ORIGINAL ARTICLE

Serosurveillance of Post vaccination SARS-CoV-2 antibodies in Covishield and Covaxin Vaccinated Subjects

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ABSTRACT

Background: Whole virion (attenuated) vaccine (Covaxin) and m-RNA of whole Spike protein (Covishield) were used as emergency measures in SARS-CoV-2 infection. **Aim & Objective:** To evaluate the antigenicity of both vaccines by measuring the quantum of antibodies. **Methods and Material:** Periodic antigenicity of both the vaccines post-vaccination i.e. after 5-6 and 11-12 months was undertaken in two categories; those who were not infected and vaccinated and secondly infected and vaccinated and got re-infected (after vaccination). **Results:** Both the categories of patients showed similar quantum of IgA/IgG/ IgM and Neutralizing antibodies generated by Covaxin. In subjects, vaccinated by Covishield, the recipients of single dose of vaccine showed higher titre of antibodies than double dose vaccinated. Less antigenicity of Covishield vaccine in infected vaccinated was seen compared to un-infected vaccinated subjects. **Conclusions:** Paper reports antigenic strength of both the vaccines during 5-6 and 11-12 months' post-vaccination and also the important point that antibodies titre was higher among cohort of single vaccinated as compared to those who received two doses of vaccines. The paper further sensitises the issue that both the vaccines were developed on the Wuhan strain whereas the virus has mutated twice, generating two new subsequent strains namely Delta and Omicron.

KEYWORDS

Covishield; Covaxin; Neutralizing Antibody; IgG; IgM Antibody

INTRODUCTION

India had faced severe pandemic of COVID-19 resulting into huge morbidity and mortality (1,2). Emergency use of m-RNA vaccine of whole-spike protein of SARS-Cov-2 (Covishield) and whole virion vaccine (Covaxin) was adopted as immediate measure in January 2021 to mitigate rampant pandemic. Immunogenicity of these emergency vaccines need to be evaluated for the long term assurance of protection of infected as well as unexposed individuals, especially when virus has shown continuous mutations during its

progression from 2020 till 2023. Inspite of mass vaccination by Covishield and Covaxin in India during 2021 and many other countries, third wave of Covid-19 caused by its Omicron strain (3) was witnessed which necessitates evaluation of current status of antigenicity of vaccines.

Earlier workers had undertaken study on antigenicity of vaccines 1-month post vaccination (2). However, realizing that virus is undergoing fast mutation in its Spike Protein (SP) and there have been subsequent pandemic waves even after vaccination, we have undertaken a 6 months and 12 months post vaccination sero surveillance of Neutralizing, IgG, IgA and IgM antibodies in the vaccinated subjects to update knowledge on the vaccine generated immunological status and have also attempted fine analysis of antibodies level among cohorts of patients in the categories of Infected & Vaccinated, Vaccinated & Infected and Uninfected and Vaccinated. Present paper reports details of work done. Studies undertaken will help in understanding post vaccination status of antibodies and its possible epidemiological impact.

Aim & Objective: To evaluate the antigenicity of Covaxin and Covishield vaccines by measuring the quantum of Neutralizing antibodies and IgA/IgG/ IgM antibodies in varied subjects.

Material & Methods

Study design& period: In this hospital-based study, a total of 175 blood samples were collected from Sharda University and its associated hospital during the period from January 2022 to March 2022 and from July 2022 to February 2023. Four age groups were categorised for this study comprising of 18- 25 years old; 26-50 years; 51-69 years and 70+ years for both vaccines i.e. Covaxin and Covishield. Record of participants who received single dose and double dose were also recorded and details of their being Infected and then Vaccinated and Not infected and Vaccinated were noted down. The participants were made aware of the study and after signing the consent form and filling the Patient Information sheet, the blood was drawn. Around 2 ml of blood was collected and brought to the laboratory under proper storage conditions. The blood was allowed to stand at Room Temperature for 2 hours and then serum sample was separated through centrifugation at 5000rpm for 10 min. The serum was then separated and taken for ELISA test employing two kits as follows: Covid 19 (IgM + IgG + IgA) Microlisa kit (m/s J. mitra and Co Pvt Ltd, India) and second Covid 19 Neutralizing Antibody Microlisa kit (m/s J. mitra and Co Pvt Ltd, India). The ELISA assay was performed as per the manufacturer's protocol and then Optical Density (OD) values were noted down. For neutralizing antibody, the percent inhibition was seen. And for IgG, IgA and IgM Antibody detection the COVID antibodies units were seen and then based on these values respectively, the positivity and negativity of the samples were determined. For Neutralizing Antibody, a total of 89 samples were processed and for IgG/A/M antibody detection, a total of 175 samples were assayed.

Data analysis: Statistical analysis of the results was also done using the Statistical Package EZR (Easy-R) version 2.4. For Descriptive statistics – Frequency and percentages were used. In Inferential statistics, Chi-square test and Fisher's Exact test was performed.

Ethics approval: The study received ethical approval on Institutional Ethical Committee, Sharda University, India (SU/SMS&R/ 76-A/2021/08 dated 2.2.21)

Results

Serosurveillance of antibodies in Covaxin vaccinated subjects: Of 36 study subjects vaccinated by whole virion inactivated vaccine (Covaxin), 24 received double dose of vaccine and 12 received single dose (Table 1a). Study of the presence of neutralizing, IgG, IgA and IgM antibodies against SARS-Cov-2 was made. Study of neutralizing antibodies in the patients during the period of 5-6 months post vaccination revealed that all were positive for neutralizing antibodies with OD Values ranging

from 0.152 to 0.54 and inhibition percentage from 92.58 to 73.5 % respectively. In the subjects' cohort of 11-12 months post vaccination, OD Values ranged from 0.18 to 2.06 and percentage inhibition ranged from 91.07 to 0.6 % respectively. Two subjects tested negative. (Table 1a& b).

