Detection of neutralizing antibody towards spike protein of SARS-CoV-2 in vaccinated subjects

Pushpa Kachari 1, Jerin Francis 1, Anuradha K 2, Deepa S 3 and Pushpa kachari 1,*

1 Department of Microbiology, Post Graduate, Mysore Medical College & research Institute, Mysuru, India.
2 Department of Microbiology, Professor & HOD, Mysore Medical College & research Institute, Mysuru, India.
3 Department of Microbiology, Associate Professor, Mysore Medical College & research Institute, Mysuru, India.

World Journal of Advanced Pharmaceutical and Life Sciences, 2023, 04(02), 001–006

Publication history: Received on 12 February 2023; revised on 22 March 2023; accepted on 25 March 2023

Article DOI: https://doi.org/10.53346/wjapls.2023.4.2.0058

Abstract

Background: The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged in December 2019 in Wuhan, China causing a severe acute respiratory disease (COVID-19). The virus has rapidly spread into a pandemic affecting over more than 40 million & mortality more than 5 million as per recent data. Due to lack of specific treatment vaccination was considered as main measures to control the pandemic. India’s drugs regulator, Drugs Controller General of India (DCGI) authorized emergency use for AZD1222/Covishield (ChAdOx1-Recombinant) and BBV152/Covaxin in January 2021 and vaccination was started beginning with healthcare and frontline workers and to all aged above 18 years.

The present study aimed to know the presence of neutralizing antibodies in vaccinated subjects with or without history of infection by SARS-CoV-2.

Materials & Methods: After obtaining Institutional Ethical Clearance 260 Vaccinated subjects who consented to participate were included for the study. Details were recorded in the proforma. After sterile precaution about 2 ml blood sample was collected in plain vacutainer tube & Serum sample was separated and stored at -20°C. The neutralizing antibody towards spike protein was detected by performing commercial ELISA (MICROLISA, J MITRA) according to the manufacturer’s guidelines. The results were interpreted by calculating Inhibition rate which is above 30% was considered as positive.

Result: Among 260 vaccinated subjects 197(75.6%) positive for neutralizing antibody. Among them 178(76.2%) were Covishield and 82(74.07%) Covaxin vaccinees. Age & sex distribution were recorded the highest in 18-40 years 35106(53.8%) & female 61(54.4%). Vaccinated and infected 151(86.2%) & only vaccinated 46(54.1%). Good antibody titre was observed in 78.5% upto 4 months of vaccination.

Conclusion: The vaccinated subjects with history of SARS-cov-2 infection showed better neutralizing antibody titre with good percentage of persistence Nab till 4th months of post vaccination period.

Keywords: SARS-C0V-2; COVID-19; Neutralizing antibody (Nab); Covishield; Covaxin

1. Introduction

The novel Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) first emerged in December 2019 has rapidly spread into a pandemic affecting over more than 40 million & mortality of more than 5 million as per recent data till the
time of writing the article. The globe has faced multiple waves of the infection since its inception. Every wave has drawn new challenges in combating the infections as the world has confronted various mutant strains of this parent virus. The challenge is both in formulating therapeutic as well as preventing measures to contain the infection. Looking back, it is realized that introducing preventing measures in terms vaccinations or other therapeutic means was the most demanding but the hardest call of that hour. However, in the grace of fast developing research works the world had been witnessed the weapon ‘vaccines’ to halt the infections. As paucity of specific treatment vaccination was considered as main tool to control the pandemic. India’s drugs regulator, Drugs Controller General of India (DCGI) authorized emergency use for AZD1222/Covishield (ChAdOx1-Recombinant) & BBV152/Covaxin in January 2021. Vaccine has been rolled out for of Indian populations till the date which was first introduced for the health care workers >18 & covered the rest of populations in phase manner.2,3 Structurally the SARS-CoV-2 contains the spike (S), matrix (M), and envelope (E) proteins. The S protein is primely selected for vaccine development as a critical target for production of neutralizing antibodies (NAbs). NAbs are crucial for virus clearance and protection against SARS-CoV-2. It prevents the interaction between the Receptor Binding Domain viral spike glycoprotein (RBD) & cell surface receptor Angiotensin Converting Enzyme-2 (ACE2). It was evidenced that serum neutralizing antibodies rapidly appear after SARS-CoV-2 infection & vaccination. The same is maintained in serum for several months.6 It is to be mentioned worthy that of late the pandemic has been plunged into a controllable stage as recent data recorded the world incidence of SARS-CoV-2 is 0.1 to 10,000 per 100,000 population in most of the regions. In this regard vaccination has the definite remarkable role. Since the introduction of vaccines against the virus, numerous studies recorded its efficacy, protective roles & adaptability with the changing trends of infective dynamics. Our study was carried out during the peak of second wave of the SARS-CoV-2 infection while the most of the health care workers had completed of having second dose of recommended vaccines. The study aimed to detect the presence of neutralizing antibodies against the spike protein of SARS-CoV-2 virus in the vaccinated subjects.

