fischer's exact test used to compare levels of neutralizing titers against the Delta psuedovirus showed differences at 12 (p = 0.001) and 24 (p = 0.001) weeks after vaccinations, **panel Bi-iii.** Specifically, at 12 weeks in group A, some albeit low neutralizing capacity was detectable in 47.6 %, 96 % in group B and 69.2 % in group C participants; high titers against delta were evident in 33.3 % of group A, 76 % of group B and 42.3 % of group C participants. By 24 weeks; anti-Delta titers were highest in group B and lowest in group C participants.

To study current SARS-CoV-2 strain in context, we tested a subset of sera for neutralizing capacity against JN.1. None of the sera collected at baseline had neutralizing activity against JN.1 at baseline, Fig. 4 **panel C.** However, after 12 weeks low level activity was evident in 8 % of group A, 20 % of group B and 15.4 % of group C vaccinees (Fig. 4, **Ci-iii**). This trend remained consistent at 24 weeks after vaccination, with sera from a few participants in each group showing some activity against JN.1.

3.6. mRNA vaccination induced greater T cell activation than inactivated vaccines

To investigate cellular immunity against SARS-CoV-2 we studied T cell IFN- γ responses using the SARS-CoV-2 QFN assay as described previously [15]. We focused on participants from vaccine groups A and B comparing responses at baseline with those after 6 weeks after vaccination. We tested 45 participants aged 33.4 ± 11.7 years. They were age and gender matched between groups, with 30 from group A and 15 from group B. SARS-CoV-2 antigen (Ag1, Ag2 and Ag3) - stimulated IFN-γ was assessed as per a positive IFN-γ responses against 'any Ag' (one or more) antigen; with IFN-γ positivity of 16 % to Ag1, 9 % to Ag 2 and 29 % to Ag3, respectively. The overall result to 'any Ag' identified T cell activation to be present in 31 % of individuals at baseline **(**Fig. 5A**).** We then compared levels of IFN-γ secretion in participants of groups A and B after 6 weeks. Here we observed that 50 % of participants in the inactivated vaccine as compared with 73 % in the mRNA vaccine group showed T cell reactivity to SARS-CoV-2 antigens. Further comparison at 6 weeks between the vaccine groups revealed that IFN-γ levels were greater in those vaccinated with BNT162b2 as compared with CoronaVac as induced by Ag2 ($p = 0.001$) and Ag3 ($p = 0.002$), Fig. 5B.

4. Discussion

Through the investigation of IgG antibody dynamics and neutralizing antibody responses against SARS-CoV-2 wildtype, Delta and JN.1 variants we compared the effectiveness of COVID-19 vaccinations of the inactivated, mRNA and vector types in population which remained healthy through the pandemic period 2021–2022. We also studied cellular immunity after COVID-19 vaccinations, comparing the effect of inactivated and mRNA vaccines.

The baseline Spike seropositivity of 81 % and RBD seropositivity of 40 % we observed fits with rising SARS-CoV-2 seroprevalence reported for this population between July 2020 and November 2021 [12]. We observed an increase with plateau of Spike IgG levels for up to 24 weeks after inactivated vaccines, as seen previously [15]. mRNA vaccination induced increase in Spike and RBD IgG antibodies followed by a decrease after the second dose fits with prior observations [18]. At 6 weeks after the first dose of vaccine, the level of IgG antibodies to RBD were highest in group B (mRNA) as compared with group A, C and D participants (inactivated type, one-dose vector and two-dose vector). Our data fits reports from Germany which found mRNA vaccines to induce higher IgG antibodies than DNA-viral vector and inactivated types [19]. The age-related lowering of antibody responses we observed in those over 50 years is also supported by earlier reports showing vaccine efficacy to be affected by older age [20,21].

Notably, of those who remained seronegative at 24 weeks after vaccinations most had received inactivated vaccines. As antibodies against SARS-CoV-2 are associated with protection against infection, lower rates of seropositivity in those who received inactivated as compared with mRNA vaccines supports reports that the latter type is more protective [22,23].

By testing sera collected from study subjects prior to vaccinations in October 2021, we observed that 38.5 % of individuals had neutralizing capability against wildtype SARS-CoV-2, with activity against Delta variants found in 34.2 % of participants tested. However, none could neutralize JN.1 omicron variants. Such baseline levels of neutralizing activity against wildtype SARS-CoV-2 are concordant with the 40 % IgG RBD seropositivity observed in the participants. After vaccination, an increase in neutralizing activity was observed against wildtype SARS-CoV-2 and this was higher in those who received mRNA as compared with inactivated and vector vaccines, as shown in other populations [19].

