ORIGINAL ARTICLE

sciendo

10.2478/AMB-2024-0005

SEROPREVALENCE OF IGG ANTIBODIES AGAINST SARS-COV-2 N PROTEIN AMONG VACCINATED AND UNVACCINATED SUBJECTS IN LAHORE, PAKISTAN

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Abstract. *The spread of SARS-CoV-2 represented a global health crisis. On May 5, 2023, the chief of the World Health Organization (WHO) officially announced the end of COVID-19 as a global health emergency. Serological assays can identify previously infected SARS-CoV-2 individuals, even if they did not go for testing while acutely ill. The current study sought to detect antibodies directed against the nucleocapsid of SARS-CoV-2 (IgG against the SARS-CoV-2 N protein) in both vaccinated and unvaccinated COVID-19 individuals. Of the 100 participants, 53 and 47 were vaccinated and unvaccinated, respectively. The vaccination status of the cohort based on gender data indicates that 41 (41%) of all participants were vaccinated males, whereas 12 (12%) were vaccinated females. We found that 42 (42%) were unvaccinated males and 5 (5%) were unvaccinated females. Of 53 vaccinated subjects, 42 and 11 participants were positive and negative for IgG against the SARS-CoV-2 N protein, respectively. Of 47 unvaccinated participants, 28 and 19 were positive and negative for IgG against the SARS-CoV-2 N protein, respectively. The average of S/P "Sample/Positive control" percentages, which correlate to levels of IgG against SARS-CoV-2 N protein, were significantly higher among the vaccinated patients (73.8%) as compared to non-vaccinated patients (57.1%), with p = 0.02. There was a downward trend in levels of IgG against the SARS-CoV-2 N protein with increasing age, except for the 60–69 age group.*

Key words: *SARS-CoV-2 N protein, COVID-19, SARS-CoV-2*

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Received: *29 May 2023;* ***Revised/Accepted:*** *10 August 2023*

INTRODUCTION

The emergence and spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the coronavirus disease-2019 pandemic (COVID-19) [1]. On May 5, 2023, the chief of the World Health Organization (WHO) officially announced the end of COVID-19 as a global health emergency: https://news.un.org/en/story/2023/05/1136367. Antibody tests are useful diagnostic assays that are mainly applied to patients in their later disease courses and to people who have been infected with the virus in the past [2, 3]. Serological assays could help in identifying previously

infected COVID-19 individuals, in spite of the fact that they did not go for testing while acutely ill [4]. Serologic testing helps in determining immunity, stratifying vaccine receipt individuals, and documenting vaccine response, which can provide details about return-to-work status and travel decisions [2, 3]. They also have a pivotal role in the evaluation of the epidemiology at local, national, and global levels [5, 6].

Serological assays normally depend on the detection of antibodies against nucleoprotein (N) or spike protein (S) because these are highly immunogenic SARS-CoV-2 proteins [7]. The Spike protein is present on the SARS-CoV-2 surface, and it has the ability to attach to ACE-2 receptors. It is a good sign of a strong immune system to detect antibodies against spike protein because anti-spike protein antibodies were found to have neutralizing effects in vitro [8, 9]. A recent study, which detected both anti-N and anti-S antibodies, found an average seropositivity duration with anti-N assay specificity comparable to anti-S [10].

COVID-19 infection can elicit antibodies against nucleoprotein and spike protein [9, 11, 12], of which, based on animal virus-challenge research, the spike protein-specific antibodies were neutralized and lined with a protected immune response [13, 14]. All of the approved COVID-19 vaccines are able to produce strong antibody responses [15]. Although there is not enough data about the persistence of antibodies induced by the effects of vaccines, infection-induced neutralizing antibodies can be detected for an average of six months after the onset of symptoms [16].

Spike-specific and neutralizing antibodies showed a remarkable rise in people who took a single shot of vaccine after SARS-CoV-2 infection, which notably surpassed levels observed with only COVID-19 infection. In fact, COVID-19 patients who are recovered and vaccinated could be the best COVID-19 convalescent plasma donors [17]. Researchers also found that the production of antibodies is higher in severe diseases than in mild ones. Previous infections and vaccination are associated with a low rate of COVID-19 infections [18-20]. The majority of previous studies examined the antibody response to the spike protein of SARS-CoV-2 [21-24]. In the present study, we aim to measure and assess the antibody level produced against the SARS-CoV-2 nucleocapsid protein in both vaccinated and unvaccinated COVID-19 subjects.

