Original Article

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A Prospective Analysis of Sustained Immunity following Covishield Vaccination

Microbiology Section

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ABSTRACT

Introduction: The outbreak of Coronavirus Disease 2019 (COVID-19) affected a large number of people worldwide within a short period of time. The mortality rate was high, and there was no specific medicine available to cure it. Therefore, the situation demanded the rapid development of a vaccine. When the vaccines were introduced, there was limited knowledge about their efficacy, side-effects, and duration of protection. Hence, a detailed study was conducted in these areas.

Aim: To study the immunological responses following Covishield vaccination and determine the duration of protection offered by the vaccine.

Materials and Methods: The present study is a prospective observational study conducted at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India from March 2021 to May 2023, involving the staff members of the hospital and college who were above 18-year-old. Individuals with immunodeficiency, those on immunosuppressive medication, or anyone with proof of COVID-19 were excluded. The total sample size was 56. Blood samples were collected before administering the vaccine, at 0, 3, and 12 months, and tested for Complete Blood Count (CBC), COVID-19-specific Immunoglobulin G (IgG), Cluster of Differentiation (CD) 45, CD3, CD4, CD8, etc. The findings were statistically analysed using Statistical Packages of Social Sciences (SPSS) software version 22.0 and STATA software version 10.

Results: A total of 154 volunteers initially provided the first blood samples. However, only 129 of them provided blood samples 2nd time and received two doses of the vaccine. Out of these, only 56 participants completed the fourth time blood test. The results consistently demonstrated a rise in IgG levels over time, with consistently higher levels observed in females. Participants above 45 years exhibited higher IgG levels. Individuals with co-morbidities also showed an increase in IgG levels. The research revealed that individuals who were initially IgG negative experienced a greater fold increase in IgG levels after the first dose of vaccination. The incidence and duration of side-effects post-vaccination reduced with each successive vaccine dose.

Conclusion: All the volunteers developed adequate IgG antibodies. Two doses of Covishield effectively resulted in lasting immunity in 94.64% of cases, and three doses achieved 100% immunity. Females and participants above 45 years consistently exhibited higher antibody levels. Individuals with co-morbidities also developed antibodies, albeit at slightly lower levels. The side-effects were mild and short-lived. No long-term after effects were detected even after two years and two months following vaccination.

Keywords: Co-morbidity, Coronavirus disease 2019, Immunoglobulin G

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) has shaken the whole world in the recent past, affecting millions of people and causing numerous deaths within a short period. Hence, there was an urgent need to develop a good vaccine to halt the spread and progression of this disease and protect the people. For this, an expedited approach was followed to give approval for COVID-19 vaccination, although a vaccine trial usually takes many years. When the first few batches of vaccines arrived, no one had a definite idea about their efficacy, side-effects, duration of action, or the dose and frequency of administration. Hence, the present study was considered a novel study.

For present study in March 2021, a thorough literature search was conducted. Prakash O et al., have stated that COVID-19 vaccine-induced IgG antibodies may not last for long [1]. Elgendy IY et al., have stated that women have about half the incidence of COVID-19 with much less disease severity and mortality compared to men due to differential regulation of Angiotensin Converting Enzyme (ACE)2 and sex hormones may also play a role [2]. Takahashi T et al., stated that female patients with COVID-19 have more abundant activated and terminally differentiated T cell populations than male patients at baseline in unadjusted analysis [3]. Luo H et al., have observed that advancing age and co-morbidities have an obvious effect on IgG1 and IgG3 [4].

After reviewing many articles at that time, adequate, suitable, and convincing references regarding the various immunological responses following COVID-19 vaccination were not found. In March 2021, the vaccination program had just started in the hospital. No one had sufficient knowledge about the vaccine's efficacy, duration of action, and side-effects. However, the media was highlighting and exaggerating the side-effects, leading to people being reluctant to get vaccinated. There were not many standard publications to refer to at that time. The available literature suggested that immunity may last for only a few weeks. In this situation, the present novel study was initiated, hoping that it would provide valuable insights into the vaccine's efficacy, duration of protection, and side-effects. This is the rationale behind the study. The aim of the study was to assess various immunological responses following Covishield vaccination and determine the duration of protection offered by the vaccine. The primary objective of the study was to determine the number of doses of Covishield required to achieve adequate immunity. Secondary objective was to investigate the side-effects of the vaccine and their association with age and IgG levels.

