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ORIGINAL ARTICLE

Evaluation of SARS-CoV-2 antibody persistence and viral spread in stool: a long-term care experience before COVID-19 vaccination

Oya Ozlem Eren-Kutsoylu[1] · Ozgur Appak[2] · Arzu Nazli-Zeka[1] · Gokcen Omeroglu-Simsek[3] · Nil Tekin[4] · Basak Bayram[5] · Ayca Arzu Sayiner[2] · Nesim Tanglay[6] · Vildan Avkan-Oguz[1]

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Abstract

Background Due to elderly residents, nursing homes/assisted living facilities were the most affected places in COVID-19 pandemic. Besides symptomatic patients, asymptomatic patients were detected during routine screening.

Aim This study aims to determine the factors that affect antibody response and viral shedding in stool samples after natural exposure to the virus in residents and staff who recovered from COVID-19 before the vaccine was available.

Methods This prospective cross-sectional study was conducted at the nation's highest-capacity Residential and Nursing Home. Blood samples were collected between December 15, 2020 and January 15, 2021 from participating residents and staff for anti-SARS-CoV-2 antibody testing. Stool samples were obtained for SARS-CoV-2 PCR testing 2 months after COVID-19. The Social Sciences (SPSS) program version 15.0 was used for statistical analysis. The Mann–Whitney *U* test compared SARS-CoV-2 antibody concentration between two groups.

Results Four hundred sixty-four (52.3%) residents and 424 (47.7%) staff participated. Entirely 259 (29.2%) participants were anti-SARS-CoV-2 IgG (+) and 255 (28.7%) were SARS-CoV-2 PCR (+). Both antibody and PCR positivity was detected in 196 (76.9%). In PCR (−) group, 63 (10.0%) participants were SARS-CoV-2 IgG (+). Antibody titers were found highest in SARS-CoV-2 PCR (+) male residents. SARS-CoV-2 IgG titers were significantly high in SARS-CoV-2 PCR (+) and hospitalized participants regardless of age. Stool samples were obtained from 61(23.9%) participants and were found negative.

Conclusion A durable SARS-CoV-2 IgG antibody response was monitored at least 9 months after the participants were diagnosed with COVID-19. SARS-CoV-2 antibody positivity was detected 76.9% in PCR (+) and 10.0% in PCR (−) participants. Knowing the duration of detectable antibodies is an important finding for developing disease prevention and public health strategies.

Keywords Antibody persistence · Natural exposure · Nursing home · Residential care · SARS-CoV-2 antibody · Stool viral shedding

Introduction

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a severe threat to global public health [1]. Due to the advanced age of the residents, nursing homes/assisted living facilities were the most affected places in this pandemic [2–4].

In assisted living facilities, COVID-19 outbreaks occurred among both residents and staff. Furthermore, residents were hospitalized and sometimes died because of severe COVID-19 [5–7]. However, during the same period,

✉ Oya Ozlem Eren-Kutsoylu
oyaeren76@yahoo.com; oya.eren@deu.edu.tr

[1] Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

[2] Division of Medical Virology, Department of Medical Microbiology, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey

[3] Department of Pulmonary Diseases, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

[4] Narlidere Nursing Home Elderly Care and Rehabilitation Center, Izmir, Turkey

[5] Department of Emergency Medicine, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

[6] Turkish Ministry of Family and Social Services, İzmir Provincial Directorate, Izmir, Turkey

some asymptomatic residents and staff found SARS-CoV-2 PCR positive during routine screening. Some had COVID-19 asymptomatically with SARS-CoV-2 PCR negative test results. Studies showed that advancing age, disease severity, and hospitalization are associated with higher SARS-CoV-2 antibody response [8–10].

Unfortunately, due to the pandemic conditions, the exact number of asymptomatic residents and staff and the incidence of COVID-19 is unknown in elderly care facilities. Also, it is still unclear how long the antibodies can be detected after the disease and whether factors affect this situation.

This study measured SARS-CoV-2 antibody levels in residents and staff of the nation's largest elderly care facility. Regardless of the clinical severity of the disease, we aimed to determine the factors that affect antibody response and viral shedding in stool after natural exposure to the virus in participants who recovered from COVID-19 in the period before the vaccine was available.