Table 1a: Status of IgA/IgM/IgG Antibodies in Subjects vaccinated by Single and Double doses of Covaxin

S.No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status		Post vaccination duration (in months)	IgG/IgA/IgM Antibody**		
				Single Dose	Double Dose		OD Value	Antibody units	Results(+ve/ -ve/ equivocal)
1	SAb/2022/41	F	51	√		11-12	3.698	153.12	+ve
2	SAb/2022/46	F	48	√		11-12	3.706	153.45	+ve
3	SAb/2022/47	M	29		√	11-12	3.627	150.18	+ve
4	SAb/2022/48	F	31	√		11-12	3.662	151.63	+ve
5	SAb/2022/49	F	42	√		11-12	3.643	150.84	+ve
6	SAb/2022/75	M	22		√	5-6	3.651	151.1	+ve
7	SAb/2022/125	M	20		√	11-12	3.625	15.010	+ve
8	SAb/2022/127	F	21	√		11-12	3.561	147.4	+ve
9	SAb/2022/129	M	20		√	11-12	3.145	141.4	+ve
10	SAb/2022/130	F	20		√	5-6	3.642	150.8	+ve
11	SAb/2022/135	M	20		√	5-6	3.623	150	+ve
12	SAb/2022/144	F	22		√	5-6	3.570	147.8	+ve
13	SAb/2022/147	M	19		√	5-6	3.635	150.5	+ve
14	SAb/2022/149	M	18		√	5-6	3.663	151.6	+ve
15	SAb/2022/150	M	18		√	11-12	3.634	150.4	+ve
16	SAb/2022/151	F	18		√	11-12	3.573	147.9	+ve
17	SAb/2022/211	F	21	√		11-12	3.717	153.9	+ve
18	SAb/2022/165	M	18		√	5-6	3.629	150.2	+ve
19	SAb/2022/177	M	20		√	5-6	3.670	151.9	+ve
20	SAb/2022/191	F	18	√		11-12	3.668	151.8	+ve
21	SAb/2022/193	M	18	√		5-6	3.702	153.2	+ve
22	SAb/2022/209	F	19	√		11-12	3.624	150	+ve
23	SAb/2022/219	F	21	√		11-12	3.649	151	+ve
24	SAb/2022/52	F	28	√		11-12	3.519	145.7	+ve
25	SAb/2022/146	F	19		√	5-6	2.159	60.22	+ve
26	SAb/2022/43	M	31		√	5-6	1.969	54.92	+ve
27	SAb/2022/45	M	49		√	5-6	1.969	54.92	+ve
28	SAb/2022/50	M	56		√	5-6	2.084	58.13	+ve
29	SAb/2022/183	M	18		√	5-6	2.185	60.94	+ve
30	SAb/2022/185	M	18		√	5-6	2.152	60.027	+ve
31	SAb/2022/193	M	18	√		5-6	2.142	59.7	+ve
32	SAb/2022/44	F	35		√	11-12	0.188	5.244	-ve
33	SAb/2022/42	F	40		√	11-12	2.061	57.48	+ve
34	SAb/2022/191	F	18		√	11-12	2.171	60.55	+ve
35	SAb/2022/51	M	34		√	11-12	2.095	58.437	+ve
36	SAb/2022/53	M	58		√	11-12	2.015	56.206	+ve

***For IgG, IgA and IgM antibody Sample OD ratio =Sample O. D./ Cut off value Cut off value = Negative Control $\bar{X}$ + 0.2*
Covid antibodies units=Sample OD ratio X 10

Table 1b: Neutralizing Antibodies in Subjects vaccinated by Single and Double doses of Covaxin

S.No.	Patient Code	Gender (M/F)	Age	Patient's vaccination Status		Post vaccination duration (in months)	Neutralizing Antibody*		
				Single Dose	Double Dose		OD Value	% inhibition*	Results
1	SAb/2022/146	F	19		√	5-6	0.181	91.16	+ve
2	SAb/2022/43	M	31		√	5-6	0.203	90.09	+ve
3	SAb/2022/45	M	49		√	5-6	0.203	90.09	+ve
4	SAb/2022/50	M	56		√	5-6	0.543	73.5	+ve
5	SAb/2022/183	M	18		√	5-6	0.193	90.58	+ve
6	SAb/2022/185	M	18		√	5-6	0.152	92.58	+ve
7	SAb/2022/193	M	18	√		5-6	0.174	91.51	+ve
8	SAb/2022/44	F	35		√	11-12	0.188	90.82	+ve
9	SAb/2022/42	F	40		√	11-12	2.061	0.6	-ve
10	SAb/2022/191	F	18		√	11-12	0.183	91.07	+ve
11	SAb/2022/51	M	34		√	11-12	1.955	4.52	-ve
12	SAb/2022/53	M	58		√	11-12	0.216	89.46	+ve

** For Neutralizing antibody NC=2.0475; PC=0.206* $\text{Percent Inhibition}=\left(1-\frac{\text{Sample OD}}{\text{Negative Control OD}}\right) \text{X } 100$ *; **For IgG, IgA and IgM antibody Sample OD ratio =Sample O. D./ Cut off value; Cut off value = Negative Control* $\bar{X}$ *+ 0.2 Covid antibodies units=Sample OD ratio X 10*

Sero-surveillance of IgG, IgA and IgM antibodies in the subjects belonging to category of 5-6 months of post vaccination showed positive test results for all the sixteen subjects. In the group of 11-12 months' post-vaccination period, except one subject, all tested positive for IgG, IgA and IgM antibodies. No difference of antibodies strength (neutralizing as well as IgG, IgA and IgM) was observed between two groups having vaccinated by single or double doses of vaccine (Table 1b).