MATERIALS AND METHODS

The present was a prospective observational study conducted at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.

The study included 154 staff members from various categories, ranging from housekeeping staff to professors. The study was conducted from March 2021 to May 2023. Institutional Ethical Committee approval was obtained from Sree Balaji Medical College Hospital (No. 002/SBMC/IHEC/2021/1528, dated 12.03.2021). Written informed consent was obtained from each volunteer for their participation in the study. During present study period, the second wave of the pandemic peaked around April 2021, and the third wave peaked around February 2022. The first wave of the pandemic had previously peaked in September 2020. The predominant strains of viruses observed were Alpha B.1.17, B.1.315, Delta B.1.617.2, and Omicron B1.1.529.

Sample size calculation: The sample size was determined based on the study publication by Mahadevaiah A et al., which stated that the efficacy of Covishield among healthcare workers was 69.67% with a 95% confidence limit and 18% relative precision of estimate [5]. The sample size calculation used the formula $(Z)^2\times(1-p)/(p)\times(e)^2$, where Z=1.96, p=0.6967, and precision e=18%. The resulting sample size (n) was calculated as follows: Sample size $(n)=(1.96)^2\times(1-0.6967)/0.6967\times(0.25)^2=3.84\times0.3033/0.0225=52$ samples.

Inclusion criteria: The volunteers above 18 years of age belonging to both the sexes and remained unvaccinated were included in the study.

Exclusion criteria: Known cases of immunodeficiency, individuals on immunosuppressive drugs, and those who already had symptoms of COVID-19/Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) proven disease were excluded from the study.

Study Procedure

All the volunteers underwent a clinical examination, and their details were recorded in a proforma. In the detailed proforma data on general demographic parameters, educational qualification, occupation, financial status, blood group, diet, co-morbid conditions, past treatments, etc., were entered.

The first blood sample was taken just before the first dose of Covishield, a chimpanzee adenovirus vector vaccine manufactured by Serum Institute of India, which was administered in the left deltoid muscle.

The blood sample was tested for various parameters including CBC, Erythrocyte Sedimentation Rate (ESR), C-reactive Protein (CRP), COVID-19-specific Immunoglobulin G and Immunoglobulin M, Clusters of Differentiation (CD) CD45, CD3, CD4, and CD8, among others.

Complete Blood Count (CBC) was performed using a 5-part Haematology Analyser (BC 6000 Mindray) based on the electrical impedance principle. ESR analysis was done using the modified Westergren method on Vescube 30 Touch. CRP was measured using the nephlometric technique.

The specific Immunoglobulin G (IgG) assay for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Biomerieux) was performed using the Enzyme-linked Fluorescent Assay (ELFA) technique. This assay helps to determine if individuals have been exposed to and infected by the virus and whether they have developed a specific anti-SARS-CoV-2 IgG immune response. The assay principle employs a two-step sandwich enzyme immune assay method with final fluorescent detection.

All the assays were automatically performed using the VIDAS instrument. In the first step, recombinant SARS-CoV-2 antigen coated on the wall captured the SARS-CoV-2 IgG. Unbound components were eliminated during the washing process. In the second step, IgG was specifically detected using anti-human IgG labelled with alkaline phosphatase. Unbound components were eliminated during washing, and a substrate was added. The fluorescence of the resulting product was measured at 450 nm. The Intensity of the fluorescence was directly proportional to the antibody level in the sample.

All the above procedures were performed in the Central Laboratory of the Institution. CD counts were conducted by flow cytometry in HCG Anderson Laboratory, Chennai, with whom the present Institution had a Memorandum of Understanding (MoU). The CD values were expressed in units of 10^6/L by the laboratory.

The number of vaccine doses and the interval between each dose were determined based on government recommendations and vaccine availability. The first blood sample was taken in March 2021 (Test 1), followed by the administration of the first dose of Covishield. The second blood sample was collected in June 2021 (Test 2), along with the administration of the second dose of the vaccine. The third blood sample (Test 3) was taken nine months after the second dose of the vaccine (12 months after the first blood sample), and the third dose of the vaccine was administered as a precautionary dose. The fourth blood sample (Test 4) was collected 14 months after the third dose of the vaccine, which was 26 months after the first dose of the vaccine. The same tests performed on the first blood sample were repeated for the second and third blood samples as well. Since, taking large amount of blood for many blood tests repeatedly each time was the main reason for many dropouts, for the fourth sample, only a small amount of blood was collected, and IgG testing was conducted.

