Research Article

EVALUATION OF NEUTRALIZING ANTIBODIES POST VACCINATION BY SURROGATE VIRAL NEUTRALIZATION TEST (sVNT) AND ITS CHANGE IN TITER WITH TIME- A SIX MONTHS STUDY

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ARTICLE INFO	ABSTRACT
	In the last two years of the COVID-19 pandemic our health care system went through major changes. A large scale screening and immunization of the community was done to overcome such pandemic situation. Virus neutralization antibodies play a crucial role in assessment of long term immunity, herd immunity, vaccine efficacy. A prospective cross sectional study was done in completely vaccinated individuals for six months in three phases. Neutralizing antibody response in individuals immunized with full two doses of vaccination were monitored by using surrogate virus neutralization test (sVNT). We found that in the second phase neutralizing antibodies has decreased with respect to the first phase. In the third phase of study (after six months of complete vaccination) the titers of the antibody fell considerably among majority of subjects. In our study the antibody titer of Covaxin was considerably more prevalent than Covishield. In our study the level of neutralizing antibodies titer fell down 3-5 months post vaccination. 42% (21) of 50 subjects were resulted negative for neutralizing antibodies during third phase of study i.e. after 6-7 months of the second dose.

INTRODUCTION

The first confirmed case of COVID-19 in India was reported on 30 January, 2020 in Kerala state, till then nearly 43 million positive cases and 0.5 million deaths are reported due to this novel SARS-CoV-2 disease [Andrews MA *et al*., 2020;Website:*www.mygov.in/covid-19*, accessed on June 6, 2022.]. During this pandemic, several preventive measures were introduced by governments and health agencies to minimize the spread of infection in the large population of the country [*Website: www.who.int/emergencies/diseases/novel-coronavirus-2019.Geneva: who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public, Geneva, WHO*]. Early diagnosis, appropriate treatment and immunization plays a key role in reducing morbidity and mortality. In the last two years of the COVID-19 pandemic our health care system went through major changes also. Due to high infectivity rate and severity of the COVID-19 virus, a large scale screening andimmunization of the community was done to overcome such pandemic situation [Lotfi, M *et al*., 2020; Md Kamal Hossain *et al*., 2021].

For COVID 19, various vaccines were approved and many are in developingor in the trail phase. These vaccines provide immunity by activating immunogenic response, which activate the antibody and cell mediated immunityin human body [Sadarangani, M, *et al*., 2021]. The effectiveness and longevity of the immunity provided by these vaccines is debatable even today. Some studies shows that there is a good immunogenic response after the vaccination but there is waning of antibody titer and effectiveness of vaccine with time and against the various SARS-CoV-2 variants. [Lianlian Bian, Fan Gao, *et al*., 202; Muena NA, *et al*., 2021].

Virus neutralization antibodies play a crucial role in assessment of long term immunity, herd immunity, vaccine efficacy etc., hence the detection of virus neutralizing antibodies is of great importance [Jiang, S, *et al*., 2020]. Various virus neutralization test like Plaque Reduction Neutralization test (PRNT), Micro Neutralization test (Micro NT) etc. are conventionally used to detect neutralizing antibodies and are considered as gold standard. Recently developed "surrogate virus neutralization tests" (sVNTs) are performed due to its high sensitivity, specificity, less time taking and also for ease of doing. The conventional virus neutralizationtest requires BSL-3 facilities just because of use of live viruses and cell lines. Important to note that these tests are time taking and unfit for large scale screening, surveillances and vaccination trials [Grigorov, B, *et al*., 2011; Alexander Krüttgen, *et al*.,2022; Katharina Müller, *et al*., 2021].

In our study, neutralizing antibody response in individuals

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immunized with full doses of either Covishield or Covaxin was monitored for six month to check the longevity of neutralizing antibodies by sVNT assay.

MATERIAL AND METHOD

A prospective cross sectional study was done for the duration of 6 months in urban area of Gwalior city. By using purposive sampling method, we included a total of 50individuals above 18 years of age who received Covid-19 vaccination (received all the recommended doses). Two vaccines are included for this study, Covishield (Research name: AZD1222) a non-replicating viral vector based vaccine produced by Astrazeneca and Serum Institute of India and Covaxin (Research Name: BBV152) which is an inactivated virus vaccine produced by Bharat Biotech.