Materials and method

Study population

This prospective cross-sectional study was conducted in Narlidere Nursing Home Elderly Care and Rehabilitation Center. We planned to assess staff and elderly individuals residing at our nation's highest-capacity residential and nursing home. There are 735 residents and 455 staff in this center.

Study design

All the staff and nursing home residents included in the study signed an informed consent form. Residents who do not have authority to sign instead, their relatives signed the consent form. We separated participants into two groups, staff and residents, to understand the effect of both infectiveness and age. The data of all consenting participants were recorded using data collection forms which included demographic data regarding age, gender, comorbidities, and information regarding COVID-19 history, SARS-CoV-2 RT-PCR test dates and results, and hospitalization because of COVID-19. A positive PCR test was accepted as a confirmed disease, regardless of disease symptoms in the participants.

There were two disease peaks in our country until the sampling process, between April–May 2020 and October–December 2020.

Since the staff in this institution worked with 10-day shifts during the pandemic, all the staff were routinely screened with PCR test every 10 days. PCR test was performed on the residents living in the institution when they had complaints or when the virus was detected in their close contacts. Blood samples were taken for SARS-CoV-2 antibody testing from residents and employees from 15 December 2020 through 15 January 2021. On 20 January, vaccination for COVID-19 started in the center. Also, stool samplings were obtained from participants who agreed in this period.

SARS-CoV-2 antibody testing

ARCHITECT SARSCoV-2 IgG II Quantitative (Abbott Laboratories, Wiesbaden, Germany) was used. The SARS-CoV-2 IgG II Quant test is a two-step chemiluminescent microparticle enzyme immunological (CMIA) technology. This test detects qualitative and quantitative IgG antibodies directed to the receptor-binding domain (RBD) of the S1 subunit of the SARS-CoV-2 spike protein in human serum and plasma. Results are expressed in arbitrary units per milliliter (AU/mL). The manufacturer reports the analytical measurement interval of the test as 21 to 40,000 AU/mL, and the positivity cutoff is $\geq$ 50 AU/mL.

Stool SARS-CoV-2 RT-PCR testing

Stool samples were obtained from participants 2 months after SARS-CoV-2 PCR positivity. Stool samples were stored in an appropriate sterile container at + 4 °C and delivered to the laboratory on the same day. A 20% suspension was prepared from the samples reaching the laboratory, centrifuged at 10,000 g for 1 min, and viral RNA extraction was performed with the supernatant using the EZ-1 virus kit (Qiagen, Hilden, Germany). Real-time RT-PCR on the Rotor-Gene Q device with the help of a commercial kit (BioSpeedy SARS-CoV-2, Bioeksen R&D Technologies, Turkey) that detects two separate viral gene regions (ORF1ab and N genes) was carried out.

Statistical analysis

Statistical analysis was performed using the 15.0 version of the Statistical Package for the Social Sciences (SPSS) program (IBM, Armonk, NY, USA). Categorical variables were analyzed using Fisher's exact test. The Mann–Whitney *U* test compared SARS-CoV-2 IgG antibody concentration between two groups. A *p* value less than 0.05 was considered statistically significant.

Ethical approval

The Republic of Turkey Ministry of Health (Approval Number: Oya Özlem Eren Kutsoylu -2020–09-20T20_48_31) and the Ethics Committee of Dokuz Eylul University, Faculty of Medicine (Approval Date:15/06/2020, No:2020/13–29) approved the study. The study was supported under DEU Scientific Research Projects (project number: 2470).

Table 1 Demographic data of the participants

	Staff $n=424$ (%)	Residential home residents $n=319$ (%)	Nursing home residents $n=145$ (%)	Total $n=$ (%)
Age mean/median ± SD (min–max years)	41.66 ± 7.39 (22–64)	80.16 ± 7.001 (60–97)	87.38 ± 7.164 (66–102)	62.96 ± 21.41 (22–102)
Female	206 (48.6)	212 (66.5)	117 (80.7)	535 (60.2)
Underlying disease	70 (%16.5)	294 (92.2)	145 (100)	509 (57.3)
Hypertension	31 (7.3)	243 (76.2)	105 (72.4)	379 (42.7)
Chronic heart disease	3 (0.7)	92 (28.8)	41 (28.3)	136 (15.3)
Diabetes mellitus	9 (2.1)	37 (11.6)	16 (11.0)	62 (7.0)
Chronic lung disease	17 (4.0)	28 (8.8)	8 (5.5)	53 (6.0)
Chronic renal failure	0 (0)	16 (5.0)	9 (6.2)	25 (2.8)
Chronic liver disease	2 (0.5)	0 (0)	0 (%0)	2 (0.2)
Chronic neurological disease	2 (0.5)	55 (17.2)	35 (24.1)	92 (10.4)
Neoplasia	2 (0.5)	7 (2.2)	2 (1.4)	11 (1.2)
Dementia	0 (0)	18 (5.6)	93 (64.1)	111 (12.5)
Hospitalization	9 (2.1)	28 (8.8)	28 (19.3)	65 (7.3)