The timeline for the blood samples and vaccines is as follows:

1st blood sample and vaccine: March-April 2021

2nd blood sample and vaccine: June-July 2021

3rd blood sample and vaccine: March-April 2022

4th blood sample: May 2023

Although there were initially 154 volunteers, only 129 volunteers attended the second blood test and received the second dose of the vaccine. Only 56 volunteers consistently participated until the end of the study and completed the fourth blood sample. The blood samples of volunteers who did not complete all four tests were utilised for other analyses related to the other studies in COVID-19 (e.g., T cell response).

STATISTICAL ANALYSIS

Demographic variables were presented in frequencies and percentages. Immunological parameters were described using mean and Standard Deviation. Age and sex-wise IgG values were compared using the Mann-Whitney test. Test-wise negativity and positivity were evaluated using McNemar's test. Repeated measures analysis of variance was conducted using the non parametric Friedman's test to assess quantitative differences between Test 1, Test 2, Test 3, and Test 4. Qualitative differences between Test 1 and Test 4 were assessed using McNemar's test. Chi-square test was performed to examine side-effects and their associations. A p-value of ≤0.05 was considered statistically significant, and two-tailed tests were used for significance testing. Statistical analysis was conducted using the SPSS version 22.0 and STATA version 10.0 software.

RESULTS

The total number of volunteers who gave the first sample of blood and had the first dose of the vaccine was 154. However, those who gave the second sample of blood and received the second dose of the vaccine were 129. The total number of volunteers who completed the study with two years and two months of follow-up and the fourth blood test was 56. Participants below 45 years of age, females, and housekeeping staff showed more interest in present research than others. In fact, female housekeeping staff was more curious to know about their immunological status after vaccination [Table/Fig-1].

The CBC conducted each time before vaccination did not reveal any significant abnormalities. During the first blood test, 74 (48%) were positive, found to have COVID-19 specific IgG antibodies (asymptomatic), while 80 (52%) were found to be negative. Females

Demographic variables		Number of participants	n (%)
Age group (in years)	18-45 years	92	59.75%
	>45 years	62	40.25%
Gender	Male	31	20.13%
	Female	123	79.87%
Occupation	Doctors	47	30.50%
	Nurses	11	7.14%
	Housekeeping staff	68	44.17%
	Others	28	18.19%

[Table/Fig-1]: Demographic variables.

consistently exhibited higher levels of antibodies than males from Test 1 to Test 4. Three months after the first dose (2nd test) of the vaccine, IgG levels increased eight times more in males and five times in females, yet the levels in females remained higher.

Nine months after the second dose of the vaccine (3rd test), IgG levels were higher compared to those found during the second test. Test 4 was conducted 14 months after the third dose of the vaccine. The IgG levels steadily increased following each dose of the vaccine.

During the 4th test, conducted two years and two months after the first dose, the IgG values were still found to be higher than the levels observed in the 3rd test. Throughout the two years and two months period, in all four tests, the IgG levels in females were consistently much higher than the levels in males at all times [Table/Fig-2].

	Gender				
	Male		Female		Mann-Whitney U-test
Assessment	Mean	SD	Mean	SD	
Test 1	11.29	20.20	84.11	154.49	Z=2.35 'p=0.02'*
Test 2	81.63	83.16	399.93	192.52	Z=3.83 'p=0.01'**
Test 3	370.00	266.38	573.51	198.70	Z=2.09 'p=0.03'*
Test 4	418.86	222.08	645.45	168.79	Z=2.64 'p=0.02'*

[Table/Fig-2]: Gender-wise IgG values (BAU/mL).
Mann Whitney U-test, *'p<0.05' significant ***'p≤0.01' highly significant
(Test 1=Baseline value before giving the 1st dose of vaccine. Test 2=3 months after giving the first dose of vaccine. Test 3=9 months after the 2nd dose of vaccine. Test 4=14 months after the 3rd dose of vaccine)

Age-wise IgG Value

The volunteers who were above 45 years had higher levels of IgG compared to those who were below 45 years. However, among individuals younger than 45 years, the increase in IgG levels from the first test to the second test (after three months of the first dose of the vaccine) was nine-fold, which is nine times higher than the initial values. In contrast, among those above 45 years the increase was only 3.5-fold.