We saw that vaccination induced the expansion of neutralizing antibodies against Delta variants, with the highest increase observed in those who received mRNA vaccines, with lower titers observed in individuals who received inactivated and vector vaccines. These data are concordant with prior data showing delta virus surges resulted in breakthrough infections after inactivated vaccine administrations [24]. Limited neutralizing effect of CanSino BIO against Delta variants found in study from Mexico [25]. Although efficacy against Delta infections was seen to be reduced as compared with earlier SARS-CoV-2 strains, mRNA vaccine have been shown to protect more against these strains [26].

We found minimal neutralizing capacity against JN.1 in sera from unvaccinated participants collected at baseline (by July 2022). Importantly, after 12 weeks of vaccination, 8–12 % of individuals showed low

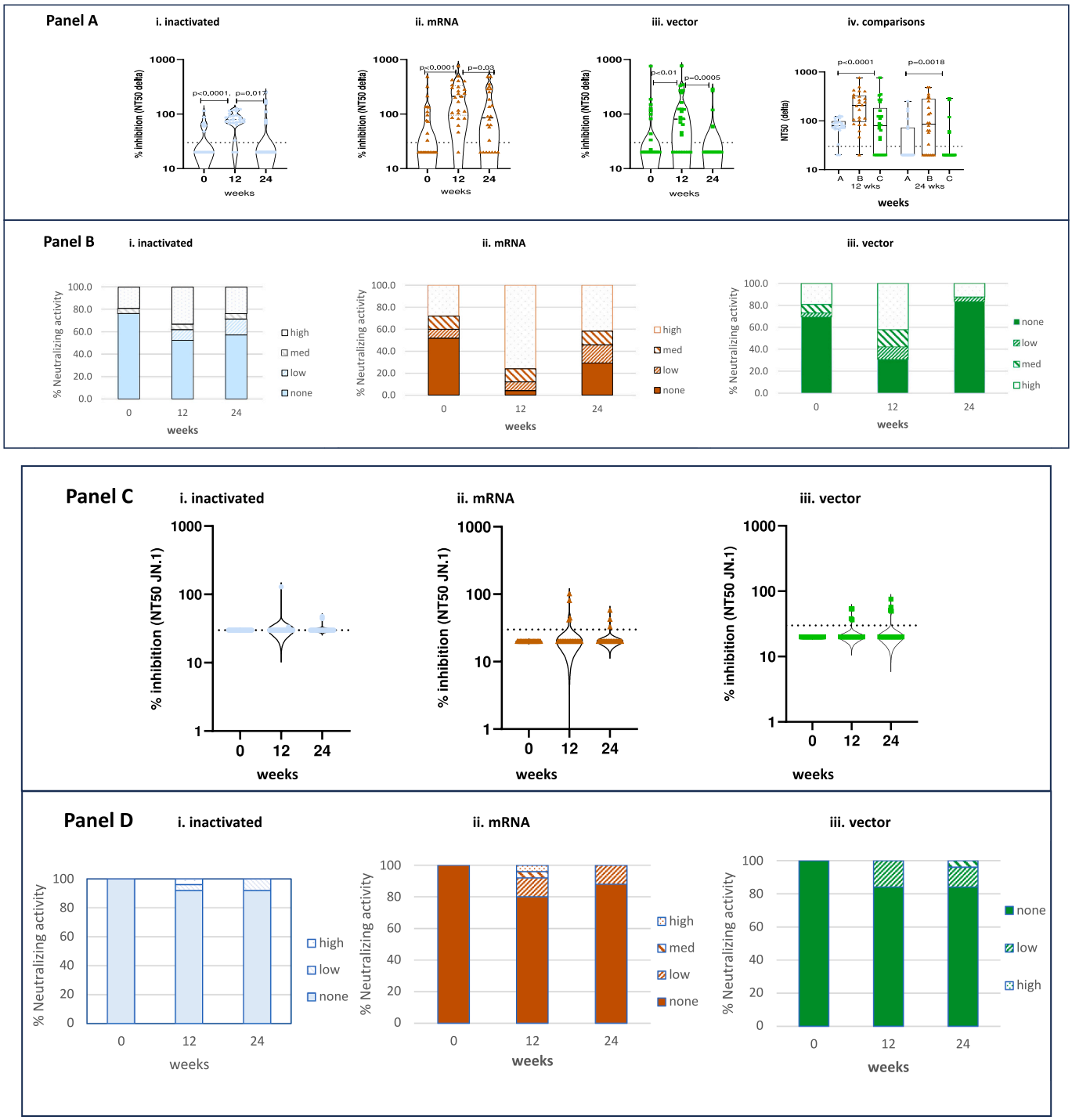


Fig. 4. Comparison of neutralizing effect against SARS-CoV-2 Delta and JN.1 Omicron types. The graphs depict SARS-CoV-2 neutralizing titers against Delta and JN.1 omicron psuedovirus (PV) in sera of individuals after enrolment (0 weeks), 12 and 24 weeks. **Panel A** The violin plot depicts in a scatter the for neutralizing titer (NT_{50}) for individuals in vaccine groups, **i**, inactivated, **ii,** mRNA and **iii,** one-dose vector types. Statistical