2. Material and methods

Institutional Ethical Clearance was obtained from the Institutional ethical review committee. A total of 260 vaccinated subjects who consented to participate were included for the study. Required details of the participated subjects were recorded in the proforma generated. The Nab detection method in brief, with sterile precaution About 2 ml blood sample was collected in plain vacutainer with sterile precaution & Serum was separated and stored at -20 °C for future reference. The neutralizing antibody towards spike protein was detected by performing commercial ELISA (MICROLISA, J MITRA) according to the manufacturer’s guidelines. Inhibition rate was calculated according to the recommendation provided by the manufacture. The neutralizing antibody was considered to be positive if the inhibition rate was > 30%.

3. Results

In the study a total of 260 vaccinated subjects participated. Among the subjects 178 were vaccinated with Covishield while another 82 subjects had taken Covaxin. The neutralizing antibody were positive in total 197(75.6%) of vaccinated subjects. Among the groups 136(76.2%) had taken Covieshield & 61(74.07%) were subjected to Covaxin.

<table>
<thead>
<tr>
<th></th>
<th>Covishield n=178</th>
<th>Covaxin n=82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>136 (76.2)</td>
<td>61 (74.07)</td>
</tr>
<tr>
<td>Negative</td>
<td>42 (23.8)</td>
<td>21 (25.93)</td>
</tr>
</tbody>
</table>

During the study period people aged above 18 years were subjected for vaccination. Age group in between 18-40 years had highest study subject representation in our study. The neutralizing antibody was seen the highest in age group 18-40 years, 106 (53.8%) with female preponderance, 112 (56.9%)
A total of 175 subjects had history of Covid-19 infection prior to or in post vaccination period. Among these vaccinated subjects with infection history 151(86.2%) showed presence of neutralizing antibody, while it was 54.1% among the subjects who did not have any history of infection during the period.

Table 3 Distribution of Neutralizing antibody among the vaccinated with & without history of infection

<table>
<thead>
<tr>
<th>Total Neutralizing Ab present</th>
<th>Vaccination &amp; Infection n=175</th>
<th>Only vaccination n=85</th>
</tr>
</thead>
<tbody>
<tr>
<td>197(75.6%)</td>
<td>151(86.2%)</td>
<td>46(54.1%)</td>
</tr>
</tbody>
</table>

It was seen that the subjects with prior history Covid-19 infection had taken vaccines mostly after 2 months infections. 3 subjects had history of infection in less than 1 month of vaccination & 9 had infection history within 1-2 months of vaccination. All the subjects were positive for neutralizing antibodies (100%).

Table 4 Distribution of Neutralizing antibody with history of infection

<table>
<thead>
<tr>
<th>Tested for Neutralizing Ab, N-175</th>
<th>Infection history, Pre vaccination, N-139</th>
<th>Post vaccination, N-36</th>
<th>Neutralizing Ab, Pre vaccination, N-121</th>
<th>Post vaccination, N-30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 month</td>
<td>NA</td>
<td>3</td>
<td>NA</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>1-2 months</td>
<td>NA</td>
<td>9</td>
<td>NA</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>2-3 months</td>
<td>9</td>
<td>12</td>
<td>9 (100%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>3-4 months</td>
<td>36</td>
<td>12</td>
<td>30 (83.3%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>4-5 months</td>
<td>30</td>
<td>NA</td>
<td>30 (100%)</td>
<td>NA</td>
</tr>
<tr>
<td>5-6 months</td>
<td>42</td>
<td>NA</td>
<td>36 (85.7%)</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>22</td>
<td>NA</td>
<td>16 (72.7%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Good percentage of population showed the presence of Neutralizing antibodies till the average duration of 4 to 6 months of post vaccination period. It was about average 78.5% of total subjects showed positive neutralizing antibodies till 4 months of vaccinations.
Table 5 Distribution & duration of persistence of Neutralizing antibody level with different candidate vaccines

<table>
<thead>
<tr>
<th>Tested for Neutralizing ab</th>
<th>No of tested N-260</th>
<th>Covishield n-178</th>
<th>Covaxin n-82</th>
<th>Positive with Covishield</th>
<th>Positive with Covaxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 month</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>NA</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>1-2 months</td>
<td>30</td>
<td>08</td>
<td>22</td>
<td>05 (66.6%)</td>
<td>22 (100%)</td>
</tr>
<tr>
<td>2-3 months</td>
<td>36</td>
<td>12</td>
<td>24</td>
<td>12 (100%)</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td>3-4 months</td>
<td>30</td>
<td>09</td>
<td>21</td>
<td>09 (100%)</td>
<td>12 (57.1%)</td>
</tr>
<tr>
<td>4-5 months</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>12 (80%)</td>
<td>NA</td>
</tr>
<tr>
<td>5-6 months</td>
<td>74</td>
<td>74</td>
<td>0</td>
<td>59 (79.1%)</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>39 (65%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