MATERIALS AND METHODS

Participant blood samples

We recruited 100 volunteers at the Institute of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Human serums from all 100 participants were collected in plastic serum separator gel blood collection tubes on April 9, 2021. Age, sex, and vaccination status were recorded for all participants. All subjects declared that they did not have any current physically identifiable COVID-19 symptoms at the time of sample collection. All participants in this study were Pakistanis and took two doses of the Sinopharm COVID-19 vaccine.

ELISA Procedure

The IgG antibody level produced against the nucleocapsid (N) protein of SARS-CoV-2 (IgG against SARS-CoV-2 N protein) in human serum was detected using indirect semi-quantitative ELISA (the ID-Screen® IgG against SARS-CoV-2 N protein indirect kit, Innovative diagnostic (ID), France). Optical densities (OD) were read and recorded at 450nm. The plates were read within 30–40 minutes as a way to avert loss of optical density.

The S/P ratio “Sample/Positive Control” for each sample was calculated and expressed as a percentage (S/P %):

$$\times\ 100 : \frac{\text{OD (Sample)-OD(Negative Control)}}{\text{OD (Postive Control)-OD (Negative Control)}} \times 100$$

As indicated by the kit, it is recommended to keep the threshold at S/P% > 60% to indicate positive for IgG against the SARS-CoV-2 N protein.

Statistical analysis

Statistical analysis was done using GraphPad Prism. Age was classified into the following five groups: 20-29 years old, 30-39 years old, 40-49 years old, 50-59 years old, and 60-69 years old. Seropositivity outcomes were analyzed in the different groups. The Student T test was used to assess the statistical significance.

RESULTS

Gender-wise vaccination status

Of 100 participants, we found that 83 (83%), and 17 (17%) were males and females, respectively. Out of the 100 participants, 53 were vaccinated and 47 participants were unvaccinated, respectively. All 53 vaccinated subjects confirmed that they received full COVID-19 vaccination shots. The subjects in each age group were as follows: 16 subjects in the 20-29 age group, 25 subjects in the 30-39 age group, 23 subjects in the 40-49 age group, 32 subjects in the 50-59 age group, and 4 subjects in the 60-69 age group. The vaccination status of the cohort based on

gender data indicates that 41 (41%) of all participants were vaccinated males, whereas 12 (12%) were vaccinated females (Figure 1). 42 (42%) were unvaccinated males, and 5 (5%) were unvaccinated females. Figure 2 shows the distribution of vaccination status and gender among all participants.

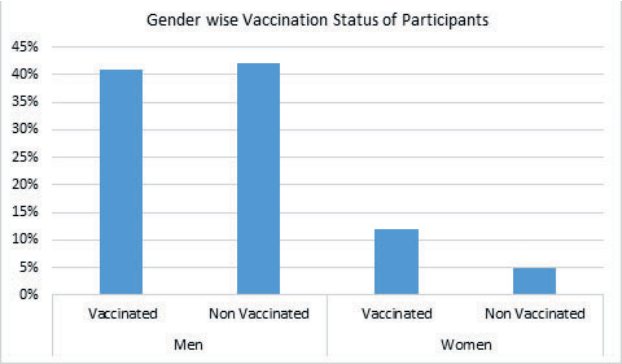

Fig. 1. Vaccination status of the cohort based on gender data

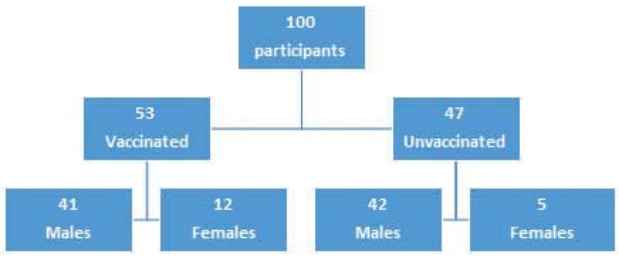

Fig. 2. Distribution of vaccination status in relation to gender among all participants

Antibody level, vaccination status, and age groups

Among all participants, 70 (70%) were positive for IgG antibodies against the SARS-CoV-2 N protein. Out of 53 vaccinated subjects, 42 and 11 participants were positive and negative for IgG antibodies against the SARS-CoV-2 N protein, respectively. Out of 47 unvaccinated participants, 28 and 19 were positive and negative for IgG antibodies against the SARS-CoV-2 N protein, respectively. Figure 3 shows the distribution of vaccination status in relation to IgG against the SARS-CoV-2 N protein among all participants. This study found that 11 out of 53 (20.7%) vaccinated participants were negative for IgG antibodies against the SARS-CoV-2 N protein, whereas 19 out of 47 (40.4%) unvaccinated participants were negative. This showed that 79.3% and 59.6% of vaccinated subjects and unvaccinated participants were positive, respectively. The average of S/P percentages, which correlate to IgG against SARS-CoV-2 N protein, were significantly higher among vaccinated patients (73.8%) as compared to non-vaccinated (57.1%), with p = 0.02 (Figure 4). There was a downward trend in IgG antibodies against the SARS-CoV-2 N protein with age, except for the 60-69 age group (Figure 5). No significant difference was detected among all the age groups with p > 0.05.