All individuals got their second dose not before less than 30 days and more than 60 days, from the day of sample collection. All participants were informed with the purpose of study and a written consent was taken from all the participants. Individuals, with partial vaccination or not willing to participate were excluded.

This study was conducted from Aug 2021-Jan 2022 in three phases. First time of sample collection is considered as first phase, the second time sampling was done after 90 days of first sample collection (first phase)and again sampling was done third time after 180 days (6 months) of first sample collection.
In this study, we used commercially available "SARS-CoV-2 Surrogate Virus Neutralization Test Kit" which was originally compared with gold standard Plaque Reduction Neutralization Test (PRNT)by the manufacturer. It is based on competitive inhibition of the recombinant human ACE2 receptorprotein with SARS-CoV-2 RBD protein by neutralizing antibodies in human sera to measure the specific neutralizing effect.

The tests were done as per the recommendation of the manufacturer and correlated accordingly. The result of the samples can be found out by finding the inhibition rate which is calculated as below:

Inhibition = (1- OD value of sample / OD value of negative control) * 100%

The cutoff interpretation was done as per the following table:

Items	Cutoff	Result	Interpretation
SARS-CoV-2 neutralizing antibody test	≥30%	Positive	SARS-CoV-2 neutralizing antibody detected
	< 30%	Negative	No detectable SARS-CoV-2 neutralizing antibody

RESULT

The percentage of inhibition calculated on the basis of optical density of the sample as per the above mentioned formulae and interpretation is given as Table:

Of the total 50 subjects 40 (80%) were males and 10 (20%) were females. A total of 17 subjects (34%) were in the age group of 18 years to 30 years whereas 29 subjects (58%) were in the age group of 31-60 years of age. More than 60 years subjects were only 4 in number (8%).

Out of total 50 individuals 44 were taken two doses of Covishield and 6 individuals were taken two doses of Covaxin.

In our study, during first phase we found 45 individuals (=90%) positive for the neutralizing antibodies for Covid-19 while 5 subject individuals (=10%) resulted negative for detectable neutralizing antibodies. Out of these 5 individual four were vaccinated with Covishield while one was vaccinated with Covaxin, It means that out of 44 subjects with full doses of Covisheld 4 subjects (9%) didn't show any antibody in first phase. For Covaxin out of 6 subjects 1 subject (17%) was negative for neutralizing antibodies in first phase. We also find that 37 subjects (82%) showed a robust response of inhibition of more than 90 only 8 subjects showed a lower immune response.

In the end of second phase we found that the antibodies has decreased with respect to first phase, in neutralizing antibodies positive subjects while those subjects which were negative in the first phase all of them showed an immune response on the second phase showing delayed type of immune reaction.

It was also observed that 17 new subjects (34%) became negative for neutralizing antibody which were positive in the first phase. This depicting, waning of robust immune response by the end of third month in few subjects. Some subjects (n=19, 38%) showed enhancement in their immune response out of which few individuals showed a remarkable increase. The remaining 14 subjects (28%) showed a decrease in the inhibition percentage but the fall was well above the cut off line, hence there was a decrease in neutralizing antibody titer but not up to the level of being negative.

In the third phase of study (after six months of complete vaccination) the titers of the antibody fell considerably among majority of subjects. 46% of the positive subjects in first phase has turned negative now. It is noteworthy in our study that the antibody titer of Covaxin was considerably more prevalent than Covishield. 6 subjects got two shots of Covaxin out of which 4 subjects (66.66%) were having detectable levels ofantibody titer and only 2 (33.4%) were turned negative it is also noteworthy that all subjects of Covaxin developed antibody response in first 6 months. Those subjects which got Covishield their titers fall considerably at the end of third phase only 25 subjects (56.8%) remainedpositive for antibody whereas 19 subjects (43.2%) showed negative antibody titers.

