in vivo *35*: 2947-2949 (2021)
doi:10.21873/invivo.12587

Nine Cases of SARS-CoV-2-PCR-positive Samples Showed No Increase of Antibodies Against SARS-CoV-2

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Abstract. *Background/Aim: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been affecting Hokkaido, Japan since late February 2020 until present. The aim of this study was to report the relationship between anti-SARS-CoV-2 antibody-positive and SARS-CoV-2 PCR-positive cases by analyzing anti-SARS-CoV-2 antibodies (IgG and total-Ig). Patients and Methods: Serum samples were collected from care workers and nurses in two nursing homes and two hospitals which underwent virus outbreak. All people were confirmed to be SARS-CoV-2-positive by RT-qPCR and their sera was analyzed for anti-SARS-CoV-2 antibodies (IgG and total-Ig). Results: Although 34 out of 43 samples (79.1%) showed enough amount of anti-SARS-CoV-2 antibodies, 9 RT-qPCR -positive samples (20.9%) showed absence of anti-SARS-CoV-2 antibodies in their sera. Conclusion: The results that 20.9% of RT-qPCR-positive samples with SARS-CoV-2 showed absence of anti-SARS-CoV-2 antibodies provides a possibility that the innate immune reaction could eliminate the virus without activating adaptive immune reaction.*

From December 2019, the entire world has been affected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that originated in Wuhan, China (1), and the World Health Organization (WHO) declared that coronavirus disease 2019 (COVID-19) a global pandemic on March 11th 2020 (2). Hokkaido, Japan, has been also affected by SARS-CoV-2 beginning late February 2020 (3). In April, May and November, two nursing homes and two hospitals had outbreak and facilities had more than 10 PCR-positive people who are care workers and nurses. All were confirmed to have COVID-19 infection by RT-qPCR for SARS-CoV-2 according to the nationally recommended protocol (4). The PCR-positive samples were analyzed for anti-SARS-CoV-2 antibodies (IgG and total-Ig) to analyze the relationship between antibody-positive and PCR-positive cases.

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Key Words: COVID-19, SARS-Cov-2, antibodies, innate immunity.

Patients and Methods

Samples. The study protocol was approved by the Institutional Review Board for Human Use of the Health Sciences, University of Hokkaido and the other four facilities (I) Nursing Home Barato Akashia-Heights (II) Chitose Daiichi Hospital (III) Kin-ikyo Chuo Hospital (IV) Nursing home Dream House. Written informed consent was obtained from all patients before study. The PCR-positive samples collected more than 30 days from the date of PCR positivity from these 4 facilities.

Measurement of antibody. The measurement of anti-SARS-CoV-2 antibodies (IgG and total-Ig) in sera were done by using the Vitros Immunodiagnostic Products anti-SARS-CoV-2 total Ig test and the anti-SARS-CoV-2 IgG test (Ortho Clinical Diagnostics) (5). The sensitivity of the anti-SARS-CoV-2 total Ig test was reported to be 100% in samples collected more than 6 days from the date of PCR positivity (5). The sensitivity of the anti-SARS-CoV-2 IgG test was also reported to be 100% in samples collected at least 15 days following initial disease manifestation (6).

Results

Thirty-four out of 43 PCR-positive samples (79.1%) showed enough amounts of IgG and total-Ig against SARS-CoV-2. However, nine PCR-positive samples (20.9%) (3 out of 11; Nursing Home Barato Akashia-Heights, 3 out of 10; Chitose Daiichi Hospital and 1 out of 8; Kin-ikyo Chuo Hospital; 2 out of 14; Nursing home Dream House) showed absence of anti-SARS-CoV-2 antibodies in their sera (Table I). The

Table I. *Results of RT-PCR and antibody test against SARS-CoV-2.*

Nursing Home Barato Akashia-Heits

ID	PCR Test date	Ab Test date	Total Ig value	IgG value
N0067A8B	4/28/2020	6/17/2020	0.07	0
N009919B	4/29/2020	6/17/2020	0.04	0
N001738B	4/28/2020	6/17/2020	0.08	0.01
N006064B	5/4/2020	6/17/2020	295.00	13.40
N001646B	5/11/2020	6/17/2020	236.00	7.78
N003894B	4/29/2020	6/17/2020	45.20	1.87
N005108B	5/5/2020	6/17/2020	249.00	8.07
N002078B	5/15/2020	6/17/2020	333.00	10.70
N003849B	4/22/2020	6/17/2020	89.80	1.30
N008549A	5/29/2020	6/30/2020	138.00	9.59
N005815A	5/22/2020	6/30/2020	110.00	5.17

Chitose Daiichi Hospital

ID	PCR Test date	Ab Test date	Total Ig value	IgG value
M08511	4/30/2020	6/30/2020	0.01	0
M06310	4/30/2020	6/30/2020	0.02	0
M00170	4/30/2020	6/30/2020	0.02	0
M08117	4/30/2020	6/30/2020	3.03	0.16
M03574	4/19/2020	6/30/2020	65.30	2.31
M09297	4/27/2020	6/30/2020	46.70	2.06
M04585	4/23/2020	6/30/2020	245.00	5.92
M01693	4/23/2020	6/30/2020	79.00	2.00
M01226	4/28/2020	6/30/2020	87.20	5.00
M04427	4/13/2020	6/30/2020	154.00	5.31

