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Research Article

Antibody response and its persistence to an inactivated SARS-CoV-2 virus vaccine in young Bangladeshi adults: a prospective study

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Abstract

Background and objectives: COVID-19 vaccination program has become a global priority to combat the worldwide pandemic. Studies claimed that severity and case fatality could be minimized by vaccination. The durability of antibodies developed after vaccination is crucial for preventing COVID-19. The purpose of this study was to investigate the dynamics of antibody responses to an inactivated SARS-CoV-2 virus vaccine over time.

Materials and method: The study was conducted from November 2021 to November 2022 among young adults. A pre-tested structured questionnaire was used to record the socio-demographic and clinical data of all the participants. All the participants were vaccinated with two doses of Sinopharm COVID-19 vaccine. Blood samples were collected for estimation of IgG antibodies to SARS-CoV-2 spike S1 protein by indirect ELISA. Biochemical parameters namely random blood sugar (RBS), lipid profile, total protein, thyroid stimulating hormone (TSH), FT4 (free thyroxin) and vitamin D levels were determined in baseline samples by standard methods.

***Result*:** Total 348 adults, aged 18-28 years, were enrolled and of which 35.3% and 64.7% were male and female respectively. Out of 348 participants, 51.7% was seropositive for anti- SARS-CoV-2 antibodies before receiving vaccination. Seropositivity was not significantly ($p > 0.05$) different in male and female participants before and after vaccination. Seropositivity at 1 month after 1st dose and 4 and 7 months after 2nd dose of vaccination increased significantly ($p < 0.05$) compared to pre-vaccination rate. Compared to pre-vaccination level, the mean anti-SARS-CoV-2 antibody levels increased significantly ($p<0.05$) at 1 month after 1st dose and 4 and 7 months following 2nd dose of vaccination. Among 41 seronegative (non-immune) individuals, seropositivity to SARS-CoV-2 increased significantly (<0.05) at 7 month after 2nd dose of vaccine compared to 1 month and 4 months following 1st and 2nd doses of vaccine respectively.

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Seropositivity was not significantly (p >0.05) different before and after vaccination in participants having adequate and insufficient/deficient vitamin D levels.

Conclusion: The study revealed that a good proportion of young adults possessed anti- SARS-CoV-2 antibody before vaccination and the seropositivity increased to over 90% following vaccination with Sinopharm COVID-19 vaccine. High level of anti- SARS-CoV-2 antibody persisted 7 months after 2nd dose of vaccine.

Introduction

The pandemic of coronavirus disease 2019 (COVID-19) is caused by a virus named severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The dreadful disease was first reported on December, 2019 in Wuhan, China. According to the report of World Health Organization (WHO) of August, 2023 more than 769 million people became infected and nearly 7 million died throughout the world. Currently, a number of COVID-19 vaccines are available worldwide. These vaccines vary in terms of side-effects, immunogenicity, efficacy, and duration of protection [1].Vaccines, recommended by WHO and other health agencies provide active acquired immunity against the virus and blocks the virus transmission, consequently reducing the number of COVID-19 cases. Vaccination induced immune response are measured mainly by serum IgG antibodies and vaccine specific effecter T cells which indicate both humoral and cellular immunity respectively. Assessment of cellular immunity on large scale is not easy. Hence, quantitative measurement of antibodies is the mainstay of evaluation of vaccine effectiveness [2]. SARS-CoV-2 virus contains spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N). Of these four structural proteins, spike protein (S) interacts with hosts' ACE2 and TMPRSS2 receptors for invading the host. Therefore regarding protection, the S protein is contemplated as the most suitable antigen for inducing effective antibody responses [3]. Several serological tests are used to measure antibody directed against the spike glycoprotein or its receptor binding domain (RBD) [4,5]. Many studies reported a significant decrease of anti-SARS-CoV-2 antibodies by 12 months after vaccination or natural infection [1-3], and eventually susceptibility to infection rises.

