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Decline of humoral immune responses after natural SARS-CoV-2 infection can be efficiently reversed by vaccination

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Abstract

Our aim was to analyze severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody level kinetics after coronavirus disease 2019 (COVID-19) infection and determine the efficiency of vaccination on SARS-CoV-2-specific antibody levels. The study included 50 SARS-CoV-2 infected and 70 uninfected cases. Levels of SARS-CoV-2-specific IgG nucleocapsid protein (IgG-NP), IgG spike protein (IgG-SP), IgM nucleocapsid protein (IgM-NP), and IgA spike protein (IgA-SP) antibodies were evaluated by an enzyme-linked immunosorbent assay in sera obtained at baseline, 1st, 3rd, and 6th month follow-up visits for infected cases and at postvaccination visits for all cases. In symptomatic cases ($n = 50$), IgG-SP levels were decreased in 6 months compared with baseline, while IgA-SP levels were significantly increased. IgG-NP levels were significantly decreased in symptomatic cases at the 6-month visit. After vaccination, IgG-SP levels were increased in symptomatic cases compared with prevaccination levels. Among subjects vaccinated with CoronaVac (the Sinovac COVID-19 vaccine), infected cases had approximately double the IgG-SP level of uninfected cases. SARS-CoV-2-specific antibody levels were higher at the baseline in symptomatic cases. Nevertheless, all infected cases showed significantly reduced IgG-SP levels at the 6th month. Vaccination effectively increased IgG-SP levels.

Key words: SARS-CoV-2, COVID-19, immunoglobulins, vaccination, neutralizing antibodies

Résumé

L'objectif des auteurs consistait à analyser la cinétique du taux d'anticorps spécifiques au SRAS-CoV-2 après une infection par la COVID-19 et de déterminer l'efficacité de la vaccination sur le taux d'anticorps spécifiques au SRAS-CoV-2. L'étude a porté sur 50 cas infectés par le SRAS-CoV-2 et 70 cas non infectés. Les niveaux d'anticorps spécifiques au SRAS-CoV-2 de type IgG-nucléocapside (IgG-NP), IgG-spicule (IgG-SP), IgM-nucléocapside (IgM-NP) et IgA-spicule (IgA-SP) ont été évalués par ELISA dans les sérums obtenus au début de l'étude, et lors des visites de suivi au 1er, 3e et 6e mois pour les cas infectés et aux visites post-vaccination pour tous les cas. Chez les cas symptomatiques ($n = 50$), les niveaux d'IgG-SP étaient diminués en 6 mois par rapport à la ligne de base, tandis que les niveaux d'IgA-SP étaient significativement augmentés. Les niveaux d'IgG-NP étaient significativement diminués lors de la visite au 6e mois chez les cas symptomatiques. Après la vaccination, les niveaux d'IgG-SP étaient augmentés chez les cas symptomatiques par rapport aux niveaux prévaccination. Parmi les sujets vaccinés avec le CoronaVac (le vaccin Sinovac COVID-19), les sujets infectés présentaient un taux d'IgG-SP environ deux fois supérieur à celui des cas non infectés. Les niveaux d'anticorps spécifiques au SRAS-CoV-2 sont plus élevés au départ chez les cas symptomatiques. Néanmoins, tous les cas infectés présentent des niveaux d'IgG-SP significativement réduits au 6e mois. La vaccination augmente efficacement les niveaux d'IgG-SP. [Traduit par la Rédaction]

Mots-clés : SARS-CoV-2, COVID-19, immunoglobulines, vaccination, anticorps neutralisants

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China at the end of 2019 and developed rapidly, causing a global pandemic (Sonsoz et al. 2021). Although the clinical syndrome caused by SARS-CoV-2 varies widely, it can cause mild symptoms to severe pneumonia,

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respiratory distress syndrome, or death (Fu et al. 2020). On the other hand, it has been reported that many people may carry the virus without showing any symptoms (Mizumoto et al. 2020).

SARS-CoV-2, an RNA virus, facilitates cellular entry by binding to receptor on the cell surface angiotensin-converting enzyme 2 (ACE2) (Jackson et al. 2022). The virus particle is structurally composed of spike, membrane, nucleocapsid, and envelope proteins. In particular, the receptor-binding domain (RBD) part of the spike protein interacts with the ACE2 receptor, allowing entry into host cells (Lan et al. 2020). Both spike and nucleocapsid proteins have been reported to be highly immunogenic, making them potential targets for serological diagnosis (Juno et al. 2020; Okba et al. 2020).