Kin-ikyo Chuo Hospital

ID	PCR Test date	Ab Test date	Total Ig value	IgG value
T01604	5/25/2020	6/30/2020	0.02	0.01
T04133	5/25/2020	6/30/2020	240.00	19.20
T09475	5/22/2020	6/30/2020	387.00	19.80
T09962	5/30/2020	6/30/2020	25.20	16.30
T03087	5/24/2020	6/30/2020	233.00	21.20
T01393	5/21/2020	7/3/2020	33.70	21.60
T07372	5/22/2020	7/3/2020	162.00	21.90
T08980	5/22/2020	7/3/2020	134.00	20.20

Nursing Home Dream House

ID	PCR Test date	Ab Test date	Total Ig value	IgG value
F02184	11/8/2020	1/14/2021	0.12	0.01
F07621	11/8/2020	1/14/2021	0.16	0.01
F04485	11/8/2020	1/14/2021	60.20	11.10
F02710	11/8/2020	1/14/2021	26.50	4.90
F01325	11/8/2020	1/14/2021	103.00	6.16
F07312	11/8/2020	1/14/2021	27.70	9.11
F00420	11/8/2020	1/14/2021	148.00	9.25
F06514	11/8/2020	1/14/2021	234.00	13.50
F01721	11/8/2020	1/14/2021	67.20	10.40
F03577	11/8/2020	1/14/2021	238.00	13.60
F01770	11/8/2020	1/14/2021	17.60	3.19
F01592	11/8/2020	1/14/2021	18.70	2.11
F07332	11/8/2020	1/14/2021	236.00	15.80
F00892	11/8/2020	1/14/2021	45.00	1.73

The anti-SARS-CoV-2 IgG and total-Ig assays were performed by VITROS XT 7600 immunoassay system (Ortho-Clinical Diagnostics, Rochester, NY, USA). The antibody values were adjusted by the calibrator and control reagents and estimated by the signal to cutoff (S/C) values of <1.00 and ≥1.00 corresponding to non-reactive and reactive results, respectively. PCR: Polymerase chain reaction; Ab: antibody; Ig: immunoglobulin.

three anti-SARS-CoV-2 antibody-absent cases in the nursing home Barato Akashia-Heights showed PCR-positive with high Ct values (32.19, 33.94, and 36.68) and calculated viral copies were less than 100 copies. These three people showed negative results of IgG values and Total Ig values again from sera collected 14 days later.

Discussion

The results of the present study showed that 9 cases out of 43 SARS-CoV-2-PCR-positive samples showed no increase of antibodies against SARS-CoV-2. These results show a possibility of innate immune reaction that could eliminate the virus without activating adaptive immune reaction involving B lymphocytes, helper T cells and plasma cells. Many reports showed an increase of NK cells in SARS-CoV-2-infected people with no symptoms, convalescence and mild symptoms, and decrease of NK cells in SARS-CoV-2-infected people with severe symptoms. (7, 8). In the present study, all cases whose antibodies against SARS-CoV-2 were measured were asymptomatic. It has been reported that the sensitivity of the anti-SARS-CoV-2 total Ig test and the anti-SARS-CoV-2IgG test is greater than 95% (5, 6, 9), and that antibodies are positive in more than 95% of PCR-positive patients. For the first time, our study examined the presence of antibody production in PCR-positive patients, and we found that innate immune response might eliminate SARS-Cov-2 in more than 20% of SARS-CoV-2 PCR-positive patients before adaptive immune system start up. Smetana *et al*. reported the role of interleukin-6 (IL6) on lung complications in patients with COVID-19 and they mentioned that inhibitors of IL6 signaling represent a promising approach that can be employed for attenuation of a cytokine storm and might be beneficial for patients with COVID-19 (10). IL-6 is involved in the regulation of B cell response into antibody producing cells (11). Presumably asymptomatic SARS-CoV-2-infected people

whose antibodies against SARS-CoV-2 were measured in the present study might produce very low levels of IL6 and might not induce enough B cell differentiation to antibody-producing cells. Further studies are needed to clarify the role of innate immunity and IL6 during production of antibodies against SARS-CoV-2 in asymptomatic SARS-CoV-2-infected people.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

All Authors contributed to the study conception and design. Materials preparation, data collection, and analysis were performed by TK, YK, TO, MT, YT, RT, OU, KN and RI. The first draft of the manuscript was written by TK, YK, YT and MK and all authors commented on previous versions of the manuscript. All Authors read and approved the final manuscript.

Acknowledgements

This work was supported, in part, by the Grant from Advanced Research Promotion Centre, Health Sciences University of Hokkaido. We thank Ortho Clinical Diagnostics to lent us Vitros XT7600 integrated system

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Received May 21, 2021
Revised June 9, 2021
Accepted June 23, 2021