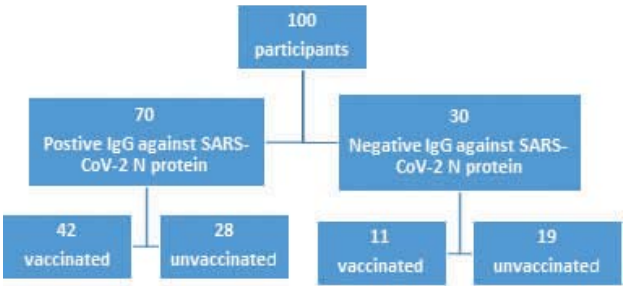

Fig. 3. Distribution of vaccination status in relation to IgG against SARS-CoV-2 N protein among all participants

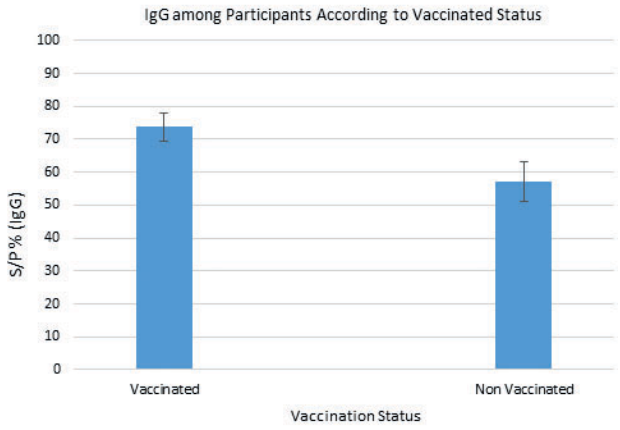

Fig. 4. The graph shows the IgG (S/P%) among vaccinated and non-vaccinated study participants. The IgG (S/P%) was significantly higher among the vaccinated patients as compared to the non-vaccinated, with p = 0.02

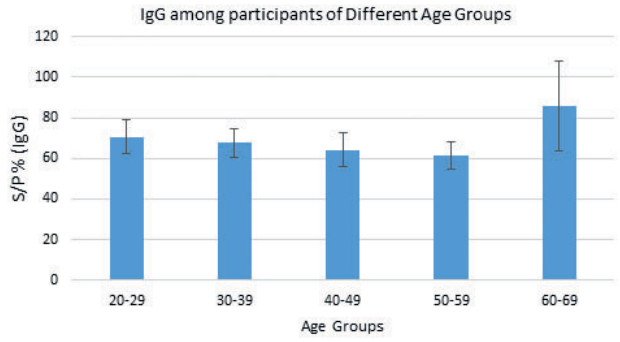

Fig. 5. The graph shows the IgG antibodies among the participants distributed among different age groups. There was no significant difference among all the age groups with p > 0.05

DISCUSSION

There is no conclusive information about antibody responses after COVID-19 infection [25-27], or among

COVID-19-vaccinated subjects [28-30]. IgG against SARS-CoV-2 N protein were detectable in the serum of infected COVID-19 people [31]. Our study reported that 28 out of 47 (59.6%) unvaccinated subjects were positive for IgG against the SARS-CoV-2 N protein, implying that they had been infected with COVID-19 before. These 28 unvaccinated individuals positive for IgG against the SARS-CoV-2 N protein did not give conclusive answers as to whether they had been infected with COVID-19 before or not. Previous study found that 76% (367/484) of recovered COVID-19 patients were positive for IgG against the SARS-CoV-2 N protein [32]. Our study found that 19 out of 47 (40.4%) unvaccinated subjects were negative for IgG against the SARS-CoV-2 N protein, giving two possibilities. The first is that they have not been infected with COVID-19 before. The second one is that they may have had COVID-19 a long time ago and the antibodies have waned. These 19 unvaccinated individuals, negative for IgG against the SARS-CoV-2 N protein, did not also give conclusive answers as to whether they had a previous infection with COVID-19 or not. A recent study reported that the rate of seroreversion at six months in mildly symptomatic or asymptomatic patients is higher than in symptomatic COVID-19 patients [33]. Waning antibody levels in previously infected COVID-19 patients have been previously reported [34], and this could make them prone to reinfection again.