Immunoglobulins are glycoprotein molecules found in blood, mucosa, and various body fluids. They are also involved in the neutralization of microorganisms and activation of the complement system. IgG is responsible for long-term immunity after infection or vaccination. IgG antibodies can persist for years after an infection, but they are not usually used as a marker to indicate an active infection (Wang et al. 2018).

Serological testing of virus-specific antibodies is one of the significant methods used to diagnose coronavirus disease 2019 (COVID-19). Since SARS-CoV-2 RNA cannot be detected in nasopharyngeal swabs of many infected individuals, it is important to detect specific antibodies developed against the spike protein of the virus (Petherick 2020). Detection of humoral immune responses is also very important for determining the effectiveness of vaccines developed against COVID-19.

A specific drug therapy for COVID-19 disease has not yet been developed; therefore, vaccination plays an important role in preventing the SARS-CoV-2 pandemic. Various vaccine types are being developed against SARS-CoV-2 based on recombinant subunits, inactivated virus, virus-like particles, and DNA and RNA containing viral epitopes (Lurie et al. 2020). It has been observed that there is a decrease in the number of cases and hospitalization rates in countries where vaccination is carried out intensively (Ledford 2021). Although the efficacy of each vaccine is different, the importance of evaluating the vaccine response in COVID-19 infected and uninfected cases is obvious.

Neutralizing antibodies produced after infection or vaccination are known to be important in protection against re-exposures to viral or bacterial pathogens (Griffin 2018). Thus, after both vaccination and COVID-19 infection, it is important to determine the neutralizing activity rather than quantification of antibodies. The reinfection of vaccinated individuals underlines the importance of the neutralizing capacity of antibodies. The follow-up of long-term antibody responses in infected cases and the evaluation of the neutralizing effects of these antibodies are important in the management of the COVID-19 pandemic. In addition, the evaluation of neutralizing antibody levels is also important in demonstrating both long-term protection and the real-life efficacy of the vaccine (Sadarangani et al. 2021).

In this study, the levels of SARS-CoV-2-specific IgA and IgG antibodies were determined at multiple time points for a follow-up duration of 6 months after the onset of disease symptoms in individuals infected with SARS-CoV-2. Additionally, to determine the efficiency of vaccination, specific IgG levels were analyzed in sera of infected and uninfected individuals after vaccination with CoronaVac (Sinovac Life Sciences, Beijing, China). Moreover, neutralizing activity of serum samples was analyzed with a pseudovirus-based assay. In this way, the determination of antibody levels was evaluated in the Turkish population as a measure of herd immunity to be developed against the anti-SARS-CoV-2 virus. Since COVID-19 has been reported to have a more severe clinical progression in male patients (Jin et al. 2020), we also compared antibody profiles of men and women with COVID-19 infection.

Materials and methods

Study subjects

Most of the samples included in the study were obtained from the nurse staff of the Istanbul Medical Faculty Hospital. Seventy cases not infected with SARS-CoV-2 (uninfected group) and 50 COVID-19 cases infected with SARS-CoV-2 and displaying typical symptoms of the infection were included in the study. SARS-CoV-2 positivity was determined according to a positive SARS-CoV-2 real time polimerase chain reaction (RT-PCR) nasopharyngeal swab test and typical chest computed tomography findings, and SARS-CoV-2 infected cases were denoted as symptomatic. All blood samples were collected after the recovery period. To avoid inclusion of asymptomatic individuals who were reinfected with COVID-19, participants who were positive for antinucleocapsid protein IgM at the recovery period were excluded. All included symptomatic cases ($n = 50$) received hydroxychloroquine, azithromycin, and favipiravir. Uninfected cases were included on the basis of absence of clinical history of COVID-19 symptoms and negativity for anti-SARS-CoV-2-specific antibody subtypes. No mortality or symptomatic reinfection was seen in this cohort of patients and donors during the 6-month follow-up period.

Evaluation of anti-SARS-CoV-2-specific IgG nucleocapsid protein, IgG spike protein, IgM nucleocapsid protein and IgA spike protein

This study was approved by the Clinical Research Ethics Committee of Istanbul University Faculty of Medicine (11/05/2020-81367), and all included volunteers read and signed the "informed voluntary consent form" accordingly.