DISCUSSION

In our study we observed neutralizing antibodies production in all 50 individuals in certain period of time,during study some subject individuals showed delayed neutralizing antibodies production, while some subject individuals showed low level of neutralizing antibodies after second dose of vaccination. Several studies on SARS-CoV-2 anti-spike binding antibody response against Covaxin and Covishield vaccination showed that a significant antibody response is observed after first and second standard dose, but after 4-5 moths of second dose there is 2 to 4 fold of decrease in anti-spike antibodies in both Covishield and Covaxin recipients[Choudhary HR, *et al*., 2021; Singh AK, *et al*., 2021]. These studies suggest waning of antibodies with time while there are very few studies on waning of neutralizing antibodies. A study conducted in Indonesia on individuals vaccinated with CoronaVac vaccine (Sinovac Biotech) showed a significant drop in the level of neutralizing antibodies titer after five and six month of second dose of CoronaVac vaccine and an infection of SARS CoV-2 is also seen in some cases after second dose of vaccination [Harapan H, *et al*., 2022].

Table Neutralizing Antibodies Titers in All The Three Phases

S.N.	Sample Code	Age	Gender	Result of 1st Phase (1st Testing) As % of Inhibition	Result of 2nd Phase (End of 3rd Month) As % of Inhibition	Result of 3rd Phase (End of 6th Month) As % of Inhibition
1	SV-01	45	M	99	24	21
2	SV-02	40	F	100	61	42
3	SV-03	55	M	98	24	13
4	SV-04	46	M	18	47	76
5	SV-05	62	F	74	28	4
6	SV-06	26	F	85	23	16
7	SV-07	49	F	88	99	85
8	SV-08	45	M	50	99	76
9	SV-09	35	F	99	23	14
10	SV-10	40	M	98	98	78
11	SV-11	39	M	99	41	26
12	SV-12	62	M	65	31	18
13	SV-13	37	M	100	99	76
14	SV-14	30	M	21	99	70
15	SV-15	51	M	98	94	85
16	SV-16	28	M	99	16	13
17	SV-17	45	M	99	14	12
18	SV-18	47	M	99	47	25
19	SV-19	56	M	99	99	88
20	SV-20	50	F	90	87	62
21	SV-21	55	M	100	20	12
22	SV-22	65	M	99	99	77
23	SV-23	58	M	99	99	77
24	SV-24	47	M	98	15	12
25	SV-25	21	M	97	25	17
26	SV-26	26	M	16	44	67
27	SV-27	28	M	99	97	81
28	SV-28	30	M	99	97	84
29	SV-29	30	M	35	97	77
30	SV-30	24	M	88	98	87
31	SV-31	50	F	99	18	8
32	SV-32	60	M	96	98	84
33	SV-33	60	M	99	98	86
34	SV-34	56	F	99	26	13
35	SV-35	28	F	99	41	28
36	SV-36	28	M	99	93	84
37	SV-37	65	M	100	74	46
38	SV-38	45	M	99	17	2
39	SV-39	23	M	99	23	11
40	SV-40	44	M	99	20	11
41	SV-41	55	M	6	97	84
42	SV-42	23	M	99	99	85
43	SV-43	25	F	99	82	71
44	SV-44	28	M	99	71	49
45	SV-45	28	M	96	99	82
46	SV-46	35	M	100	20	7
47	SV-47	27	M	23	100	85
48	SV-48	34	M	100	82	59
49	SV-49	33	M	80	77	64
50	SV-50	33	M	97	28	18

Another studies on the efficacy of Covishield after two dosesconcluded that, two doses of Covishield does not satisfactorily prevent the infection this might be because of the down fall of neutralizing antibodies after 3-4 months of 2nd dose. Many reinfection and breakthrough cases were reported during this pandemic after the 2nd dose of vaccination but the symptoms were mild and infection was not severe. These studies demonstrate the waning of immune response in naïve Yadav PD, et al., 2022].

On the basis of our study and available literature, we can conclude that the antibodies production after the standard second dose of the vaccines is remarkable and it also lower the severity and symptoms of infection. After the 3-5 months of vaccination a down fall is observed in the neutralizing antibodies titer which could lead to a possibility of infection. Few cases were reportedeven after complete vaccination.

Also in our study the level of neutralizing antibodies titer fell down 3-5 months post vaccination. 42% (21) of 50 subjects were resulted negative for neutralizing antibodies during third phase of study i.e. after 6-7 months of the second dose. And nearly 10% (5) of individuals were near at the cut off level indicating low titers. Since the number of subject individuals was less in our study we feel more elaborative study would have given a better picture. Our study also highlighted the importance of booster dose as reported by others authors also.

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