The antibodies strength of neutralizing antibodies in subjected vaccinated by Covaxin (whole virion) were compared between those who were Uninfected - Vaccinated and Infected – Vaccinated. Study showed more OD Values (ranging from 0.1 to 1.9) in Infected – Vaccinated group as compared to Uninfected-Vaccinated group. However, no difference of IgG, IgA and IgM antibodies was observed between these two study groups (Table 2a & 2b).

Table 2a: Relative binding strength of Covaxin generated antibodies in infected-vaccinated and uninfected vaccinated subjects

S.No.	Patient Code	Gender	Patient's vaccination Status		Post Vaccination duration (in months)	Neutralizing Antibody**	
		(M/F)	Infected Vaccinated	Uninfected Vaccinated		OD Value	Results
1	SAb/2022/193	M		√	05-Jun	0.174	+ve
2	SAb/2022/191	F		√	11-Dec	0.183	+ve
3	SAb/2022/146	F	√		05-Jun	0.181	+ve
4	SAb/2022/43	M	√		05-Jun	0.203	+ve
5	SAb/2022/45	M	√		05-Jun	0.203	+ve
6	SAb/2022/50	M	√		05-Jun	0.543	+ve
7	SAb/2022/183	M	√		05-Jun	0.193	+ve
8	SAb/2022/185	M	√		05-Jun	0.152	+ve
9*	SAb/2022/51	M	√		11-Dec	1.955	-ve
10*	SAb/2022/53	M	√		11-Dec	0.216	+ve

**- Infected Vaccinated Re-infected ** For Neutralizing antibody; NC=2.0475; PC=0.206*

$\text{Percent Inhibition}=\left(1-\frac{\text{Sample OD}}{\text{Negative Control OD}}\right) \text{X } 100$

Table 2b: Relative binding strength of Covaxin generated antibodies in infected-vaccinated and uninfected vaccinated subjects

S.No.	Patient Code	Gender	Patients' vaccination Status		Post Vaccination Duration	IgG/IgA/IgM Antibody***	
		(M/F)	Infected Vaccinated	Uninfected Vaccinated	(in months)	OD Value	Results (+ve/ -ve/ equivocal)
1	SAb/2022/193	M		√	05-Jun	2.142	+ve
2	SAb/2022/191	F		√	11-Dec	2.171	+ve
3	SAb/2022/146	F	√		05-Jun	2.159	+ve
4	SAb/2022/43	M	√		05-Jun	1.969	+ve
5	SAb/2022/45	M	√		05-Jun	1.969	+ve
6	SAb/2022/50	M	√		05-Jun	2.084	+ve
7	SAb/2022/183	M	√		05-Jun	2.185	+ve
8	SAb/2022/185	M	√		05-Jun	2.152	+ve
9*	SAb/2022/51	M	√		11-Dec	2.095	+ve
10*	SAb/2022/53	M	√		11-Dec	2.015	+ve
11	SAb/2022/41	F		√	11-Dec	3.698	+ve
12	SAb/2022/46	F		√	11-Dec	3.706	+ve
13	SAb/2022/47	M		√	11-Dec	3.627	+ve
14	SAb/2022/48	F		√	11-Dec	3.662	+ve
15	SAb/2022/49	F		√	11-Dec	3.643	+ve
16	SAb/2022/75	M		√	05-Jun	3.651	+ve
17	SAb/2022/125	M		√	11-Dec	3.625	+ve
18	SAb/2022/127	F		√	11-Dec	3.561	+ve
19	SAb/2022/129	M		√	11-Dec	3.145	+ve
20	SAb/2022/130	F		√	05-Jun	3.642	+ve
21	SAb/2022/135	M		√	05-Jun	3.623	+ve
22	SAb/2022/144	F		√	05-Jun	3.57	+ve
23	SAb/2022/147	M		√	05-Jun	3.635	+ve
24	SAb/2022/149	M		√	05-Jun	3.663	+ve
25	SAb/2022/150	M		√	11-Dec	3.634	+ve
26	SAb/2022/151	F		√	11-Dec	3.573	+ve
27	SAb/2022/211	F		√	11-Dec	3.717	+ve
28	SAb/2022/165	M		√	05-Jun	3.629	+ve
29	SAb/2022/177	M		√	05-Jun	3.67	+ve
30	SAb/2022/191	F		√	11-Dec	3.668	+ve
31	SAb/2022/193	M		√	05-Jun	3.702	+ve
32	SAb/2022/209	F		√	11-Dec	3.624	+ve
33	SAb/2022/219	F		√	11-Dec	3.649	+ve
34	SAb/2022/52	F		√	11-Dec	3.519	+ve

**- Infected Vaccinated Re-infected; ***For IgG, IgA and IgM antibody Sample OD ratio =Sample O. D./ Cut off value Cut off value = Negative Control $\bar{X}$ + 0.2 Covid antibodies units=Sample OD ratio X 10; Negative Control $\bar{X}$=0.1585, Cut off value=0.3585; Positive Control= 2.066*

Sero-surveillance of Covishield generated antibodies in subjects: Sero-surveillance of Covishield generated antibodies among 5-6 months' post vaccination subjects and 11- 12 months showed presence of neutralizing antibodies in 73 (94.8%) subjects (Table 3a) and IgG/IgA/IgM antibodies in 134(96.40%) (Table 3b). Out of total 175 subjects examined, no difference of antibody strength for neutralization as well as IgG, IgA and IgM antibodies was observed among subjects belonging to 5-6 months and 11-12 months' post vaccination period (Table 3a and 3b).