Serum samples were collected at the time of first negative PCR test after SARS-CoV-2 infection (baseline) and 1, 3, and 6 months after the baseline time point from symptomatic cases and 28 days after the second CoronaVac vaccination from all subject groups. Serum samples were portioned and stored at −20 °C until use. Anti-SARS-CoV-2-specific IgG nucleocapsid protein (IgG-NP), IgG spike protein (IgG-SP), IgM nucleocapsid protein (IgM-NP), and IgA spike protein (IgA-SP) (Cat. No. Er 2606-9601) antibodies in sera were evaluated by an enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN Medizinische Labordiagnostika AG, Germany). As no quantified international reference serum exists for

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antibodies against SARS-CoV-2, the calibration is performed in ratios that are a relative measure for the concentration of antibodies in serum or plasma. Calibrator optical density (OD) was obtained from a standard calibrator solution found in the kit. The ratio/index value for the results was calculated (serum OD/calibrator OD). The ratio was considered negative if <0.8, limiting if ≥0.8 to <1.1, and positive if ≥1.1 for all parameters. All included uninfected cases showed negative results before vaccination.

Pseudovirus-based neutralization assay

SARS-CoV-2 spike gene with a 19-amino acid truncation to remove the endoplasmic reticulum retention signal (Spike△19) was amplified from pTwist-EF1-Alpha-SARS-CoV-2-S-2xStrep (Addgene plasmid 141382) and cloned into the pCMV-VSV-G plasmid (Addgene plasmid 8454) using BamHI restriction site to remove the VSV-G coding region. The D614 variant of this pCMV-Spike△19 plasmid was generated by site-directed mutagenesis and used for pseudovirus production. For the expression of SARS-CoV-2 E and N proteins, pCMV-SARS-CoV-2-E and pCMV-SARS-CoV-2-N vectors were constructed by amplifying the respective genes from pLVX-EF1alpha-SARS-CoV-2-N-2xStrep-IRES-Puro (Addgene plasmid 141391) and pLVX-EF1alpha-SARS-CoV-2-E-2xStrep-n IRES-Puro (Addgene plasmid 141385) vectors and cloning into the pCMV-VSV-G vector (Addgene plasmid 8454) using BamHI and EcoRI sites to remove the VSV-G gene.

For the lentiviral expression of secreted embryonic alkaline phosphatase (SEAP), the LeGo-SEAP-iG2 vector was constructed by amplification of the SEAP gene from the pNiFty3-N-SEAP (InvivoGen, San Diego, CA, USA) plasmid and cloned into the LeGo-iG2 (Addgene plasmid 27341) vector with BamHI and EcoRI sites before the IRES region.

For production of spike-pseudotyped lentiviral particles, 293FT cells (5×10^6) were seeded in a poly-L-lysine-coated 100 mm tissue culture dish, and when cells reached 70% confluency on the following day, 7.5 μg of lentiviral vector plasmid encoding the SEAP reporter gene (LeGo-SEAP-iG2) was cotransfected using calcium phosphate precipitation along with 3.75 μg of pMDLg/pRRE (Addgene plasmid 12251), 2.25 μg of pRSV-Rev (Addgene plasmid 12253), 0.5 μg of pCMV-Spike△19 (D614G) (encoding for spike protein carrying the D614 mutation and lacking the 19 amino acids in its C terminal tail), 0.25 μg of pGBW-m4134096 (encoding for SARS-CoV-2 M protein) (Addgene plasmid 152039), 0.25 μg of pCMV-SARS-CoV-2-N (encoding for SARS-CoV-2 N protein), and 0.25 μg of pCMV-SARS-CoV-2-E (encoding for SARS-CoV-2 E protein) plasmids. After 8–10 h, the medium was changed, and the supernatants containing pseudovirus particles carrying SARS-CoV-2 S, M, N, and E proteins were collected at 48 h after changing the medium, filtered through a 0.45 μm syringe filter, divided into aliquots, and stored at –80°C.