Results

A total of 464 (52.3%) residents and 424 (47.7%) staff participated in the study. Out of 464 residents, 145 (31.25%) were nursing home residents, and 319 (68.75%) were residential home residents. Demographic data of the participants were given in Table 1. Two hundred fifty-nine (29.2%) participants had SARS-CoV-2 IgG, and 255 (28.7%) had viral RNA detected by the RT-PCR assay (Table 2).

Antibody-positive, PCR positive group

Antibodies were detected in 196 (76.9%) participants who had positive PCR tests. The proportion of the residents was 102/136 (75%), and the staff was 94/119 (79%) in this group (Table 2). Regardless of age, the antibody titers of those with positive PCR test was found to be significantly higher ($p=0.0001$). Mean antibody titer was significantly higher in PCR positive resident group than in PCR positive staff group (205.54 ± 387.25 AU/mL and 129.80 ± 352.05 AU/mL, respectively, $p=0.029$).

There was a statistical difference in antibody titers between 48 hospitalized and 88 non-hospitalized PCR positive residents (371.73 ± 573.97 AU/mL and 114.89 ± 176.54 AU/mL, respectively, $p=0.000$). Likewise, a significant antibody titer difference was observed between nine hospitalized and 110 non-hospitalized PCR positive staff (597.86 ± 1011.07 AU/mL and 91.51 ± 198.93 AU/mL, respectively, $p=0.001$). Antibody titers were significantly higher in hospitalized participants without considering age ($p=0.000$).

There was no significant difference in antibody titers between PCR positive male and female groups ($p=0.810$); between genders in residents ($p=0.136$) and staff ($p=0.916$). Similarly, no significant difference was determined in antibody titers between PCR positive female residents and staff (174.36 ± 359.68 AU/mL and 107.97 ± 230.61 AU/mL, respectively, $p=0.217$). However, the difference was significant between PCR positive male residents and staff (280.37 ± 442.35 AU/mL and 145.62 ± 419.69 AU/mL, respectively, $p=0.037$).

Table 2 SARS-CoV-2 antibody and PCR results of the participants

SARS-CoV-2 antibody	Staff PCR $n=424$ (%)		Residents' PCR			
			Residential home R $n=319$ (%)		Nursing home R $n=145$ (%)	
	Positive $n=119$ (%)	Negative $n=305$ (%)	Positive $n=89$ (%)	Negative $n=230$ (%)	Positive $n=47$ (%)	Negative $n=98$ (%)
Antibody positive	94 (79.0)	26 (8.5)	66 (74.2)	27 (11.7)	36 (76.6)	10 (10.2)
Antibody negative	25 (21.0)	279 (91.5)	23 (25.8)	203 (88.3)	11 (23.4)	88 (89.8)
Total	119 (100)	305 (100)	89 (100)	230 (100)	47 (100)	98 (100)

The presence of underlying disease in the residents and staff did not make a difference in antibody titers ($p=0.097$). Also, no statistical difference was observed in antibody titers when the underlying diseases were analyzed individually and in groups.

Antibody-negative, PCR positive group

Fifty-nine (9.4%) participants were SARS-CoV-2 IgG negative, although their PCR tests were positive. Thirty-four (57.6%) were residents and 25 (42.4%) were the staff. Forty (67.8%) of them had the PCR test positivity in the first pandemic peak (April–May 2020), while the rest in the second peak (October–December 2020). Except for three hospitalized residents, all other residents and staff members were asymptomatic.

Out of 34 residents, 33 (97.1%) were PCR (+) at the first peak. Because of symptomatic disease, three nursing home residents were hospitalized in April 2020. All three residents were female, had dementia and chronic heart disease. When the underlying diseases were analyzed, no statistical difference was observed in antibody titers.