Table 3a: Neutralizing Antibodies in Subjects vaccinated by Single and Double doses of Covishield

S. No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status		Post vaccination duration (in months)	Neutralizing Antibody OD Value	Results
				Single Dose	Double Dose			
1	SAb/2022/12	F	36	√		05-Jun	0.192	+ve
2	SAb/2022/91	F	22	√		05-Jun	0.178	+ve
3	SAb/2022/114	F	26	√		05-Jun	0.157	+ve
4	SAb/2022/140	F	18	√		05-Jun	0.176	+ve
5	SAb/2022/189	F	18	√		05-Jun	0.132	+ve
6	SAb/2022/153	F	19	√		05-Jun	0.242	+ve
7	SAb/2022/70	F	24	√		05-Jun	0.198	+ve
8	SAb/2022/187	F	19	√		05-Jun	0.169	+ve
9	SAb/2022/168	F	18	√		05-Jun	0.538	+ve
10	SAb/2022/121	M	25	√		05-Jun	0.179	+ve
11	SAb/2022/152	M	19	√		05-Jun	0.149	+ve
12	SAb/2022/173	M	19	√		05-Jun	0.159	+ve
13	SAb/2022/174	M	18	√		05-Jun	0.187	+ve
14	SAb/2022/178	M	18	√		05-Jun	0.158	+ve
15	SAb/2022/181	M	18	√		05-Jun	0.31	+ve
16	SAb/2022/108	M	27	√		05-Jun	0.247	+ve
17	SAb/2022/110	M	22	√		05-Jun	0.183	+ve
18	SAb/2022/111	M	22	√		05-Jun	0.137	+ve
19	SAb/2022/112	M	20	√		05-Jun	0.165	+ve
20	SAb/2022/117	M	24	√		05-Jun	1.76	-ve
21	SAb/2022/182	M	19	√		05-Jun	0.149	+ve
22	SAb/2022/186	M	18	√		05-Jun	0.148	+ve
23	SAb/2022/203	F	18	√		05-Jun	0.237	+ve
24	SAb/2022/167	F	18		√	05-Jun	0.193	+ve
25	SAb/2022/169	F	18		√	05-Jun	0.21	+ve
26	SAb/2022/176	F	19		√	05-Jun	1.674	-ve
27	SAb/2022/188	F	19		√	05-Jun	0.547	+ve
28	SAb/2022/190	F	20		√	05-Jun	0.288	+ve
29	SAb/2022/11	F	67		√	05-Jun	0.181	+ve
30	SAb/2022/57	F	27		√	05-Jun	0.369	+ve
31	SAb/2022/109	F	35		√	05-Jun	0.187	+ve
32	SAb/2022/141	F	21		√	05-Jun	0.183	+ve
33	SAb/2022/01	M	66		√	05-Jun	0.221	+ve
34	SAb/2022/27	M	41		√	05-Jun	0.79	+ve
35	SAb/2022/143	M	20		√	05-Jun	0.169	+ve
36	SAb/2022/66	M	28		√	05-Jun	0.196	+ve
37	SAb/2022/133	M	20		√	05-Jun	0.193	+ve
38	SAb/2022/154	M	19		√	05-Jun	0.115	+ve
39	SAb/2022/171	M	19		√	05-Jun	0.126	+ve
40	SAb/2022/199	M	21		√	05-Jun	0.14	+ve
41	SAb/2022/224	F	25		√	05-Jun	0.205	+ve
42	SAb/2022/107	F	20		√	05-Jun	0.215	+ve
43	SAb/2022/90	F	23		√	05-Jun	0.172	+ve
44	SAb/2022/92	F	29		√	05-Jun	0.145	+ve
45	SAb/2022/234	F	21		√	05-Jun	0.21	+ve
46	SAb/2022/94	F	29		√	05-Jun	0.163	+ve
47	SAb/2022/100	F	22		√	05-Jun	0.181	+ve
48	SAb/2022/93	F	28		√	05-Jun	0.207	+ve
49	SAb/2022/145	M	22		√	05-Jun	0.268	+ve
50	SAb/2022/06	F	31		√	05-Jun	0.271	+ve
51	SAb/2022/09	M	25		√	05-Jun	2.198	-ve

S. No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status Single Dose	Double Dose	Post vaccination duration (in months)	Neutralizing Antibody OD Value	Results
52	SAb/2022/103	F	25		√	05-Jun	0.191	+ve
53	SAb/2022/34	M	40	√		05-Jun	0.236	+ve
54	SAb/2022/55	F	30	√		05-Jun	0.181	+ve
55	SAb/2022/123	M	18	√		05-Jun	0.176	+ve
56	SAb/2022/56	F	32	√		05-Jun	0.185	+ve
57	SAb/2022/63	F	23	√		05-Jun	0.153	+ve
58	SAb/2022/33	M	30	√		11-Dec	0.192	+ve
59	SAb/2022/113	M	26	√		11-Dec	0.246	+ve
60	SAb/2022/36	F	45		√	11-Dec	0.155	+ve
61	SAb/2022/194	F	19		√	11-Dec	0.359	+ve
62	SAb/2022/7	F	26		√	11-Dec	0.285	+ve
63	SAb/2022/105	F	19		√	11-Dec	0.151	+ve
64	SAb/2022/236	F	25		√	11-Dec	0.178	+ve
65	SAb/2022/10	F	26		√	11-Dec	0.168	+ve
66	SAb/2022/87	F	24		√	11-Dec	0.296	+ve
67	SAb/2022/88	F	22		√	11-Dec	0.171	+ve
68	SAb/2022/89	F	21		√	11-Dec	0.152	+ve
69	SAb/2022/98	F	20		√	11-Dec	0.19	+ve
70	SAb/2022/226	F	23		√	11-Dec	0.234	+ve
71	SAb/2022/230	F	21		√	11-Dec	2.011	-ve
72	SAb/2022/38	F	27		√	11-Dec	0.288	+ve
73	SAb/2022/04	F	25		√	11-Dec	0.164	+ve
74	SAb/2022/104	F	22	√		11-Dec	0.221	+ve
75	SAb/2022/102	F	18	√		11-Dec	0.212	+ve
76	SAb/2022/106	F	23	√		11-Dec	0.216	+ve
77	SAb/2022/101	M	21	√		11-Dec	0.18	+ve