For a pseudovirus-based neutralization assay, 16 h prior to infection, 7.5×10^3 293FT-hACE2 cells were seeded in 100 μL full growth media Dulbecco's Modified Eagle's Medium (DMEM) + 10% fetal bovine serum (FBS) into flat bottom 96-well plates. Plasma samples were thawed to room temperature, heat inactivated at 56°C for 30 min, and mixed with pseudovirus supernatants at various dilutions ranging from 1:10 to 1:10 000 in triplicates in the wells of a fresh 96-well plate and incubated at 37°C for 1 h. At the end of the incubation, these mixtures were transferred to wells seeded with 293FT-hACE2 cells and incubated overnight in the presence of 8 μg/mL protamine sulfate. The medium was changed at 24 and 48 h after infection, and the collected supernatant at 72 h was used to assay for SEAP expression using QUANTI-Blue substrate as per the manufacturer's instructions (InvivoGen, San Diego, CA, USA).

The results of neutralization assays were plotted by normalization to samples where no plasma was used, and the half-maximal inhibitory concentration (IC_{50}) was calculated using four-parameter nonlinear regression. The results of regression analysis were only deemed acceptable when the R^2 value was above 0.9. The dilution factor corresponding to the calculated IC_{50} was calculated as $1/IC_{50}$ used as neutralizing titer value.

ELISA-based neutralization assay

SARS-CoV-2-specific neutralizing antibody activity in sera was determined by NeutraLISA (EUROIMMUN Medizinische Labordiagnostika AG, Germany; Cat. No. EI 2606-9601-4). The test was performed according to the manufacturer's procedure. The neutralization assay provides semiquantitative detection of neutralizing antibodies that prevent SARS-CoV-2 S1/RBD from binding to the ACE2 receptor. Neutralizing antibodies compete with ACE2 for binding in wells coated with S1/RBD. The results were evaluated with a photometric measurement of color intensity at a wavelength of 450 nm (reference wavelength 620–650 nm) with a spectrophotometer device (ELx800, BioTek Instruments, Inc.). The calculation of the percentage of inhibition (IH%) was based on the mean value of the blank measurement. In the obtained serum results, the rate was accepted as negative if IH% was <20; IH% ≥20 to <35 was considered borderline and IH% ≥35% as positive.

Statistical analyses

One-way ANOVA (nonparametric) was used between multiple groups, and a nonparametric Mann–Whitney test was used for paired group comparisons. GraphPad Prism 9 program was used for analysis and graphs. $P < 0.05$ was considered statistically significant.

Results

Demographic data

The demographic characteristics of the donors participating in the study are presented in Table 1. Of the 120 cases included in the study, 47 were male and 73 were female. The symptomatic group consisted of 19 men and 31 women. Seventy uninfected cases were included in the study; 28 of them were men and 42 of them were women. Fever and fatigue (32%) were the most common symptoms in infected cases (Table 1). Table S1 presents the number of symptomatic cases evaluated in terms of IgA-SP, IgG-SP, and IgG-NP antibody levels at the baseline, 1st, 3rd, and 6th month follow-up time points and 28 days after second vaccination.

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Table 1. Demographic characteristics, initial signs, and symptomatology.

	Symptomatic cases ($N = 50$)	Uninfected cases ($N = 70$)
Gender	31 Females	42 Females
	19 Males	28 Males
Age [median (min.–max.)]	40 (18–58)	40 (27–63)
Initial symptoms		
Fever	32%	
Diarrhea	18%	
Fatigue and myalgia	32%	
Cough	26%	
Dyspnea	12%	
Anosmia/dysgeusia	30%	
Nausea	8%	
Comorbid conditions		
Hypertension	10%	
Diabetes mellitus	2%	
Chronic obstructive pulmonary disease (COPD) or asthma	4%	
Coronary artery disease	4%	
Congestive heart failure	None	
History of solid malignancy	None	
History of hematologic malignancy	None	
Bacillus Calmette-Guerin (BCG) vaccine	28%	
Treatment		
	Hydroxychloroquine, azithromycin, favipiravir	

IgG-SP antibody levels are decreased in infected subjects by the 6th month

Approximately 75% of all antibodies in serum were IgG-type immunoglobulins with a long half-life (Schroeder and Cavacini 2010). Sera IgG-SP antibody levels measured at the 6th month in symptomatic cases were found to be significantly lower compared with baseline, 1st month, and 3rd month ($P < 0.001$, $P < 0.001$, and $P = 0.005$, respectively (Fig. 1A). The IgG-SP levels were decreased in symptomatic cases by 49% at 6 months as compared with baseline.