Therefore, the current study aimed to assess the anti-SARS-CoV-2 antibody response and its persistence up to 7 months period after receiving two doses of Sinopharm vaccine (an inactivated SARS-CoV-2 virus vaccine) in young Bangladeshi adults.

Materials and methods

This prospective study was conducted over a period of one year from November 2021 to November 2022.The study was approved by the Institutional Ethical Review Board of Ibrahim Medical College. Informed written consent was obtained from all participants after explaining the nature and purpose of the study.

Study population, vaccination and collection of blood: Previously non-vaccinated adults, aged 18 - 28 years, were selected as study participants irrespective of their history of COVID-19 infection. A pre-tested structured questionnaire (closed ended) was used to record the socio-demographic and clinical data of all the participants. All the participants were vaccinated with two doses of Sinopharm COVID-19 vaccine (Sinopharm Group Co., Ltd, China). The vaccine is an inactivated virus vaccine using SARS-CoV-2 viruses and has an efficacy rate of 78.1% [6]. Each participant received two doses of vaccine. First and second dose of vaccine was administered at the time of enrollment and 1 month after the first dose respectively. Before administering the first dose of vaccine 5 ml of venous blood was collected aseptically to determine the IgG antibody level against SARS-CoV-2 and to estimate some biochemical parameters. Second blood sample (3 ml) was collected 1 month after the first dose prior to the administration of 2nd dose of vaccine. Third and 4th samples (each 3 ml) were collected 4 and 7 months after the second dose of vaccination respectively.

Estimation of IgG antibodies to receptor binding domain (RBD) of SARS-CoV-2: IgG antibodies to RBD of SARS-CoV-2 spike protein S1 (anti-RBDS1 IgG) was determined in serum by ELISA using DRG

ELISA kit (EIA-6150; Marburg, Germany). ELISA test was performed according to manufacturer's instruction. Concentration of anti-RBDS1 IgG antibody was expressed in DU/ml. Any sample showing antibody concentration above the cut off value of 5.4 DU/ml (1DU/ml=5.15IU/ml) was considered as positive.

Biochemical tests: Biochemical parameters namely random blood sugar (RBS), lipid profile, total protein, thyroid stimulating hormone (TSH) and FT4 (free thyroxin) levels were determined in baseline samples by standard methods. Total 25-OH vitamin D level was estimated by DRG-25-OH vitamin D ELISA kit (Marburg, Germany). A cut off value of 30 to 100 ng/ml was considered sufficiency of vitamin D concentration.

Statistical analysis: Data were analyzed and expressed in frequencies, mean, standard deviation and 95% confidence interval (CI). Association between baseline biochemical characteristics and antibody status of participants were determined by chi-square and student's t-tests. Kruskal-Wallis test was done to compare the mean antibody levels measured in samples taken at four different time points. Mann-Whitney U test was done to find out which pairs of groups were significantly different.

Results

Serum anti-SARS-CoV-2 spike IgG antibodies against COVID-19 were assessed at various intervals before and after vaccination. A total of 348 participants were enrolled in the study of which 123 (35.3%) and 225 (64.7%) were male and female respectively. A baseline sample was taken from 348 participants to check for the presence of SARS-CoV-2 IgG antibody as well as other biochemical parameters that might be associated with antibody responses against SARS-CoV-2. Table-1 shows the detail biochemical profile of the enrolled participants. Only 14.9% of participants had adequate levels of vitamin D (> 30 ng/ml).