Out of 25 staff, seven (28.0%) were PCR (+) at the first peak, the remaining 18 (72.0%) were PCR positive in the second peak, and six (33.3%) of these staff members' PCR positivity was determined 3 to 4 weeks before the antibody blood testing at the second peak. Two had hypertension, and one had diabetes mellitus as an underlying disease.

Antibody-positive, PCR negative group

Among 633 PCR negative participants, 63 (10.0%) had SARS-CoV-2 antibodies. Thirty-seven (58.7%) were residents, and 26 (41.3%) were staff. Four out of 63 (6.35%) were hospitalized with pneumonia. Non-hospitalized participants in this group were all asymptomatic.

Although their PCR test results were negative, four residents were diagnosed with COVID-19 pneumonia and were hospitalized. All the hospitalized patients were female, had dementia and chronic heart disease. Three of them were nursing home residents. Of 26 staff, none of them was hospitalized. Only two male employees had hypertension.

Antibody negative, PCR negative group

Among 633 PCR negative participants, 570 (90.0%) were also SARS-CoV-2 antibody negative. Two hundred and ninety-one (%51.1) were residents, and 279 (48.9%) were staff.

Antibody titers of all the participants, according to PCR and antibody status, were given in Table 3.

Stool samples were obtained from 47 residents and 14 staff members. Stool sampling could only be obtained from 23.9% of participants. They all had positive SARS-CoV-2 PCR tests from nasopharyngeal swabs in the second month of the proven disease. None of the stool samples was PCR positive.

Discussion

This elderly care and rehabilitation center study demonstrated that anti-SARS-CoV-2 antibodies were detected in 76.9% of participants with PCR-confirmed infection, and SARS-CoV-2 PCR positivity affects antibody titer levels. Antibody titers were high in PCR-confirmed diseases regardless of age. According to the antibody titer results, 29.2% of the residents and staff encountered the virus. Higher antibody titers were detected in residents with PCR confirmed infections. Antibody titers were found to be highest in PCR-positive male residents. In 9.4% of the PCR-positive participants, the anti-SARS-CoV-2 antibodies were not detected. In addition, 24.3% of PCR-negative participants had anti-SARS-CoV-2 antibodies.

In the present study, advancing age was associated with higher IgG response to SARS-CoV-2. Moreover, male residents had the highest antibody levels. This is consistent with the previous studies as male sex in advanced age groups are associated with a higher risk for severe COVID-19, and this group is more likely to be hospitalized [8, 11]. Antibody titers were significantly increased, supporting previous research [10].

As determined in many studies, regardless of age, SARS-CoV-2 IgG titers were significantly high in hospitalized participants in our research [9, 12, 13].

Table 3 Antibody titers according to SARS-CoV-2 PCR and antibody status of the participants

Mean SARS-CoV-2 antibody titers (AU/mL)	Antibody positive		Antibody negative	
	PCR (+)	PCR (−)	PCR (+)	PCR (−)
Resident/staff	273.76/164.04*	115.34/52.78**	0.86/1.08	0.55/0.39
Female resident/staff	235.46/128.46***	0.55/0.38	0.82/0.41	120.30/46.94[a]
Male resident/staff	361.48/192.77[b]	0.55/0.40	0.98/1.40	101.96/56.43

*$p=0.000$; **$p=0.029$; ***$p=0.001$

[a]$p=0.017$

[b]$p=0.003$

Although there are different answers regarding gender dominance in COVID-19, it has been shown in subsequent studies that there is no difference between genders, similar to our research [14, 15]. Even though in some studies, it was noted that males older than 60 were more affected, the authors determined that in some studies, the total number of older males was less than females, which could indicate a higher incidence of the disease among older males [16].

Comorbidities such as diabetes, hypertension, chronic respiratory diseases, cancer, and cardiovascular diseases increase the risk of hospitalization and death due to COVID-19. Karuna et al. found that neutralizing antibody levels were higher in individuals recovering from severe COVID-19, with diabetes and lower in people with hypertension [17]. In a study searching for the predictors of high titer of anti-SARS-CoV-2 antibody of convalescent plasma donors, the authors pointed out that although high antibody titers were present, the antibody titer was not affected by comorbidities of donors [18].