	Age group				
	18-45 years (n=30)		>45 years (n=26)		Mann-Whitney U-test
Assessment	Mean	SD	Mean	SD	
Test 1	35.56	88.20	120.52	184.87	Z=3.70 p=0.001***
Test 2	306.84	200.33	421.64	209.17	Z=2.05 p=0.05**
Test 3	486.23	238.14	619.42	165.27	Z=2.15 p=0.03*
Test 4	563.13	177.61	679.42	187.06	Z=2.58 p=0.01**

[Table/Fig-3]: IgG values in different ages (BAU/mL).
Mann-Whitney U-test, *p<0.05 significant **p≤0.01 highly significant ***'p≤0.001' very highly significant

Furthermore, in individuals below 45 years, the rise in IgG levels from the first test to the fourth test (2 years and 2 months after the first dose of the vaccine) was 16-fold, while in those above 45 years, the increase was approximately 6-fold. However, the IgG levels remained consistently higher in those above 45 years of age at all times. Despite the greater rate of increase in those below 45 years of age, the absolute levels of IgG consistently remained high in individuals above 45 years of age [Table/Fig-3].

The COVID-19-specific Immunoglobulin G level has steadily increased after each dose of the vaccine, and it has remained high even after two years and two months. The mean levels of CD45+, CD3+, CD4+, and CD8+ have remained within normal limits. However, the mean level of CD4+ has gradually decreased compared to the level observed before administering the vaccine [Table/Fig-4].

Co-morbidities and IgG Values

After each dose of the vaccine, there was a significant rise in the level of IgG both in those without co-morbidities and in those with co-morbidities. Individuals with co-morbidities such as diabetes mellitus, hypertension, asthma, etc., also experienced an increase in IgG levels following each dose of the vaccine, but the rise was less compared to those without co-morbidities [Table/Fig-5].

There is a significant difference in the IgG status from test 1 to test 4. When the study began in March 2021, 74 (48%) volunteers were already positive with high levels of IgG, while 80 (52%) were negative. The positivity rate increased with each vaccine dose. When the blood was tested nine months after the 2nd dose of the vaccine, i.e., in March-April 2022, 94.64% of the volunteers had become positive. Following the 3rd dose of the vaccine, when the blood was tested approximately 14 months later, i.e., in May 2023, all the volunteers (100%) had become positive [Table/Fig-6].

When the IgG levels was compared in those who were initially negative for IgG with those who were initially positive, it was found that in the initially negative cases the rise three months after the first dose of the vaccine was around 15-fold compared to the initial positive cases in whom it was only 3-fold. Nine months after the second dose of the vaccine, in the initial negative group the rise from the first test level was 28-fold compared to those who were initially positive, for whom the rise was only 3.8-fold [Table/Fig-7].

When the 4th blood test was analysed following three doses of the vaccine (after two years and two months of starting vaccination), the absolute level of IgG was much higher in those who were above 45-year-old than in those who were below 45-year-old. Females had higher levels of antibodies than males. In some females above 60 years, the maximum level reached 1008, while in males, it reached up to 927 after two years and two months. Individuals without co-morbidities had much higher levels of antibodies compared to those with co-morbidities. However, all of them had higher levels of antibodies even after two years and two months [Table/Fig-8].

Name of test	Test 1 (baseline)		Test 2 (3 months after 1st dose)		Test 3 (9 months after 2nd dose)		Test 4 (14 months after 3rd dose)		Repeated measures Friedman's test
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
IgG	75.00	146.51	360.14	210.68	540.45	212.23	622.23	177.09	χ^2=116.17 'p=0.001' *** (S)
CD45	2519.09	799.86	2671.57	1274.46	2836.14	1198.04	-		χ^2=1.83 'p=0.16' (NS)
CD3	1938.50	1002.73	1737.18	579.32	1737.18	579.32	-		χ^2=0.25 'p=0.88' (NS)
CD4	1093.68	498.99	1052.80	498.81	840.43	358.47	-		χ^2=7.99 'p=0.001' *** (S)
CD8	649.91	385.57	580.45	243.39	623.82	381.79	-		χ^2=1.11 'p=0.38' (NS)