Out of 348 participants, blood samples were obtained from 211 participants 1 month after 1st dose of vaccination and from 207 and 123 participants after 4 and 7 months following 2nd dose of vaccination respectively (Table-2). Out of 348 participants, 51.7% was seropositive for anti-SARS-CoV-2 antibodies prior to any vaccination. Seropositivity rates at 1 m after 1st dose of vaccination (74.4%) and 4 and 7 months after 2nd dose of vaccination increased (81.2% and 95.1%) significantly ($p < 0.05$) compared to pre-vaccination state (51.7%). The mean anti- SARS-CoV-2 antibody (anti-RBDS1 IgG) level increased significantly ($p<0.05$) 1 month after 1st dose of vaccination and 4 and 7 months following 2nd dose of vaccination

Table-1: *Baseline biochemical characteristics of the study population*

Variables	Number	Mean ± SD	95% CI
RBS mmol/L)	347	5.79 ± 0.9	5.7, 5.9
Cholesterol (mg/dl)	348	180.5 ± 42.4	176, 184.9
HDL (mg/dl)	348	55.1 ± 9.3	54.1-56.1
LDL (mg/dl)	348	90.2 ± 38.9	86.2, 94.3
TG (mg/dl)	348	176.4 ± 54.8	170.6, 182.2
Total protein (gm/dl)	348	7.4 ± 0.6	7.3, 7.4
Albumin (gm/dl)	348	3.7 ± 0.2	3.6, 3.7
Globulin (gm/dl)	348	3.6 ± 0.5	3.6, 3.7
FT4 (mIU/L)	348	1.2 ± 0.4	1.2, 1.3
TSH (mIU/L)	344	1.9 ± 1.1	1.8, 2
Vit- D (ng/ml)	347	13.8 ± 7.3	13, 14.6

Note: RBS-random blood sugar, HDL-high density lipoprotein, LDL-low density lipoprotein, TG-triglyceride, FT4-free thyroxine, TSH-thyroid stimulating hormone, A/G ratio-albumin/globulin ratio, SD-standard deviation, Vit-D - 25-OH vitamin D

Table-2: *Seropositivity and anti- SARS-CoV-2 antibody levels in study participants before and at different time intervals following vaccination*

Vaccination status	Number of participant	Anti-RBDS1 IgG antibody			
		Positive n (%)	Concentration DU/ml Mean ± SE	Negative n (%)	Concentration DU/ml Mean ± SE
Before vaccination	348	180 (51.7)[a]	27.6 ± 1.9[a]	168 (48.3)	2.3 (0.1)
1month after 1st dose	211	157 (74.4)[b]	46 ± 2.6[b]	54 (25.6)	2.4(0.2)
4 months after 2nd dose	207	168 (81.2)[bc]	35.7 ± 2.5[bc]	39 (18.8)	3.6 (0.2)
7 months after 2nd dose	123	117 (95.1)[d]	98.9 ± 3[d]	6 (4.9)	3 (0.7)

Note: Separate letters are assigned if the seropositivity and antibody levels of study participants differ significantly (p<0.05) from each other at different time points. Mann-Whitney test was used to calculate the significant differences among the groups

Table-3: *Seropositivity and anti- SARS-CoV-2 antibody levels in 86 participants from whom all four samples were obtained at defined time interval*

Study population and vaccination status	Number	Anti-RBDS1 IgG antibody	
		Positive n (%)	Concentration DU/ml Mean ± SE
Before vaccination	86	45 (52.3)[a]	12.9 ± 2.1[a]
1month after 1st vaccination	86	61 (70.9)[b]	33.5 ± 2.3[b]
4 months after 2nd vaccination	86	67 (77.9)[bc]	30.6 ± 0.4[bc]
7 months after 2nd vaccination	86	81 (94.2)[d]	92 ± 1.7[d]

Note: Separate letters are assigned if the seropositivity and antibody levels of study participants differ significantly (p<0.05) from each other at different time points. Mann-Whitney test was used to calculate the significant differences among the groups.

compared to pre-vaccination level. The mean anti-SARS-CoV-2 antibody levels were not significantly (p>0.05) different following 1month after 1st dose and 4 months after the 2nd dose of vaccination (Table-2). There was a steep rise of anti- SARS-CoV-2 antibody level at 7 month after the 2nd dose of vaccine compared to 4 month level (98.9 DU/ml from 35.7 DU/ml). Out of 348 enrolled participants, 86 provided all four blood samples at the defined time points. Out of 86, 52.3% participants were seropositive prior to vaccination and the positivity rate became 94.2% at 7 months after 2nd dose of vaccination. Table-3 shows the detail seropositivity rates and anti- SARS-CoV-2 antibody levels of 86 participants before and after vaccination up to 7 month following 2nd dose of vaccination.