** For Neutralizing antibody NC=2.0475; PC=0.206*

$$\text{Percent Inhibition} = \left(1 - \frac{\text{Sample OD}}{\text{Negative Control OD}}\right) \times 100$$

Table 3b: Status of IgA/IgM/IgG Antibodies in Subjects vaccinated by Single and Double doses of Covishield

S.No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status Single Dose	Double Dose	Post vaccination duration (in months)	IgG/ IgA/IgM Antibody** OD Value	Results
1	SAb/2022/12	F	36	√		05-Jun	2.067	+ve
2	SAb/2022/91	F	22	√		05-Jun	2.117	+ve
3	SAb/2022/114	F	26	√		05-Jun	2.064	+ve
4	SAb/2022/140	F	18	√		05-Jun	2.146	+ve
5	SAb/2022/189	F	18	√		05-Jun	2.113	+ve
6	SAb/2022/153	F	19	√		05-Jun	2.123	+ve
7	SAb/2022/70	F	24	√		05-Jun	2.111	+ve
8	SAb/2022/187	F	19	√		05-Jun	1.991	+ve
9	SAb/2022/168	F	18	√		05-Jun	2.082	+ve
10	SAb/2022/121	M	25	√		05-Jun	1.896	+ve
11	SAb/2022/152	M	19	√		05-Jun	2.043	+ve
12	SAb/2022/173	M	19	√		05-Jun	1.987	+ve
13	SAb/2022/174	M	18	√		05-Jun	1.977	+ve
14	SAb/2022/178	M	18	√		05-Jun	1.966	+ve
15	SAb/2022/181	M	18	√		05-Jun	1.949	+ve
16	SAb/2022/108	M	27	√		05-Jun	2.208	+ve
17	SAb/2022/110	M	22	√		05-Jun	2.061	+ve

S.No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status		Post vaccination duration (in months)	IgG/ IgA/IgM Antibody**	
				Single Dose	Double Dose		OD Value	Results
18	SAb/2022/111	M	22	√		05-Jun	2.143	+ve
19	SAb/2022/112	M	20	√		05-Jun	2.101	+ve
20	SAb/2022/117	M	24	√		05-Jun	2.171	+ve
21	SAb/2022/182	M	19	√		05-Jun	2.044	+ve
22	SAb/2022/186	M	18	√		05-Jun	2.289	+ve
23	SAb/2022/203	F	18	√		05-Jun	0.237	-ve
24	SAb/2022/167	F	18		√	05-Jun	2.184	+ve
25	SAb/2022/169	F	18		√	05-Jun	2.185	+ve
26	SAb/2022/176	F	19		√	05-Jun	2.242	+ve
27	SAb/2022/188	F	19		√	05-Jun	2.241	+ve
28	SAb/2022/190	F	20		√	05-Jun	2.287	+ve
29	SAb/2022/11	F	67		√	05-Jun	2.079	+ve
30	SAb/2022/57	F	27		√	05-Jun	2.133	+ve
31	SAb/2022/109	F	35		√	05-Jun	2.14	+ve
32	SAb/2022/141	F	21		√	05-Jun	2.277	+ve
33	SAb/2022/01	M	66		√	05-Jun	1.925	+ve
34	SAb/2022/27	M	41		√	05-Jun	1.909	+ve
35	SAb/2022/143	M	20		√	05-Jun	2.072	+ve
36	SAb/2022/66	M	28		√	05-Jun	1.99	+ve
37	SAb/2022/133	M	20		√	05-Jun	2.185	+ve
38	SAb/2022/154	M	19		√	05-Jun	1.991	+ve
39	SAb/2022/171	M	19		√	05-Jun	2.208	+ve
40	SAb/2022/199	M	21		√	05-Jun	2.318	+ve
41	SAb/2022/224	F	25		√	05-Jun	2.105	+ve
42	SAb/2022/107	F	20		√	05-Jun	2.026	+ve
43	SAb/2022/90	F	23		√	05-Jun	2.027	+ve
44	SAb/2022/92	F	29		√	05-Jun	2.117	+ve
45	SAb/2022/234	F	21		√	05-Jun	2.183	+ve
46	SAb/2022/94	F	29		√	05-Jun	2.165	+ve
47	SAb/2022/100	F	22		√	05-Jun	0.181	-ve
48	SAb/2022/93	F	28		√	05-Jun	0.207	-ve
49	SAb/2022/145	M	22		√	05-Jun	1.986	+ve
50	SAb/2022/06	F	31		√	05-Jun	2.033	+ve
51	SAb/2022/09	M	25		√	05-Jun	1.713	+ve
52	SAb/2022/103	F	25		√	05-Jun	2.125	+ve
53	SAb/2022/34	M	40	√		05-Jun	1.947	+ve
54	SAb/2022/55	F	30	√		05-Jun	1.88	+ve
55	SAb/2022/123	M	18	√		05-Jun	2.09	+ve
56	SAb/2022/56	F	32	√		05-Jun	2.1	+ve
57	SAb/2022/63	F	23	√		05-Jun	2.064	+ve
58	SAb/2022/33	M	30	√		11-Dec	1.917	+ve
59	SAb/2022/113	M	26	√		11-Dec	2.128	+ve
60	SAb/2022/36	F	45		√	11-Dec	2.169	+ve
61	SAb/2022/194	F	19		√	11-Dec	2.194	+ve
62	SAb/2022/7	F	26		√	11-Dec	2.169	+ve
63	SAb/2022/105	F	19		√	11-Dec	2.066	+ve
64	SAb/2022/236	F	25		√	11-Dec	2.143	+ve
65	SAb/2022/10	F	26		√	11-Dec	2.063	+ve
66	SAb/2022/87	F	24		√	11-Dec	1.91	+ve
67	SAb/2022/88	F	22		√	11-Dec	1.963	+ve
68	SAb/2022/89	F	21		√	11-Dec	2.04	+ve
69	SAb/2022/98	F	20		√	11-Dec	2.048	+ve
70	SAb/2022/226	F	23		√	11-Dec	0.234	-ve

