Immunogenetics: Open Access

Immunochromatographic SARS-CoV-2 IgG Antibody Assay: A Cross-Sectional Study Conducted at Wakayama Medical University in Japan

Sadahiro Iwabuchi[1], Masahiro Katsuda[2,3], Yusuke Koizumi[4], Mayuko Hatai[1], Mitsue Kojima[4], Nahomi Tokudome[5], Shinobu Tamura[6], Machiko Nishio[4,7], Toshikazu Kondo[8], Masaya Hironisi[9], Chiemi Kakutani[10], Hiroki Yamaue[2], Shinichi Hashimoto[1*]

[1]Department of Molecular Pathophysiology, Wakayama Medical University, Wakayama, Japan;[2]Department of Surgery, Wakayama Medical University, Wakayama, Japan;[3]Clinical Study Support Center, Wakayama Medical University, Wakayama, Japan; [4]Department of Infection Prevention and Control Wakayama Medical University, Wakayama, Japan;[5]Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan;[6]Department of Hematology/Oncology, Wakayama Medical University, Wakayama, Japan;[7]Department of Microbiology, Wakayama Medical University, Wakayama, Japan;[8]Department of Forensic Medicine, Wakayama Medical University, Wakayama, Japan;[9]Kihoku Branch Hospital, Wakayama Medical University, Wakayama, Japan;[10]Nursing Department, Wakayama Medical University, Wakayama, Japan

ABSTRACT

Asymptomatic patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection must be quickly identified and isolated to prevent the spread of the virus. The number of asymptomatic healthy people is completely unknown because they remain untested. Detection of specific SARS-CoV-2 antibodies has been widely accepted as a diagnostic test, and an immunochromatographic test, which is simpler and relatively cheaper than other methods, is becoming the gold standard for identifying healthy people who had been infected with SARS-CoV-2 in the past. In this study, 1,528 volunteers who worked at a particular hospital were subjected to an immunochromatographic IgG test for SARS-CoV-2 to determine the ratio of asymptomatic people. Only 12 volunteers (0.79%) were IgG⁺, with no significant background differences in the sex, age, profession, experiences of working at the emergency department or caring for coronavirus disease 2019 patients. If this IgG⁺ ratio was to be extrapolated to Wakayama city's population, 2,780 out of 3,54,063 people may be asymptomatic for SARS-CoV-2. The results imply that anyone may get infected with SARS-CoV-2 but remain asymptomatic.

Keywords: SARS-CoV-2; COVID-19; Asymptomatic; SARS-CoV-2-infected healthy people; Immunochromatographic test

ABBREVIATIONS

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2); COVID-19: Coronavirus Disease 2019; SCV-IHP: SARS-Cov-2-Infected Healthy People

INTRODUCTION

Cases of the novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been reported worldwide. A total of 13,852 positive cases and 389 deaths were reported between January 16 and April 29, 2020 [1]. In June 2020, the number of COVID-19 patients seemed to reduce in Japan; however, in early July 2020, a sudden spike in SARS-CoV-2-infected healthy people (SCV-IHP) was reported. Until January 20, 2021, approximately 340,989 cases and 4,680 deaths had been reported in Japan, and this was recognized as the third waves of infection.

Some people with confirmed infection may be asymptomatic but can infect others; this feature is different from that reported

during the SARS-CoV infection that emerged in 2003. According to a retrospective study conducted in Beijing around Feb 10, 2020, which was the early phase of SARS-CoV-2 infection, 17.6%, 73.3%, 4.2%, and 5.0% of the COVID-19 patients transferred to hospitals for special treatment had severe, mild, non-pneumonia-like, and no symptoms, respectively [2]. On April 7, 2020, 1,095 of 1,453 Chinese COVID-19 patients (75.3%) were asymptomatic [3], and approximately 50%–75% of Italian COVID-19 patients were asymptomatic [4]. Thus, it is meaningful to investigate the ratio of asymptomatic COVID-19 patients as well as the ratio of SCV-IHP in the city.

Therefore, an immunochromatographic IgG test for SARS-CoV-2 was used. This study was limited to volunteers who worked at Wakayama Medical Hospital or its branch; these volunteers had no symptoms of COVID-19 on the testing day at least, and a total of 238 confirmed cases and 4 deaths had been reported in Wakayama city on the last testing day.

MATERIALS AND METHODS

Samples

Volunteers--doctors, nurses, pharmacists, medical technicians, clerks, researchers, and other professionals (childminders, secretaries, medical interns, etc. working at Wakayama Medical Hospital or its branch (medical university) having asymptomatic COVID-19 were enrolled. These volunteers sometimes visited the main hospital daily and/or communicated with the main hospital's staff as the medical university lies adjacent to the main hospital on the same site. The testing days at the main hospital were as follows: 7–9, 14–16, and 30–31 July, 2020. In contrast, the testing days at the branch hospital were 14–15 September, 2020, because some COVID-19 patients were hospitalized until the end of August 2020 in the branch hospital.

This study was approved by the research ethics committee of Wakayama Medical University (approval no: 2960), and informed consent was obtained from all volunteers by signature to the document.