IgG-SP antibody levels increased after vaccination

Whether the COVID-19 vaccines create antibodies to SARS-CoV-2 in individuals is very important in terms of determining the effectiveness of the vaccine. Sera IgG-SP levels of all uninfected cases were measured before vaccination; there was no IgG-SP positivity (Fig. S1). After the second dose of CoronaVac, which is an inactivated COVID-19 vaccine, 66 of 70 uninfected cases had detectable SARS-CoV-2-specific IgG. These results showed that efficiency for humoral response was 94.2% in uninfected cases.

IgG-SP levels of COVID-19 infected and uninfected cases were also comparatively evaluated in sera obtained 28 days after second CoronaVac vaccination. IgG-SP antibody levels after vaccination were found to be significantly higher in previously infected cases compared with those who were previously uninfected ($P = 0.008$) (Fig. 1A).

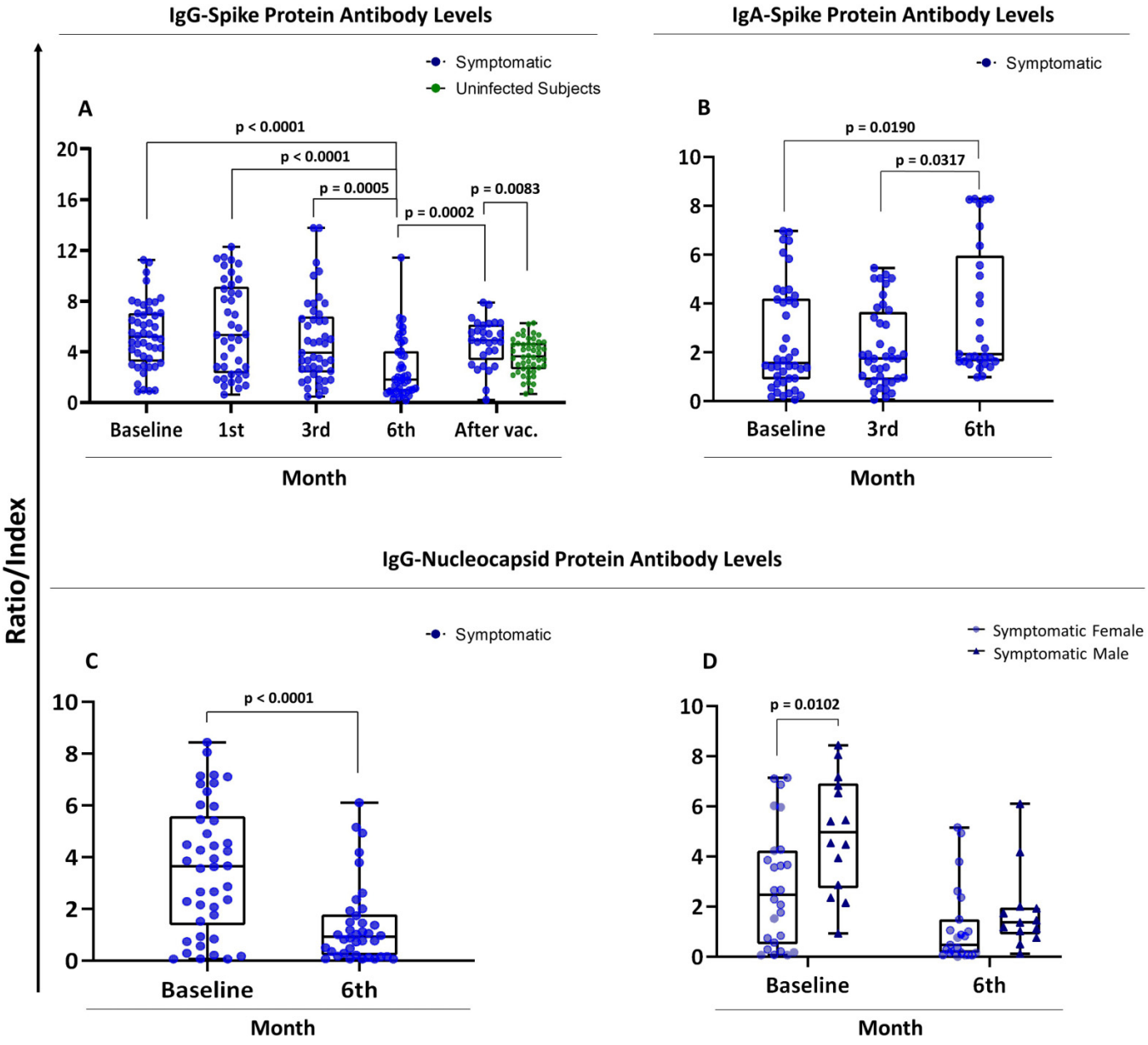
Furthermore, in previously infected cases, IgG-SP levels that were significantly reduced after 6 months successfully increased after the second dose of vaccination ($P = 0.002$) (Fig. 1A). After vaccinations, IgG-SP levels were significantly increased and almost attained the baseline levels (baseline levels before vaccination) compared with the 6th month (11% lower than the baseline). Thus, the time-dependent reduction in IgG-SP levels was reversed by two vaccinations.