S.No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status		Post vaccination duration (in months)	IgG/ IgA/IgM Antibody**	
				Single Dose	Double Dose		OD Value	Results
71	SAb/2022/230	F	21		√	11-Dec	2.011	+ve
72	SAb/2022/38	F	27		√	11-Dec	0.288	-ve
73	SAb/2022/04	F	25		√	11-Dec	2.139	+ve
74	SAb/2022/104	F	22	√		11-Dec	2.193	+ve
75	SAb/2022/102	F	18	√		11-Dec	2.133	+ve
76	SAb/2022/106	F	23	√		11-Dec	2.054	+ve
77	SAb/2022/101	M	21	√		11-Dec	1.969	+ve
78	SAb/2022/291	M	49		√+	05-Jun	3.778	+ve
79	SAb/2022/287	M	24		√	11-Dec	3.719	+ve
80	SAb/2022/243	M	57		√	05-Jun	3.621	+ve
81	SAb/2022/268	F	57	√		11-Dec	3.741	+ve
82	SAb/2022/270	F	73		√	11-Dec	3.666	+ve
83	SAb/2022/281	M	44		√+	05-Jun	3.727	+ve
84	SAb/2022/284	M	30		√+	05-Jun	3.73	+ve
85	SAb/2022/282	M	71		√+	05-Jun	3.703	+ve
86	SAb/2022/283	M	70		√+	05-Jun	3.636	+ve
87	SAb/2022/280	M	67		√+	05-Jun	3.655	+ve
88	SAb/2022/244	M	68		√	05-Jun	3.747	+ve
89	SAb/2022/249	M	51		√	05-Jun	3.736	+ve
90	SAb/2022/250	M	62		√+	05-Jun	3.775	+ve
91	SAb/2022/252	M	60		√	05-Jun	3.706	+ve
92	SAb/2022/245	F	60		√	11-Dec	3.718	+ve
93	SAb/2022/265	M	48		√	11-Dec	3.705	+ve
94	SAb/2022/264	M	36		√	11-Dec	3.697	+ve
95	SAb/2022/247	F	67		√	11-Dec	3.627	+ve
96	SAb/2022/255	M	75		√+	05-Jun	3.725	+ve
97	SAb/2022/253	F	65		√	11-Dec	3.778	+ve
98	SAb/2022/266	F	55		√	11-Dec	3.737	+ve
99	SAb/2022/254	M	82		√+	05-Jun	3.726	+ve
100	SAb/2022/262	M	70		√+	05-Jun	3.763	+ve
101	SAb/2022/269	M	61		√	11-Dec	3.705	+ve
102	SAb/2022/292	M	66		√	11-Dec	3.696	+ve
103	SAb/2022/02	F	40		√	05-Jun	3.71	+ve
104	SAb/2022/03	F	39		√	05-Jun	3.707	+ve
105	SAb/2022/16	M	45		√	05-Jun	3.578	+ve
106	SAb/2022/08	F	29		√	05-Jun	3.713	+ve
107	SAb/2022/13	F	34		√	05-Jun	3.654	+ve
108	SAb/2022/14	M	36		√	05-Jun	3.66	+ve
109	SAb/2022/05	F	26		√	11-Dec	3.731	+ve
110	SAb/2022/15	M	36		√	05-Jun	3.665	+ve
111	SAb/2022/ 17	M	30		√	05-Jun	3.738	+ve
112	SAb/2022/18	F	26		√	05-Jun	3.741	+ve
113	SAb/2022/24	M	28		√	05-Jun	3.686	+ve
114	SAb/2022/59	F	31		√	05-Jun	3.688	+ve
115	SAb/2022/65	M	26		√	05-Jun	3.66	+ve
116	SAb/2022/259	F	53		√	11-Dec	3.642	+ve
117	SAb/2022/201	M	19		√	05-Jun	3.558	+ve
118	SAb/2022/19	M	23		√	05-Jun	3.672	+ve
119	SAb/2022/23	M	24		√	05-Jun	3.671	+ve
120	SAb/2022/35	F	25		√	05-Jun	3.657	+ve
121	SAb/2022/77	M	22		√	05-Jun	3.618	+ve
122	SAb/2022/79	F	21		√	05-Jun	3.582	+ve
123	SAb/2022/80	F	19		√	05-Jun	3.609	+ve