[Table/Fig-4]: Immunological status before and after vaccination.
Friedman's test, NS: Not significant 'p>0.05' not significant S=Significant; *'p≤0.05' significant ***'p≤0.01' highly significant ****'p≤0.001' very highly significant

Test	Co-morbidity				Mann Whitney U-test
	Absent (n=47)		Present (n=9)		
	Mean	SD	Mean	SD	
Test 1	81.53	157.52	40.94	57.84	Z=0.19 p=0.85 (NS)
Test 2	364.19	209.88	339.00	226.38	Z=0.49 p=0.62 (NS)
Test 3	567.68	210.40	398.22	167.35	Z=2.42 p=0.02*
Test 4	660.98	147.30	419.89	189.61	Z=3.29 p=0.001***

[Table/Fig-5]: Co-morbidities and IgG values (BAU/mL).
Mann-Whitney U-test, NS: not significant, *p<0.05 significant ***p≤0.01 highly significant

IgG	Negative		Positive		McNemars' test
	n	%	n	%	
Test 1	29	51.79%	27	48.21%	χ^2=29.0 'p=0.001'***
Test 2	15	26.79%	41	73.21%	
Test 3	3	5.36%	53	94.64%	
Test 4	0	0.00%	56	100.00%	

[Table/Fig-6]: IgG status in initially positive vs negative volunteers.
McNemars' test, ***'p≤0.001' very high significance

IgG	IgG negative		IgG positive		Mann-Whitney U-test
	Mean	SD	Mean	SD	
Test 1	10.04	24.03	144.77	187.13	z=5.13 p=0.001***(S)
Test 2	146.20	142.35	438.41	174.62	t=4.59 p=0.001***(S)
Test 3	280.15	308.91	555.18	199.59	t=2.26 p=0.05*(S)
Test 4	-	-	622.23	177.09	-

[Table/Fig-7]: Mean values of IgG in initially negative and positive volunteers.
Mann-Whitney U-test, *'p≤0.05' significant, **'p≤0.01' highly significant, ***'p≤0.001' very highly significant; S=significant

Demographic variables		IgG levels in 4th test			Mann-Whitney U-test
		Mean	Standard deviation	N	
Age group (in years)	18-45 years	557.80	165.98	30	z=3.01 'p=0.01'**
	>45 years	696.58	162.19	26	
Gender	Male	487.43	236.23	7	z=1.97 'p=0.05'*
	Female	641.49	161.02	49	
Co-morbidity	No	660.98	147.30	47	z=3.29 'p=0.01'**
	Yes	419.89	189.61	9	

[Table/Fig-8]: IgG levels after two years and two months (BAU/mL).
Mann Whitney U-test
*'p≤0.05' significant **'p≤0.01' highly significant

The side-effects of the vaccine were mild, and as the number of vaccine doses increased, the incidence of side-effects decreased [Table/Fig-9].

As the number of vaccine doses increased, the duration of side-effects decreased [Table/Fig-10].

IgG levels were comparatively higher in those volunteers who had side-effects following Covishield vaccination [Table/Fig-11].

Volunteers in the younger age group had more side-effects than those who were older [Table/Fig-12].

Number vaccinated	Side-effects nil		Side-effects present		Chi-square
	n	%	n	%	
Vaccine 1 (n=154)	85	55.19%	69	44.81%	χ^2=14.09 'p=0.001'***
Vaccine 2 (n=129)	91	70.54%	38	29.46%	
Vaccine 3 (n=56)	45	80.36%	11	19.64%	

[Table/Fig-9]: Side-effects after Covishield vaccination.
Chi square, ***'p≤0.001' very highly significant

Side-effects	Vaccine 1		Vaccine 2		Vaccine 3		Repeated measures Friedman's test
	Mean	SD	Mean	SD	Mean	SD	
Fever	35.62	24.11	37.37	28.75	22.50	4.24	χ^2=2.00 'p=0.37' (NS)
Body pain	11.91	29.07	8.31	25.84	5.28	17.68	χ^2=6.22 'p=0.05' * (S)
Tiredness	7.38	17.73	9.47	24.00	2.88	9.25	χ^2=4.43 'p=0.11' (NS)
Others	4.91	14.57	2.63	22.78	0.71	3.73	χ^2=13.51 'p=0.001' *** (S)