We also investigated whether there were differences in IgG-SP antibody levels of symptomatic female and male cases. However, there was no significant difference in the gender comparison of IgG-SP antibody levels at baseline, 1st, 3rd, and 6th month time points, and after vaccination. Likewise, no difference was found in terms of IgG-SP levels among male and female cases of the uninfected participants (data not shown). In addition, there was no significant difference between the IgG-SP levels of participants with the age of $\geq$50 versus <50 (data not shown).

IgA-SP antibody levels are increased in infected subjects by the 6th month

IgA found in intestinal and respiratory tract mucus secretions and epithelial surface is an antibody subtype that plays an important role in COVID-19 disease by neutralizing the virus and preventing its binding to lung epithelial cells (Chao et al. 2020). We observed that serum IgA-SP levels of symptomatic cases were increased in the 6th month compared with baseline and 3rd month levels ($P = 0.019$ and $P = 0.031$,

Fig. 1. Serological investigation of SARS-CoV-2-specific circulating IgG-SP (A), IgA-SP (B), and IgG-NP (C, D) antibody levels in symptomatic cases. ELISA screening of SARS-CoV-2-specific IgG-SP (A), IgA-SP (B), IgG-NP antibody levels (C), and IgG-NP levels of females and males (D) was analyzed in symptomatic cases. Graphs showing ratio/index values of antibody levels at baseline, 1st, 3rd, and 6th month, and after vaccination. *P* values were determined with an unpaired Mann–Whitney *U* test.



respectively) (Fig. 1B). Compared with the baseline levels, the IgA-SP levels were increased by 43% in symptomatic cases 6 months after the baseline time point. There was no significant difference in IgA-SP antibody levels between symptomatic female and male patients (data not shown).

IgG-NP antibody levels are higher in symptomatic cases

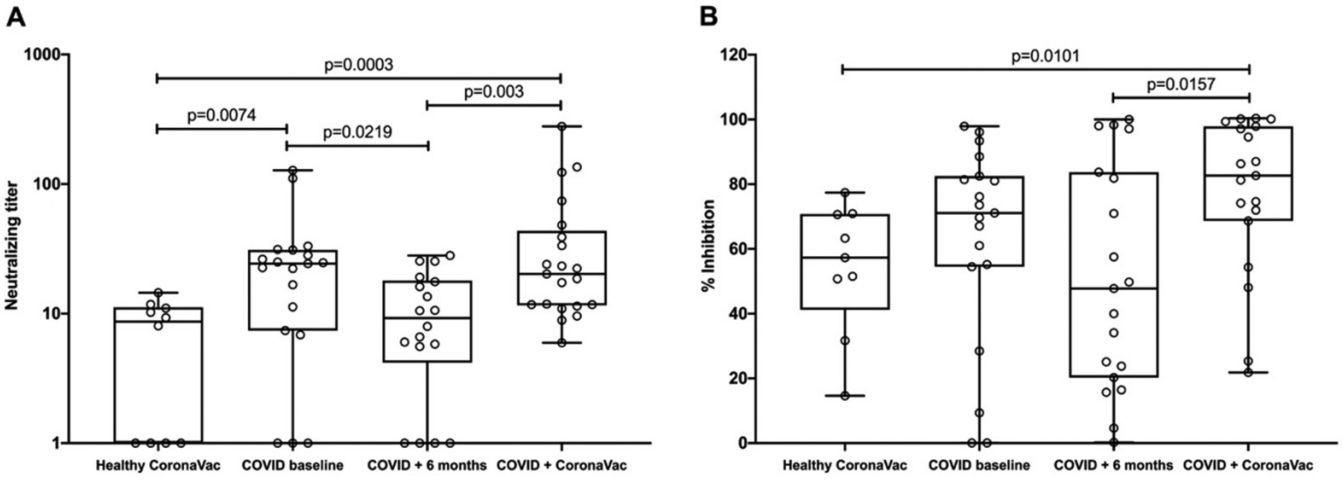
The SARS-CoV-2 genome consists of various structural proteins. Among them, NP plays essential roles in replication, viral genome packaging, and virion assembly and is an important antigenic segment. NP has been shown to be immunogenic at the time of infection (Surjit and Lal 2008). When SARS-CoV-2 NP-specific antibody responses were evaluated in sera of subjects, IgG levels were found to be lower at the 6th month for symptomatic cases compared with baseline levels ($P < 0.0001$) (Fig. 1C). Average IgG-NP levels in symptomatic cases were decreased by 62% at 6 months after baseline. IgG-NP antibody levels were found to be lower in female than male subjects at baseline ($P = 0.010$) but not at the 6th month (Fig. 1D).