Out of 41 seronegative (non-immune) individuals, 39% became seropositive 1month after the 1st dose of vaccine while 53.7% and 87.8% became seropositive by 4 and 7 months after 2nd dose respectively (Table-4). The seropositivity at 4 and 7 months following 2nd dose of vaccine were 37.7% and 63.5% respectively. The seropositivity increased significantly ($p < 0.05$) at 7 months after 2nd dose of vaccine compared to 1 month and 4 months following 1st and 2nd doses of vaccine.

No significant ($p > 0.05$) difference was observed between seropositivity and gender before and after vaccination (Table-5). Seropositivity according to the vitamin D status of the study population before and after vaccination is depicted in Table-6. Seropositivity before vaccination and 1 month after 1st dose, and 4 and 7 months following 2nd dose of vaccination were not significantly ($p > 0.05$) different in participants having adequate and insufficient/deficient vitamin D levels.

Table-4: *Seropositivity among non-immune (seronegative) participants at different time intervals following vaccination with Sinopharm COVID-19 vaccine*

Study population	Number	Seropositivity		
		1 month after 1st dose	4 month after 2nd dose	7 month after 2nd dose
		n (%)	n (%)	n (%)
Seronegative (Before vaccination)	41	16 (39)	22 (53.7)	36 (87.8)

Note: p > 0.05, 1 month versus 4 month; p < 0.05, 1 month versus 7 months; p <0.05, 4 months versus 7 months

Table-5: *Anti- SARS-CoV-2 IgG antibody of the study population at different time points according to the gender*

Study population and vaccination status	Number	Seropositive* n (%)	p value
Before vaccination			
Male	123	68 (55.3)	p > 0.05
Female	225	112 (49.8)	
1 month after 1st dose			
Male	64	51 (79.7)	p > 0.05
Female	147	106 (72.1)	
4 month after 2nd dose			
Male	74	61 (82.4)	p > 0.05
Female	133	107 (80.5)	
7 month after 2nd dose			
Male	34	32 (94.1)	p > 0.05
Female	89	85 (95.5)	

*Note: *Seropositive - Anti-RBDS1 IgG antibody positive; p value calculated by by Chi-sq test*

Table-6: *Anti- SARS-CoV-2 IgG antibody status according to the vitamin D status of the study population before and after vaccination*

Study population status	Vitamin D level	Number	Seropositive* N (%)	P value**
Pre-vaccination	Adequate	52	23 (44.2)	p > 0.05
	Insufficient/Deficient	296	157 (53)	
1 month after 1st dose	Adequate	27	19 (70.4)	p > 0.05
	Insufficient/Deficient	184	138 (75)	
4 month after 2nd dose	Adequate	33	26 (78.8)	p > 0.05
	Insufficient/Deficient	174	142 (81.6)	
7 month after 2nd dose	Adequate	16	15 (93.8)	p > 0.05
	Insufficient/Deficient	107	102 (95.3)	

*Note: *Seropositive - Anti-RBDS1 IgG antibody positive; vitamin D* Adequate - *> 30 ng/ml, Insufficient/Deficient - < 30 ng/ml **Calculated by Chi-sq test.*