S.No.	Patient Code	Gender (M/F)	Age	Patients' vaccination Status		Post vaccination duration (in months)	IgG/ IgA/IgM Antibody**	
				Single Dose	Double Dose		OD Value	Results
124	SAb/2022/81	F	21		√	05-Jun	3.592	+ve
125	SAb/2022/82	F	20		√	05-Jun	3.506	+ve
126	SAb/2022/83	F	23		√	11-Dec	3.738	+ve
127	SAb/2022/84	F	22		√	05-Jun	3.64	+ve
128	SAb/2022/29	M	26		√	05-Jun	3.673	+ve
129	SAb/2022/31	F	31	√		11-Dec	3.658	+ve
130	SAb/2022/32	F	29		√	11-Dec	3.644	+ve
131	SAb/2022/69	M	31		√	11-Dec	3.676	+ve
132	SAb/2022/142	F	28		√	05-Jun	3.648	+ve
133	SAb/2022/134	M	22		√	05-Jun	3.624	+ve
134	SAb/2022/242	F	38		√	05-Jun	3.601	+ve
135	SAb/2022/248	F	42		√	05-Jun	3.627	+ve
136	SAb/2022/290	M	75		√	11-Dec	3.668	+ve
137	SAb/2022/261	M	76	√		11-Dec	3.633	+ve
138	SAb/2022/137	M	19		√	05-Jun	3.68	+ve
139	SAb/2022/138	M	21		√	05-Jun	3.613	+ve

***For IgG/IgA/IgM antibody Sample OD ratio =Sample O. D./ Cut off value Cut off value = Negative Control $\bar{X}$ + 0.2 Covid antibodies units=Sample OD ratio X 10; Negative Control $\bar{X}$=0.1585, Cut off value=0.3585; Positive Control= 2.066*

Table 4a shows OD values of Neutralizing Antibodies generated by Covishield among subjects vaccinated by single dose and double dose of vaccines. In the category of OD values between 0.1-0.2, maximum number of subjects shows neutralizing antibodies. 91.17% of single dose vaccinated subjects showed Neutralizing Antibodies in this category as compared to 69.76% in subjects who received double dose of vaccine. However, difference was found statistically insignificant (p<0.139). Very few subjects showed Neutralizing Antibodies titres in OD values range more than 0.2. In OD range of more than 0.2, 5.88% subjects were positive in category of single vaccinated as compared to 20.93% of double vaccinated subjects in this category. In category of OD values more than 0.5 more number, only one subject was observed positive in category of double vaccinated and none in the category of single dose vaccinated. In OD range of more than 1, also only 4 subjects showed Neutralizing Antibodies (Table 4a) was observed. No significant statistical association (p > 0.05) was observed between doses of vaccination and Neutralizing Antibodies (OD Values). (Table 4a).

Table 4a: Binding values (OD) of Covishield generated neutralizing antibodies in subjects vaccinated by Single and Double Dose of vaccine

Vaccination Status	Neutralizing Antibodies (OD Values)				Chi square test	P value
	0.1-0.2 (0.1-0.25)	>0.2(0.26-0.55)	>0.5(0.56-1.5)	>1(1.6-2.1)		
Single Dose Vaccination	31/34 (91.17%)	2/34 (5.88%)	0/34 (0%)	1/34 (2.94%)		
Double Dose Vaccination	30/43 (69.76%)	9/43 (20.93%)	1/43 (.32%)	3/43 (6.97%)	5.49	0.139

The binding affinity of Covaxin generated neutralization antibodies showed 100% of single vaccinated subjects showing OD in range 0.1- 0.2 as against only 72.72% showing binding in this range, by the double vaccinated subjects. In OD range of more than 0.2, 9.09% of double vaccinated subjects were found positive as compared to single vaccinated category. In OD range of more than 1,18.18% of double vaccinated subjects were observed positive as compared to single vaccinated category. Table 4 (b) shows the association

between vaccination doses and Neutralizing Antibodies (OD Values), which reveals no significant association (p > 0.05) found between doses of vaccination and Neutralizing Antibodies (OD Values) (Table 4 b).

Table 4b: Binding values (OD) of Covaxin generated neutralizing antibodies in subjects vaccinated by Single and Double Dose of vaccine

Vaccination Status	Neutralizing Antibodies (OD Values)				P value
	0.1-0.2 (0.1-0.25)	>0.2 (0.26-0.55)	>0.5 (0.56-1.5)	>1 (1.6-2.1)	
Single Dose Vaccination	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	
Double Dose Vaccination	8/11 (72.72%)	1/11 (9.09%)	0/11 (0%)	2/11 (18.18%)	1

The binding affinity of Covishield generated IgG/IgA/IgM antibodies showed 91.89% of single vaccinated subjects showing OD in range of less than 2 as against only 42.15% showing binding by double vaccinated subjects. In OD range of more than 2, 8.10% subjects were positive in category of single vaccinated as compared to 57.84% of double vaccinated subjects. Table 5a shows the association between vaccination doses and IgG/IgA/IgM Antibodies (OD Values), which reveals significant statistical association (p < 0.05) observed between doses of vaccination and IgG/IgA/IgM Antibodies (OD Values) (Table 5a).