[Table/Fig-10]: Duration of side-effects after Covishield vaccination (in hours).
Friedman's test, NS: not significant 'p>0.05' not significant S: Significant
*'p≤0.05' significant ** 'p≤0.01' highly significant ***'p≤0.001' very highly significant

DISCUSSION

The immunological status of 154 staff volunteers was assessed from study Institute following Covishield vaccination. However, only 56 of these volunteers remained committed to present research until the end and attended regular reviews and follow-ups for a period of two years and two months, providing four blood samples. Out of this group, 48% of the volunteers had significantly high levels of COVID-19-specific IgG antibodies even before receiving the first dose of the vaccine, suggesting that they may have already had asymptomatic infections.

It was observed that IgG values were consistently higher in females, although the exact reason for this is unclear. Additionally, the absolute levels of IgG at the end of the 26-month period were higher in individuals above 45 years of age. However, the increase in IgG levels after the first dose of the vaccine, compared to baseline, was greater in individuals below the age of 45 (9-fold increase).

Fischinger S et al., in their review article, mentioned that females develop higher antibody levels and experience more adverse events following vaccination than males [6]. Similarly, in the study, higher antibody levels in females compared to males were observed. Additionally, it was found that the incidence of side-effects was higher in individuals with higher levels of IgG.

Feikin DR et al., observed that antibody levels following vaccination decline after six months [7]. Levin EG et al., also reported a substantial decrease in humoral response, especially among men and individuals above 65 years, six months after the second dose of the vaccine [8]. However, in the study, antibody levels steadily increased with each dose and remained high even nine months after the second dose and 14 months after the third dose. Elderly individuals also exhibited reasonably high levels of antibodies. In some females above 60 years of age, the maximum level reached 1008, and in males, it reached 927 after two years and two months of follow-up. Additionally, in another study by the John Solomon et al., it was observed that the T-cell response following Covishield vaccination is also equally good [9].

Verma A et al., conducted a prospective cohort study with Covishield vaccine and reported that vaccine-induced antibodies start to decline five months after the second dose [10]. Gil MS et al., suggested that women may be more resistant to SARS-CoV-2 infection compared to males [11]. Naaber P et al., have reported that after six months following vaccination the antibody levels declined to levels like those who had one dose or those who convalesce following infection. In their studies, 93% of cases experienced side-effects, and age had an influence on this. They also observed a direct relationship between the severity of side-effects and the level of antibody rise [12]. In present study, it was also observed that side-effects were more common in individuals with high levels of IgG and in younger age groups, but they occurred in only 44.81% of vaccinated individuals following the 1st dose [Table/Fig-9]. The incidence of side-effects decreased as the number of vaccine doses increased. Similarly, the duration of these symptoms also decreased with an increasing number of doses. The exact reasons for these observations are unclear. Importantly, even after a 2-year and 2-month follow-up, no long-term or serious side-effects were found following Covishield vaccination.

Side-effects		Test 1		Test 2		Test 3		Repeated measures Friedman's test
		Mean	SD	Mean	SD	Mean	SD	
Side-effects after Vaccine 1	No	72.70	153.20	210.51	277.48	308.92	318.23	χ^2=34.69 'p=0.001' *** (S)
	Yes	70.15	139.86	350.92	320.59	502.55	297.54	χ^2=51.86 'p=0.001' *** (S)
Side-effects after Vaccine 2	No	62.54	133.28	230.07	301.67	352.83	341.11	χ^2=50.21 'p=0.001' *** (S)
	Yes	97.25	176.51	382.69	290.11	503.19	258.89	χ^2=33.78 'p=0.001' *** (S)
Side-effects after Vaccine 3	No	60.53	152.35	300.17	344.44	381.44	322.87	χ^2=32.21 'p=0.001' *** (S)
	Yes	70.17	178.32	298.93	350.79	500.71	306.27	χ^2=29.90 'p=0.001' *** (S)

[Table/Fig-11]: Association between side-effects and IgG values (BAU/mL).
Friedman's test, S=Significant ***'p≤0.001' very highly significant