4. Discussion

The study was carried out during the period of surge of the second wave of Covid-19 infections. In the study among 260 subjects 197 (75.6%) were positive for neutralizing antibody against the SARS Cov-2 spike proteins. Study by Alexis R Demobrenre showed higher neutralizing antibodies >95% after second dose of vaccinations.7 While in another study by Xiaoguang Li et al among 127 vaccinated participants, 66 (51.97%) were positive for Nab.8 Study by Annika Fendler et al suggested the Nab to different strain of SARS-Cov-2 as 83% for wild type, 61%, 53%, 54% alpha, beta & delta type after the second dose of vaccinations.9 However, in the refereed study subjected vaccine was different from us.8,9

Study by Awadhesh Kumar Singh et al showed 95% of seropositivity after second dose of vaccines with Covishield & Covaxin vaccines. The same study recorded 98.1% and 80.0% seropositivity with individual Covishield & Covaxin vaccine respectively.10 Our study recorded the neutralizing antibody positivity to Covishield & Covaxin (76.2 Vs 74.07%). A relatively low percentage of Nab in our study probably because of lower number of study subjects.

In this context study by Vidya Arankalle et al reported lower titre of Nab in both the pre & post vaccination infective individual in comparison to Covishield Vs Covaxin vaccinees, the study group found significant(p<0.001) in this. Though it was mentioned that the study group among Covaxin vaccinees was comparatively lower.11

In the present study the highest Nab was detected in the age group 18-40 years with female preponderance to male (56.9%Vs 53.8%). Study by Xiaoguang Li et al found that the positive rate of neutralizing antibody was 47.22% (17/36) in men and 53.85% (49/91) in women, which is highly concordant to our study finding.

In the present study the vaccinated subjects yielded higher neutralizing antibodies percentage with accompanied history of infection with COVID-19 than those without infection which was 86.2% Vs 54.1%. Similar finding was recorded in the study by Jorge Hernandez Bello et al.12 According to the study the percentage of neutralizing antibodies in subjects with prior covid-19 infection was 98% Vs 72% in subjects with no history of infection. Therefore, a natural infection prior vaccination induces higher neutralizing antibodies percentage than immunized individuals without prior COVID-19. This finding suggests that a vaccination enhances and optimizes the neutralization capacity in vaccinated individuals with prior history of infections. The observation of higher basal neutralization percentage of the group with prior COVID-19 in comparison to the vaccinated individuals with no history of COVID-19 is suggestive of introducing next dose of vaccines for further. Thus, the third dose of vaccines has also been rolled out among the populations to cover up the lag as well as to cope up the emerging variants of SARS-Cov-2 vaccine of late.

It is important to determine how long neutralizing antibodies persists in vaccinated subjects. Study by Xiaoguang Li et al found the positive neutralizing antibody rate100.00%, 60.00%, 58.33%, 55.56%, 43.14%, 28.57%, and 0.00% at 2–4, 5–8, 9–12, 13–16,17–20, 21–24, and >24 weeks, respectively.8 However, the present study observed an average 78.5% positive neutralizing antibody rate till the 6 months of vaccination. Another study by Vidya Arankalle et al found that at 6 months of post vaccination, 71.7% COVISHIELD and 0% COVAXIN recipients were Nab-positive.11 The variation of persistence of Nab could probably because of the efficacy variation of the subjected vaccine candidates & demographic dynamics of the study populations.
Though at present the Covid-19 pandemic level over the globe has reached a controllable stage, however the stringent screening concern is still on need to trace the infective dynamics of SARS-CoV-2. Our study was an effort to learn the impact of the vaccine candidates in providing Nab in terms of demographic & infective patterns of the studied population.

**Limitations**

One of the major limitations of our study was limited study population in comparison to other studies. Among the study population Covaxin vaccinees was also less in number to that of covishield vaccinees.

**5. Conclusion**

The study showed that there is no difference in the antibody production in both types of vaccines. The infection prior or after the vaccination showed good antibody level. The protective level was seen up to 4-6 months. The vaccination may need to be administered repeatedly for its effectiveness. The time, type and dose of administration need to be determined by large population study in different epidemiological scenario.

**Compliance with ethical standards**

**Acknowledgments**

At the outset of the study we would like to convey our heartfelt gratitude to J.Mitra group for their aid in providing the required ELISA kit for the study.

**Disclosure of conflict of interest**

The present study does not bear any conflict in regards of writing & carrying out the study.

**Statement of ethical approval**

The present study has been approved by Institutional Ethics Committee (IEC), Mysore Medical College & Research Institute, Mysuru. The Ethical approval letter is being furnished as supplementary document with the main article manuscript submitted.

**Statement of informed consent**

Informed consent was obtained from all individual participants included in the study.

**References**

[1] WHO Corona Virus Disease (Covid-19) Pandemic Dashboard


[3] Indian council of Medical Research (ICMR) website. https://www.icmr.gov.in