analysis between groups was run using the Mann-Whitney *U* (MWU) test, '*', $p < 0.05$. **iv**, compares the neutralizing titers in vaccine groups A, B and C at 6, 12 and 24 weeks respectively. Statistical analysis was conducted using the ANOVA test. The horizontal dotted line marks the cut-off $NT_{50} = 30$, negative titers are plotted with an arbitrary value $NT_{50} = 20$.
Panel B compares the quality of anti-Delta PV titer induced by vaccine types i, A, ii, B or iii, C in sera of participants at 0, 12 and 24 weeks of vaccination. The % of participants with none (<30, filled colour), low (30–75, left stripe), medium (76–100, right stripe) and high (> 100, dotted) NT_{50} values against delta PV are depicted in the histogram.
Panel C depicts titers against JN.1 omicron infection after i, inactivated, ii, mRNA and iii, one-dose vector vaccinations. **Panel D** compares the quality of anti-JN.1 PV titer induced by vaccine types i, A, ii, B or iii, C in sera of participants at 0, 12 and 24 weeks of vaccination. The % of participants with none (<30, filled colour), low (30–75, left stripe) and medium (76–100, right stripe) NT_{50} values against Omicron PV are depicted in the histogram. The % of participants with none (<30, filled colour), low (30–75, left stripe), medium (76–100, right stripe) and high (> 100, dotted) NT_{50} values against Delta PV are depicted in the histogram.