We found that 11 vaccinated participants were negative for IgG against the SARS-CoV-2 N protein, which could be due to taking vaccines for more than 6 or 12 months prior to conducting the experiment. Subsequent antibody waning after vaccine administration is expected, increasing the possibility of reinfection if “booster” doses are not taken [35]. Seroprevalence generally indicates the number of patients who had values greater than the sensitivity threshold of the serological test used in the specific research and does not indicate the real number of patients who have totally lost their immunity or antibodies [34, 36]. A recent study reported a decline in antibody levels at twelve weeks and six months after vaccine administration, implying that the immunity waned with time [37], and this underscores the urgent need for monitoring possible booster vaccines.

Dong and colleagues reported that there is an inverse correlation between IgG titers to COVID-19 infection and age for those older than 18 years of age but a direct correlation with age in adults [38]. Moreover, 44-66-year-old adults are likely to have high IgG values, compared to younger adults (18-44-year-olds), within six months following the onset of symptoms, yet there is no difference seen at twelve months after the onset of symptoms [39]. We noticed that there was a downward trend in IgG against the SARS-CoV-2 N protein with age, except for the 60–69 age group. The reason for the high level of IgG against SARS-CoV-2 N protein in the 60-69 age group could be due to the fact that only 4 subjects participated in this study, and 3 out of them were positive (S/P % > 90%). Seroprevalence in 65-year-olds or older persons was usually lower than in 18- to 49-year-old persons [40]. Previous study found that the SARS-CoV-2 IgG values exhibited a positive correlation with age in adults and an inverse correlation with age in pediatric populations [41]. The strongest point in our data is that vaccinated people had higher levels of antibodies compared to unvaccinated people. Previous study reported that there were high levels of antibodies in 98% of the vaccinated cohort [42]. A recent study found that 46% of patients with hematological malignancy who received two mRNA vaccine doses did not produce SARS-CoV-2 IgG and were therefore vaccine non-responders [43].

The main strength of our study includes a clear indication of the immune responses and protection level against COVID-19 through measuring the IgG antibodies among vaccinated and non-vaccinated COVID-19 subjects. However, the present study also has limitations. First, we do not have the timeline of COVID-19 patients or vaccinated COVID-19 people. This means that we do not know how long they had COVID-19 infection or were vaccinated, or whether they had the vaccine before or after COVID-19. Previous studies showed that spike-targeting and neutralized antibodies showed a remarkable rise in people who administered a single vaccine shot after COVID-19 infection, which notably surpassed values seen in people who had only COVID-19 infection. Second, the sample was also limited to volunteers at the Institute of Microbiology at the University of Veterinary and Animal Sciences (UVAS) in Lahore and cannot give a clear picture of the Lahore population or the wider population of Pakistan. Third, there was underrepresentation of the female population, and conclusions on gender differences in both infection and vaccine responses require more studies. The study focused on measuring the IgG antibody level against nucleocapsid (IgG against SARS-CoV-2 N protein). In future studies, IgG antibodies targeting the SARS-CoV-2 spike, IgM antibodies, and viral nucleic acids should be assessed to study the kinetics of the SARS-CoV-2 prevalence.

CONCLUSION

The average of S/P “Sample/Positive control” percentages, which correlate to levels of IgG against

SARS-CoV-2 N protein, were significantly higher among the vaccinated patients (73.8%) as compared to the non-vaccinated (57.1%), with p = 0.02. There was a downward trend in levels of IgG against the SARS-CoV-2 N protein with age, except for the 60-69 age group.

Disclosure summary: *The authors have nothing to disclose.*

Acknowledgments: *This paper is part of the Ph.D. research work of the first author (Ahmed Donia), who is extremely thankful to Professor Habib Bokhari for supervising this work. He would like to warmly thank all staff at The Biosafety Level-3 Laboratory for Emerging Pathogens, Institute of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, for their extreme support in carrying out this work. He would also like to warmly thank The World Academy of Science (TWAS), Microbiology and Public Health Laboratory, COMSATS University Islamabad (CUI), Pakistan, and Kohsar University Murree.*

Author contributions: *AD and HB conceived and designed the study. AD, MFS, MN, RS coordinated, carried out the experiments, and analyzed the data. AD drafted the original manuscript. AD, MFS, RS, AJ, MN, TY, and HB did necessary editing of the manuscript. All authors read and approved the manuscript.*

Funding: *This research received no external funding*

Ethical Approval: *The study was approved by the internal review board of UVAS (with Approval No. 391). Consent was taken from all participants enrolled in the study.*

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