Discussion

In the current study, changes in antibody responses to Sinopharm vaccine against SARS-CoV-2 were evaluated from pre-vaccination up to 7 months after the 2nd dose of vaccine. It was observed that about half of the participants had anti- SARS-CoV-2 IgG antibodies before vaccination. Before vaccination, the mean antibody level in seropositive individuals was about 12 times higher (mean 27.6 DU/ml) than that of seronegative counterparts (mean 2.3 DU/ml). Seropositivity profile observed in our study is in agreement with previously reported studies [7-9]. In the current study, post vaccination anti- SARS-CoV-2 IgG antibody level was found higher among those who were seropositive before vaccination. A previous study showed that antibody levels after two doses of vaccine were similar to one dose in convalescent patients [10,11]. Though in the present study, a decrease in antibody concentration was observed 4 months after the 2nd dose of vaccine but the decline was not statistically significant. However, two separate studies also reported a steep decrease in anti- SARS-CoV-2 IgG at six months post-boost [1,2]. But no such decrease of anti-SARS-CoV-2 IgG antibodies was found by other investigators [12-15]. Antibody level starts to decline 3 and 6 months after vaccination indicating a waning immunity [10] and increases the potential of contracting infection over time. Khoury DS et al. [16] mentioned that the titer decreases by 50% every 108 days post-vaccination. According to Wheeler et al [17] waning of anti-RBD antibodies begins on day 45 following vaccination. Waning of antibodies depend on vaccine immunogenicity, as well as, other multiple factors like demography, co-morbidity, and the initiation and maintenance of memory cells [18,19]. Different vaccines may also induce different levels of antibody responses. Moreover, a substantial degree of heterogeneity exists in various immunoassays [20], with some focusing on investigation of nucleocapsid antibodies and others on spike antibodies.

The persistence of anti- SARS-CoV-2 IgG also depends on whether the infections were symptomatic or not. Lower persistence of antibody was reported among asymptomatic individuals [21]. In the current study, a robust immune response after 7 months of the 2nd dose was observed, and the antibody titer was found to be significantly associated with the positive family history of infection of the participants. On inquiry it was revealed that 42% of participants had a positive family history of infection after varying times of post-boost and showed increased antibody titres (mean103.72 DU/ml). However, the participants who had no prior infection history also exhibited elevated antibody titres (mean 84.04 DU/ml). This increase in antibody titers could be attributed to the possibility of an asymptomatic infection. This finding underscores the significance of considering asymptomatic infection in understanding the immune response dynamics in a population. In our study about half of the non-immune individuals did not develop anti- SARS-CoV-2 IgG antibodies 4months after the second dose of vaccine. A study conducted in Bangladesh, also had the similar findings. In that study 24% of participants didn't show any seroconversion after 1st dose of AstraZeneca vaccine [22]. Al-Momani et al [23] obtained the same findings in a study conducted in Jordan. They reported that the overall efficacy of the vaccine was only 67%. According to Parry et al., 13% of participants were non-reactive after 1st dose of AstraZeneca vaccine in elderly population [24]. The failure of development of antibody is might be due to geographical and ethnic variation. Appropriate maintenance of cold chain during and after transportation of the vaccine might also play a significant role. The results underline the importance of monitoring and research to assess the effectiveness of the vaccines, especially in the context of emerging variants.

The study has some limitations. The dropout rate was very high in the current study. The study was initiated with 348 participants, and all four samples could be collected from only 86 participants. A particular group of participants with similar ages were chosen and their antibody status was evaluated for seven months; hence, the findings cannot be generalized.

Conclusion

The current study has shown that individuals who received two doses of the Sinopharm SARS-CoV-2 vaccine exhibited elevated levels of antibodies. However, it is crucial to note that a proportion of

vaccinated individuals failed to produce antibodies despite receiving both doses of the vaccine. These findings suggest the necessity for additional vaccine doses and a careful and thorough evaluation of antibody levels at a defined time intervals in a large population groups.

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Authors' contribution

NT: Protocol writing, data collection, data entry, data analysis and manuscript writing; MFAS: research idea and data collection; SD, SA: protocol writing, data collection and data entry; NA, MMT, RM: data collection and data entry; SM, SPS, RK, FR: laboratory work; MSAJ: research idea and laboratory work; NH: research idea; MM: research idea, study design, data analysis, manuscript writing.

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