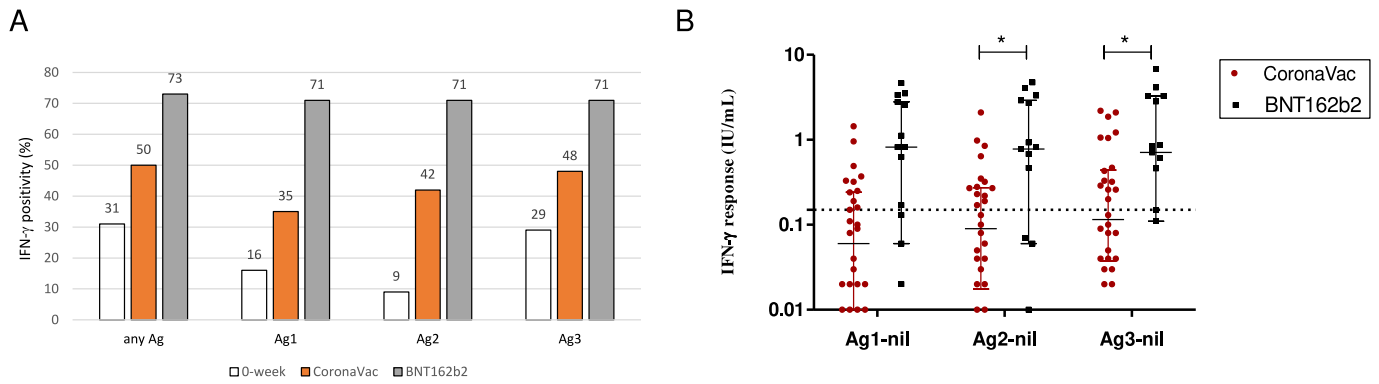


Fig. 5. Higher IFN-γ responses to SARS-CoV-2 antigens after mRNA as compared with inactivated COVID-19 vaccines. We studied participants who received inactivated (CoronaVac) or mRNA (BNT162b2) vaccines and compared T cell IFN-g release to SARS-CoV-2 Spike using the QuantiFERON SARS-CoV-2 assay (comprising Ag1, Ag2 and Ag3 tubes. Responses were measured prior to vaccination and after 6 weeks. A, The percentage of individuals with positive IFN-γ responses to (any) Ag or, Ag1, Ag2 or Ag3, respectively is shown. Graphs depict data for individuals prior to vaccination (0-week) and then 6 weeks after receiving CoronaVac or BNT162b2. B, IFN-γ responses measured 6 weeks after vaccination is compared between CoronaVac and BNT162b2 groups. Data shown is after subtraction of 'nil' values, showing the median line and IQR. A horizontal dotted line identifies the positive cut-off at 0.15 IFN-γ IU/mL. Significance of difference between antigens were determined by Mann Whitney *U* tests ($p < 0.05$, *).

level neutralizing activity against JN.1, with no difference in capacity observed between inactivated, mRNA and vector group participants.

Reduced protection against emerging SARS-CoV-2 variants resulted in the recommendation of booster doses of mRNA vaccinations especially, upon the emergence of omicron variants. It was observed that mRNA vaccines were effective against the B.1.1.529 omicron variant [27] however, there was reduced neutralization against newer omicron strains [28,29]. XBB1.5 vaccines were found to boost cross-variant neutralizing antibodies against newer XBB and EG.1 subvariants [30]. Studies from South Africa showed that XBB infections delayed JN.1 related wave in Brazil due to cross-boosting [31], indicating a protective role of cross-recognition of related viruses. Importantly, ancestral SARS-CoV-2 specific T cells are shown to cross-recognize the omicron variant [32]. This suggests that expansion of omicron variant-specific antibodies in the population is likely due to both vaccinations as well as exposure to earlier omicron variants.

Our reports here of positive T cell IFN-γ response to SARS-CoV-2 antigens in one-third of study subjects prior to vaccination fits with previous work showing antigen recognition in individuals from the pre-pandemic and early pandemic periods [33]. Notably, of the three targets in the SARS-CoV-2 QFN assay, Ag3 representing whole genome antigens elicited the highest IFN-γ response in participants at baseline. Cross-recognition of epitopes may enhance immunity induced by natural exposure to viruses as well as after COVID-19 vaccinations [17]. Hence, it is likely that both B and T cell responses may be enhanced through cross-reactive epitopes to SARS-CoV-2.

We found that more individuals who received mRNA vaccination showed SARS-CoV-2 -induced IFN-γ activation than those who received inactivated vaccines. Further, the magnitude of CD4+ and CD8+ T cell induced IFN-γ responses was significantly lower in those vaccinated with CoronaVac as compared with BNT162b2. CoronaVac, is a Vero-cell based inactivated SARS-CoV-2 vaccine and studies from Chile, showed it to activate humoral responses and IFN-γ producing cells for up to 90 days post immunization [34]. The BNT162b2 vaccine is an mRNA vaccine encoding a mutant S protein and trials showed that it induced SARS-CoV-2 S-specific neutralizing antibodies and poly-specific CD4+ and CD8+ T cells in 90 % of the healthy adults within one week of the second dose with maintenance up to 90 days [35]. The lower levels of IFN-γ reactivity to Spike antigens we observed in the CoronaVac group fits with previous reports showing higher antibody and T cell responses in response to mRNA as compared with inactivated vaccines [36,37].