Neutralizing antibody levels

Antibodies targeting the viral RBD of the SARS-CoV-2 spike protein have been shown to exhibit virus-neutralizing capacity. Blocking RBD with specific antibodies prevents the virus

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Fig. 2. Neutralizing activity against SARS-CoV-2. (A) Neutralizing titer measured by a cellular assay using lentiviral vectors pseudotyped with SARS-CoV-2 S, M, N, and E proteins. (B) Inhibition of ACE2 binding measured by an ELISA-based assay. *P* values were determined with an unpaired Mann–Whitney *U* test.



from entering the cell. Therefore, it is important to define the neutralizing activity of antibodies produced after infection or vaccination. The neutralizing activity of collected sera was measured by both a pseudovirus-based and an ELISA-based neutralization assay (Fig. 2). Analysis of neutralizing activity against a lentiviral vector pseudotyped with SARS-CoV-2 S, M, N, and E proteins showed that uninfected individuals vaccinated with CoronaVac displayed moderate levels of neutralizing activity that was slightly lower than that of individuals recovering from natural SARS-CoV-2 infection (Fig. 2A). It was also observed that COVID-19 infected individuals had a significant drop in neutralizing activity as shown 6 months after the initial infection when no further vaccination was carried out. Corroborating the results from serum IgG-SP antibody measurements, vaccination following natural infection effectively boosted the humoral immune responses and these individuals showed the highest neutralizing activity within this cohort. Similar results were observed using the ELISA-based neutralization assay (Fig. 2B). Furthermore, no difference was found between neutralizing antibody activity in the male–female comparison (data not shown).

Discussion

SARS-CoV-2 is an infection that affects the whole world and causes serious deaths (Ozbalak et al. 2021). While most patients show mild disease that does not require hospitalization, some patients can present with moderate and severe forms of the disease. The results of a meta-analysis displayed that the pooled rate of asymptomatic COVID-19 cases during the course of infection ranged from 28.0% to 31.4% (Alene et al. 2021). This situation affects the determination of the actual frequency of individuals suffering from COVID-19 disease in the community.

Vaccines developed against SARS-CoV-2 are very important for protection against the disease and herd immunity (Fontanet and Cauchemez 2020). It is important to determine the humoral immune response to SARS-CoV-2 and to evaluate the neutralizing activity in the sera of individuals who have had the disease or have been vaccinated. It is also extremely important to detect the change in antibody levels over time to follow up protection of the vaccine and evaluate the effect of booster shots.

We showed that IgG responses directed against different antigens of the SARS-CoV-2 virus are gradually diminished after the first contact with the virus and are significantly diminished within 6 months. However, in long-term follow-up, we found that the level of IgA increased over time, in contrast with IgG. Similarly, it has been reported that IgA immune response remains for a longer period than IgG in a fraction of COVID-19 patients (Ivanov and Semenova 2021). An important underlying cause might be asymptomatic reinfections with SARS-CoV-2, since samples were collected during the peak period of the pandemic in Turkey. Reinfections with low viral doses may putatively cause a local IgA response in the respiratory system without causing systemic symptoms and elevated antiviral IgG responses as proposed previously. The validity of this notion needs to be further studied. Cross-reaction due to concomitant infections with other coronavirus types and prolonged production of certain IgA-inducing cytokines (such as TGF-β) might be other potential reasons for this IgA–IgG response discrepancy (Lv et al. 2020; Ferreira-Gomes et al. 2021).