Table 5a: Binding values (OD) of Covishield generated IgG/IgA/IgM antibodies in subjects vaccinated by Single and Double Dose of vaccine

Vaccination Status	IgG, IgA and IgM antibodies (OD Values)		Chi square test	P ue
	<2 (0.2-2.5)	>2 (2.6-3.7)		
Single Dose Vaccination	34/37 (91.89%)	3/37 (8.10%)		
Double Dose Vaccination	43/102 (42.15%)	59/102 (57.84%)	27.18	0.001

The binding affinity of Covaxin generated IgG/IgA/IgM antibodies showed 8.33% of single vaccinated subjects showing OD in range of less than 2 as against only 45.83% showing binding, by double vaccinated subjects. In OD range of more than 2, 91.66% subjects were positive in category of single vaccinated as compared to 54.16% of double vaccinated subjects. Table 5b shows the association between vaccination doses and IgG/IgA/IgM Antibodies (OD Values), which reveals no significant association (p > 0.05) found between doses of vaccination and IgG/IgA/IgM Antibodies (OD Values) (Table 5b).

Table 5b: Binding values (OD) of Covaxin generated IgG/IgA/IgM antibodies in subjects vaccinated by Single and Double Dose of vaccine

Vaccination Status	IgG, IgA and IgM antibodies (OD Values)		Chi square test	P value
	<2 (0.2-2.5)	>2 (2.6-3.7)		
Single Dose Vaccination	1/12 (8.33%)	11/12 (91.6%)		
Double Dose Vaccination	11/24 (45.83%)	13/24 (54.16%)	3.51	0.06

Table 6a shows binding values of Covishield generated neutralizing antibodies among subjects who were infected and then vaccinated and among those who were not infected and vaccinated. In both the categories of binding values (<OD 0.2 and >0.2 O.D.) the

antibodies showed more binding in the subjects who were not infected and vaccinated. However, the difference was not found statistically significant (p>0.05).

Table 6a: Binding values of Covishield generated neutralizing antibodies among infected vaccinated and uninfected vaccinated subjects

Category	Binding values (01) (n=67)		Chi- square test	P value
	<0.2 (0.1-0.25)	>0.2 (0.26-2.1)		
Infected Vaccinated	18/21 (85.7%)	3/21 (14.28%)		
Uninfected Vaccinated	36/46 (78.26%)	10/46 (21.7%)	0.14	0.7

Table 6b shows binding values of Covaxin generated neutralizing antibodies among subjects who were infected and then vaccinated and among those who were not infected and vaccinated. In both the categories of binding values (<OD 0.2 and >0.2 OD) the antibodies showed more binding in the subjects who were infected and vaccinated. The difference was found statistically insignificant (p>0.05).

Table 6 b: Binding values of Covaxin generated neutralizing antibodies among infected vaccinated and uninfected vaccinated subjects

Category	Binding values (01) (n=67)		P value
	<0.2 (0.1-0.25)	>0.2 (0.26-2.1)	
Infected Vaccinated	6/8 (75%)	2/8 (25%)	
Uninfected Vaccinated	2/2 (100%)	0/2 (0%)	1

DISCUSSION

It was interesting to observe that among 5-6 months and 11-12 months post vaccination individuals, both the Covaxin as well as Covishield vaccines have shown antigenicity in terms of neutralization as well as IgM, IgA and IgG antibodies against spike protein of SARS-Cov-2. Our observations are in conformity with other reports wherein post vaccination antibodies have been observed among vaccinated population (4,5,6,7,8).

We resolved our observations with respect to the response of vaccine in people having given single dose and double dose of vaccine. Our observations report that immunological response of both the vaccines in terms of neutralization, IgG, IgA and IgM antibodies was more pronounced in single dose vaccinated cohort. Contrary to the general expectations that the double dose of vaccine may provide stronger immunity, our observations did not show such a trend. We therefore report interesting knowledge that repeat dose of vaccine may not be imparting additional immunological strength. Our data showed no difference of immune response among different age groups as well as in both the gender vaccinated by single or double dose of vaccines.

Another important observation we report is that immunological response of vaccination was more pronounced in those who did not have history of Covid-19. It was an important observation as infected subjects on account of being already exposed to viral protein should have shown aggressive immunological response against vaccines (9), but our study showed no significant difference.

CONCLUSION

Present paper reports few important observations in terms of persistence of vaccine generated response, both in terms of neutralization as well as IgG, IgA and IgM antibodies among subjects even after 12 months' of vaccination. However, the important points to mention here is that mere presence of antibodies does not ensure the protection of patient against subsequent infections, the real interaction required is that vaccine triggered antibodies should bind well with the viral epitopes. Our earlier studies have shown that vaccine developed against spike protein of Wuhan strain may not generate

antibodies which can bind with mutated strains viz; Delta and Omicron (10). Perhaps this could be the reason why large number of vaccinated patients in India got reinfected during third wave of Covid-19 which was caused by Omicron variant.

Recommendation

The Sero-surveillance of subjects having vaccinated by Covishield and Covaxin vaccines has shown presence of Neutralizing, IgM, IgG and IgA antibodies after 5-12 months' post vaccination. The single and double dose of vaccine did not have much difference of Quantum of antibodies. More research is needed to ensure whether vaccine generated antibodies are neutralizing the mutated spike protein.

Limitation of the Study

Present study has to comprehended in terms of binding suitability of vaccine generated antibodies with all the variants of SARS-CoV-2. Post vaccination studies as reported in present paper will just sensitize this area of research in context with mutated strains of SARS-COV-2.

Relevance of the Study

The study is very much relevant to the recent pandemic caused due the coronavirus. The study is unfolding post vaccination scenario from immunological stand point.

Authors Contribution

All authors had equally contribution.

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Conflict of Interest

There are no conflicts of interest.

Declaration of Generative AI and AI Assisted Technologies in the Writing Process

The authors haven't used any generative AI/AI assisted technologies in the writing process.

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