Our study had some limitations such as, history of prior COVID-19 in study subjects as the symptoms and clinical history were self-reported. Likely COVID-19 infections were not reported due to limited access to testing. We were unable differentiate natural infection or vaccination-induced or, hybrid immunity in the cohort. We could not test neutralizing activity in group D participants, who received a ChAdOx or Sputnik (two-dose vector) vaccines but these were the smallest and least representative group of COVID-19 vaccinations administered in the Pakistani population. Further, we could not measure Delta and JN.1 specific IgG RBD levels to match neutralizing activity measured. Our sample size for T cell activation studies was relatively small due to the limited availability of SARS-CoV-2 QFN assay kits. Further, whilst COVID-19 was not reported amongst the study participants, sub-clinical infections could not be ruled out. The Government of Pakistan and Ministry of Health were successful in procuring COVID-19 vaccines from February 2021 onwards, but their quantity was limited, and the types of vaccines was varied [6]. Overall, the COVID-19 pandemic brought to light disparities between countries based on their access to vaccines. LMICs such as Pakistan were limited by their dependence on supplies from external sources and donations as well as difficulties in maintaining appropriate cold chains for vaccine storage and mobilization. Transporting and storing mRNA vaccines is difficult and more costly due to its low temperature storage requirement (-70 °C) whilst, inactivated vaccines formulations require storage temperatures of 2 °C - 8 °C and have a longer shelf life [38]. In Pakistan, booster vaccinations were given with both inactivated as well as mRNA vaccines. The latter were found to induce higher antibody levels than inactivated vaccines [39]. mRNA vaccines were used as boosters particularly for older individuals, healthcare workers and as per international travel advisories. Updated COVID-19 omicron vaccines have not been available. Despite this, population level COVID-19 mortality remained below 2 % throughout the pandemic.

Our study adds important insights into the immunity against SARS-CoV-2 in the population as well as immunogenicity of the COVID-19 vaccinations used. In summary, in a large low-resource population, whilst repeated vaccination of large cohorts may not be feasible, due consideration may be given for the availability and targeted delivery of updated vaccines to high-risk groups. Additionally, our data shows whilst vaccinations with inactivated and vector types induce lower levels of immunity than mRNA vaccines, they are effective in inducing protection, as observed through population-wide data through the pandemic. This supports the use of different vaccine formulations as per availability and need of the population. Further long-term studies are necessary to investigate the longevity of immunity against SARS-CoV-2 in the local context are required.

Contributors

ZH, MJA, AH, ZAB and YW designed the research study. SQ, AK, FM, MA, JI, PF, SH, KB, KF, SM, AA, AAM, KIM and MY conducted experiments. SQ, AK, FM, MA, YW, SB, SH, KB, KF, AS, AH, AA, KIM, MY and MA analysed data. SQ, SB, JI, YW, SH, KB, KF, SM, AH, AA, AK collected samples. ZH, MJA, RH, KIM and ZAB wrote the initial draft with input from all other co-authors. All authors approved the final version. All authors had final responsibility for the decision to submit the manuscript for publication.

Data sharing

All data that underlie the results reported in this Article (including the study protocol) on individual participants will be made available to researchers providing a methodologically sound proposal to the corresponding author, who will review the proposal.

CRediT authorship contribution statement

Zahra Hasan: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Kiran Iqbal Masood:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Shama Qaiser:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Akbar Kanji:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Fridah Mwenda:** Writing – review & editing, Visualization, Methodology, Investigation. **Marta Alenquer:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Junaid Iqbal:** Writing – review & editing, Supervision, Project administration, Methodology. **Filipe Ferreira:** Methodology, Investigation. **Yaqub Wassan:** Supervision, Project administration, Methodology. **Sadaf Balouch:** Writing – review & editing, Methodology, Formal analysis. **Maliha Yameen:** Methodology, Investigation, Formal analysis. **Shahneel Hussain:** Methodology, Investigation. **Kehkashan Begum:** Project administration, Methodology. **Khalid Feroz:** Methodology, Formal analysis, Data curation. **Sajid Muhammad:** Methodology, Formal analysis, Data curation. **Ayesha Sadiqa:** Writing – review & editing, Formal analysis. **Mishgan Akhtar:** Writing – review & editing, Methodology, Formal analysis. **Atif Habib:** Supervision, Project administration, Methodology, Investigation. **Syed Muhammad Areeb Ahmed:** Writing – review & editing, Visualization, Methodology. **Afsar Ali Mian:** Writing – review & editing, Resources, Methodology. **Rabia Hussain:** Writing – review & editing, Writing – original draft, Formal analysis. **Maria Joao Amorim:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Zulfiqar A. Bhutta:** Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the Chancellors Fund, Aga Khan University as well as the Higher Education Commission, Pakistan (Grand Challenges Fund grant no. 913). We thank the Matiari Research Laboratory, Department of Pediatrics, Aga Khan University (AKU) for their support of the field study. Thanks to the Juma Research Laboratory, Center for Regenerative Medicine, and Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, AKU for use of their facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127270.

Data availability

Data will be made available on request.

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