Side-effects		Age group				Chi-square test
		18-45 years		>45 years		
		n	%	n	%	
Side-effects after Vaccine 1	No	44	51.76%	41	48.24%	χ^2=5.02 'p=0.03'*(S)
	Yes	48	69.56%	21	30.44%	
Side-effects after Vaccine 2	No	48	52.75%	43	47.25%	χ^2=4.85 'p=0.03'*(S)
	Yes	28	73.68%	10	26.32%	
Side-effects after Vaccine 3	No	19	42.22%	26	57.78%	χ^2=3.29 'p=0.07'(NS)
	Yes	8	72.73%	3	27.27%	

[Table/Fig-12]: Association between age and side-effects.
Chi-square test, NS: not significant 'p>0.05' not significant *'p≤0.05' significant; S: Significant

Demaret J et al., observed that individuals with prior COVID-19 infection had a better antibody response regardless of age. They also suggested that a repeat vaccination could be effective in boosting immune response in older populations, leading some countries to recommend a third dose for older individuals [13]. Faro VJ et al., also suggested that immunity after a single vaccine dose decreases with increasing age [14]. Arankalle V et al., stated in their article that humoral and cellular responses in the Indian population sharply decreased at six months post Covishield vaccination [15]. However, the present study shows a steady increase in IgG levels following each vaccine dose, with no decline even 14 months after the third dose. Jeewandara C et al., reported that 93.4% of individuals seroconverted following one dose of AZD1222 vaccine (Covishield) [16], while in the present study group 73.21% seroconverted after the first dose of Covishield [Table/Fig-6]. Shete AM et al., mentioned in their article that hybrid immunity declined to very low levels at 7 months post-second vaccination, leading to reinfections [17]. However, authors did not observe a decline in immunity after the second dose of the vaccine in present cases. Bhuiyan TR et al., stated in their article that co-morbidities did not appear to have a negative effect on mounting immune responses after Covishield vaccination [18]. However, in the present study, although the response to vaccination was good, the IgG levels were slightly lower in individuals with co-morbidities compared to those without co-morbidities.

Dundar B et al., stated in their research article that the seropositivity rate after two doses of the vaccine is 97.5%. They also noted that seropositivity decreased with increasing age in both genders [19]. In the present study, a seropositivity rate of 94.64% was found with two doses of the vaccine and 100% following three doses of the vaccine.

Seban RD et al., reported that vaccinated individuals in their series exhibited lymphopenia following vaccination [20]. However, in the present study this finding was not observed.

Sadoff J et al., reported common side-effects of fever, fatigue, headache, and myalgia following COVID-19 vaccination [21]. In the present study, fever, body pain, and tiredness were found to be the common side-effects. An association between side-effects, age, and IgG levels was also observed. Importantly, none of the volunteers in the present study experienced any long-term side-effects after a two-year and two-month follow-up.

Regev YG et al., stated in their article that individuals with high IgG levels above 500 BAU/mL are largely protected against infection with the delta SARS-CoV-2 variant of concern [22]. Based on this statement, it is possible to say confidently that almost all the participants under follow-up have protective levels of IgG. None of these individuals developed clinical symptoms or signs of COVID-19 during the two-year and two-month follow-up period.

Limitation(s)

When the study was initiated in March 2021, 154 staff members volunteered to participate, all of whom were asymptomatic for COVID-19 at that time. However, the first test revealed that 48% of them tested positive with significantly high levels of antibodies. Among them, 56 individuals remained committed to the research until the end. After a two-year and two-month follow-up period, all of these participants exhibited high levels of IgG antibodies. It is important to note that during the course of present study, three waves of COVID-19 occurred. Therefore, the possibility cannot be rule out that some of the volunteers may have had asymptomatic infections following the first and subsequent doses of the vaccine, which could have slightly influenced their antibody levels.

CONCLUSION(S)

All 100% of the participants in present study group had high levels of COVID-19-specific IgG antibodies at the end of 26 months. Females consistently exhibited higher antibody levels compared to males. Furthermore, individuals above 45 years of age had higher antibody levels than those below 45 years. The participants with co-morbidities such as diabetes, hypertension, and asthma also developed high levels of antibodies, although the increase in antibody levels was slightly lower compared to those without co-morbidities. The side-effects experienced following vaccination were mild and short-lived. Importantly, none of the participants in the present study developed any long-term side-effects after a two-year and two-month follow-up period. There appeared to be an association between the level of IgG antibodies, age, and the incidence of side-effects.

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