Antibody response to COVID-19 is closely related to the disease severity. Previous studies have also shown that the antibody response is weaker in asymptomatic and mild disease patients than those with severe disease [19–21].

In our SARS-CoV-2 IgG (−) group, although 56 participants did not have symptoms, they were found positive in the PCR test performed due to contact history or shift entry. Three reasons can explain this situation. Firstly, lower antibody titers were observed in asymptomatic patients. Secondly, the performing time of the SARS-CoV-2 IgG test can be a reason for this situation. In 40/59 participants, antibody test was performed almost 9 months after COVID-19. Thirdly, six asymptomatic employees had COVID-19 in the second peak, and the antibody test was performed 3 to 4 weeks after the PCR positivity. Three hospitalized antibody-negative residents had COVID-19 in the first peak and had mild symptoms. So, their antibody titers could have become negative because of timing.

Because of contact history or shift entry, residents and staff had SARS-CoV-2 PCR tests several times in this center. In SARS-CoV-2 PCR-negative participants, 10% were detected positive for anti-SARS-CoV-2 IgG, 93.6% were asymptomatic. Four residents were hospitalized with COVID-19 pneumonia diagnosis despite negative PCR test results. Only nasopharyngeal swabs were obtained from these residents. Previous studies showed that tracheal aspirate sampling could reduce the false-negative rate in patients with pneumonia. As these residents were not intubated, tracheal aspirates could not be obtained. So, this may have led to false negativity [22, 23]. The fact that all hospitalized residents were female may suggest that the disease was more severe in females in this study. Still, it should be considered that 80.7% of the residents living in the nursing home were female.

Stool sampling could only be obtained from 23.9% of participants in the second month of the proven disease. Although the number of analyzed stool samples was low, we did not detect SARS-CoV-2. Viral shedding had not been determined. Prolonged viral shedding in feces was considered in numerous studies in resolved and convalescent COVID-19 patients. In several studies, viral shedding from the gastrointestinal tract has been determined with a higher viral load and a longer duration than from the respiratory tract [24–26].

Some problems were encountered during the conduction of the project. 1—Not all of the participants agreed to give stool samples. 2—However, unfortunately, the required permissions for ethical approval had been delayed due to the global COVID-19 outbreak. As a result, the participants could not be followed individually from when they had the disease. Blood samples could be obtained from the participants only in the 9th month. Also, there was no time to further follow up on antibody titers as the vaccination program would start.

This is the first article to study long-term post-illness antibody titers in nursing home residents and employees to the best of our knowledge. According to our study, older males had a higher antibody response, and underlying diseases did not affect antibody responses. This study strongly upholds the existence of long-term antibodies to SARS-CoV-2 in naturally infected individuals. We detected and analyzed the SARS-CoV- 2 IgG antibody titers in our country's largest elderly care and rehabilitation center. Antibodies were detected in 76.9% of the participants with positive PCR tests. We observed the presence of long-term IgG antibodies after SARS-CoV-2 infection.

In conclusion, our results demonstrate a durable anti-spike SARS-CoV- 2 IgG antibody response at least 9 months after the participants were diagnosed with COVID-19. Knowing the duration of detectable antibodies is an important finding for developing disease prevention and public health strategies.

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Author contribution OOEK, NT, and VAO participated in the study protocol design, data collection, and drafting of the manuscript. OOEK, OA, and AAS participated in the laboratory analysis and laboratory protocol design. OOEK and VAO participated in the data analysis, manuscript drafting, and revision. OA, ANZ, GOS, and BB participated in the data collection and manuscript revision and approval. OOEK, VAO, NT, AAS, and NT participated in the study protocol design and manuscript revision and approval. All the authors read and approved the final manuscript.

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Availability of data and materials The data cannot be shared publicly because there was no such approval in the study protocol. The datasets used and analyzed during the study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate All the staff and nursing home residents included in the study signed an informed consent form. Residents who do not have authority to sign instead, their relatives signed the consent form. The Republic of Turkey Ministry of Health (Approval Number: Oya Özlem Eren Kutsoylu -2020–09-20T20_48_31) and the Ethics Committee of Dokuz Eylul University, Faculty of Medicine (Approval Date: 15/06/2020, No: 2020/13–29) approved the study.

Conflict of interest The authors declare no competing interests.

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