In some studies, no significant changes were found in IgG levels in patients with COVID-19 during long-term follow-up (6 months) (Alzaabi et al. 2021), while others reported that COVID-19-specific IgG levels decreased for up to 22 weeks in convalescent plasma donors (Steenhuis et al. 2021). The reduction in IgG levels and differences between individuals are important in evaluating seroprevalence.

Thus, 6 months after the symptom onset appears to be a suitable time for vaccination of individuals with past COVID-19 disease to prevent reinfection. Our results showed that in-

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fected cases with SARS-CoV-2 produced higher titers of IgG antibodies and had higher neutralizing activity than uninfected cases after vaccination. This was presumably due to activation of SARS-CoV-2-specific memory B cells in previously infected patients, as shown in other viral infections (Roltgen and Boyd 2021).

Inactive virus vaccines are widely used in vaccination worldwide, and such vaccines have been found to be harmless and effective in preventing various diseases such as polio and influenza virus (Vellozzi et al. 2009). CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine developed against SARS-CoV-2 (Stefanelli et al. 2020) in China. In our study, antibody titers and neutralization capacity of antibodies were evaluated in COVID-19 symptomatic and uninfected cases after two doses of CoronaVac vaccination. In symptomatic cases included in the study, postvaccination IgG levels after two doses of CoronaVac were found to be significantly higher than prevaccine 6-month IgG levels. Our findings showed that vaccination to both infected and uninfected cases increased the level of IgG antibodies and neutralizing capacity.

The main goal in vaccination is to ensure the development of virus-specific neutralizing antibody and T-cell response and to target long-term protection against SARS-CoV-2 infection (Dai and Gao 2021). Therefore, it is important to examine the presence of neutralizing antibody responses in COVID-19 patients and their persistence in SARS-CoV-2 infected individuals. Together with measurement of serum antibody levels, we also analyzed neutralizing activity in the sera of vaccinated individuals (previously infected or uninfected), demonstrating similar results. While neutralizing activity was significantly diminished at 6 months after infection, individuals who received vaccination after infection demonstrated a significant elevation in neutralizing activity. Also, in some studies, a decrease in neutralization titer was detected up to approximately 1 year after SARS-CoV-2 infection (Gaebler et al. 2021; Wheatley et al. 2021). Similar to our results, a study indicated that uninfected individuals ($n = 90$) had no detectable antibodies or neutralizing antibodies in any of them at baseline, but 7 days after the second dose of vaccine, the individuals' neutralizing antibody titers were above the positivity threshold. In addition, they found that neutralizing antibodies were always statistically higher in infected cases compared with uninfected cases after administration of the second dose of vaccine in time periods (Favresse et al. 2021).

It is reported that COVID-19 disease affects men more than women (Jin et al. 2020). In our study, there was no difference between symptomatic men and women in terms of IgA-SP and IgG-SP parameters, whereas IgG-NP levels were higher in men than women. Recent studies showed that there is a correlation between IgG-NP level and poor clinical outcome (Batra et al. 2021). Although all the patients enrolled in our study showed mild symptoms, our findings are consistent with these data. However, for more reliable and statistically robust results, the number of samples should be increased, and different test parameters should be examined.

Conclusion

In summary, we found that the majority of recovered COVID-19 patients showed detectable SARS-CoV-2-specific IgG 6 months after symptom onset, regardless of disease severity, but this decreased over time. We similarly found that neutralizing antibody titers decreased relatively over time. In addition, neutralizing antibody activity was higher after vaccination in individuals infected with COVID-19 compared with uninfected individuals. These results indicate the importance of boosting the immune response after natural infection by additional vaccination.

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Competing interests

The authors have declared that no competing interests exist.

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Ethical statement

All procedures performed in this study adhered to the amendments of the Helsinki Declaration. The study protocol was approved by the Clinical Research Ethics Committee of Istanbul University Faculty of Medicine (11/05/2020-81367).

Informed consent

A written informed consent was obtained from all study participants.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjm-2022-0003.

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