Immunochromatographic test

Immunochromatographic IgG tests were performed using a commercially available kit [SARS-CoV-2 antibody detection kit (IgG), RF-NC002, Kurabo Industries Ltd., Neyagawa, Osaka, Japan]. The product accepts serum, plasma, and whole blood as samples and has a sensitivity, specificity, and accuracy of 76.4%, 100%, and 94.2%, respectively. According to the manufacturer's instructions, 10 ㎕L of whole blood was added to the wells of the test strips, followed by dilution buffer. Blood was collected using a lancet (NIPRO, Kitaku, Osaka, Japan). The IgG response was evaluated after 15 min, 60 min, and 3 h by three researchers. The volunteers, who were IgG$^+$ at first round of testing, were tested again within 2 weeks to verify the false positive rate and positive predictive value. Thirty-two volunteers who were IgG$^+$ at the first round of testing were re-evaluated using the same kit, 22 of which were confirmed false positives. The percentage of false

positives and the positive predictive value were 1.59% and 31.2%, respectively.

Data analysis

The statistical analysis was performed by the analysis of variance (ANOVA), followed with unpaired Student's t-test. The 95% confidence interval (CI) and chi-squared test results were analyzed using BellCurve for Excel.

RESULTS

A total of 1,528 volunteers with no confirmed COVID-19 diagnosis were enrolled (median age was 37 (19–77) years, and average age was 40.5 ± 12.0 (mean ± S.D.) years). No significant differences were observed in the volunteers' sex and age. The number of IgG$^+$ volunteers in the main and branch hospital was 12 and 0, respectively (Table 1), and the ratio of IgG$^+$ volunteers was 0.79% (95% CI: 0.34%-1.22%). Of the 12 IgG$^+$ volunteers in the main hospital, 4 were males and 8 were females, and their median age was 36 (34–61) and 38.5 (24–60) years, respectively. The average age of the IgG$^+$ volunteers was not significantly different from that of the IgG- ones in total (p = 0.36). Of the IgG$^+$ volunteers, 3.3% were pharmacists, 1.4% were clerks, 0.6% were nurses, and 0.8% were other professionals; further, there was no significant difference in the incidence of IgG$^+$ results based on the volunteers' professions (p=0.42).

	Total	Main hospital	Branch hospital
# of volunteers	1528	1379	149
Sex, male (%)/ female (%)	486 (31.8%)/ 1042 (68.1%)	434 (31.5%)/945 (68.5%)	52 (34.8%)/97 (65.1%)
Age, male/ female, Mean ± S.D.[a]	40.5 ± 12.0/38.2 ± 11.2	40.3 ± 11.9/37.7 ± 11.1	42.7 ± 13.0/42.7 ± 11.5
Median (range)	38 (20−77)/37 (19−64)	38 (20−77)/36 (19−64)	39.5 (23−68)/43 (22−64)
Male; Doc,Nur,Pha,Med,Cle,Res,Oth	174, 67, 14, 66, 101, 44, 20	158, 62, 13, 47, 91, 44, 19	16, 5, 1, 19, 10, 0, 1
IgG positive	0, 2, 0, 0, 1, 0, 1	0, 2, 0, 0, 1, 0, 1	0, 0, 0, 0, 0, 0, 0
% in each profession[b]	0, 3.0, 0, 0, 1.0, 0, 5.0	0, 3.2, 0, 0, 1.1, 0, 5.3	0, 0, 0, 0, 0, 0, 0
Female;Doc,Nur,Pha,Med,Cle, Res,Oth	74, 533, 31, 42, 215, 24, 123	72, 468, 28, 30, 205, 24, 118	2, 65, 3, 12, 10, 0, 5
IgG positive	0, 3, 1, 0, 3, 0, 1	0, 3, 1, 0, 3, 0, 1	0, 0, 0, 0, 0, 0, 0

% in each profession	0, 0.6, 3.3, 0, 0, 0.6, 1.4, 0, 0.8	3.6, 0, 1.5, 0, 0.9	0, 0, 0, 0, 0, 0, 0
% of IgG positive in total	0.79% (95%CI; 0.34−1.22%)		
% of IgG negative in total	99.21% (95%CI; 98.8−99.7%)		

Table 1: Summary of COVID-19 IgG test in Wakayama Medical University.

A more detailed analysis of the differences in results based on the locations was performed. In the main hospital, only one patient with COVID-19 symptoms had been hospitalized until the end of July 2020, and no hospitalization was recorded on each testing day. The IgG+ rate was 0.87% in total (12/1379 cases); 5 of 530 nurses, 1 of 41 pharmacists, 4 of 296 clerks, and 2 of 209 other professionals were IgG+. Two out of five nurses in the medical university had experience working in the emergency department, and one nurse also had experience treating COVID-19 patients; in contrast, the number of nurses that had experience working in the emergency department or treating COVID-19 patients in the main hospital was 60 and 27, respectively. Thus, every 1 of 27 nurses (3.7%) who had cared for COVID-19 patients was IgG+. The number of doctors or medical technicians who had cared for COVID-19 patients was 15 and 3, respectively, but none were IgG+.

Forty-two patients were hospitalized at the branch hospital until September 15, 2020, and one patient was hospitalized and treated even on the day of the inspection (September 14–15, 2020). The number of doctors, nurses, medical technicians, clerks, and other professionals who had cared for COVID-19 patients was 9, 39, 26, 2, and 1, respectively.

DISCUSSION

Real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 is the primary tool for detecting the infection; however, the number of inspections was limited during the first wave of infection because of the expensive equipment and limited well-trained technicians. Improvements in the inspection systems contributed to increased RT-PCR coverage, and an increased number of asymptomatic cases and a decreased number of severe COVID-19 cases was reported during the recent wave of infection. On January 21st 2021, outsourced real-time PCR for COVID-19 has become relatively popular, and it is getting easier to know whether people are currently infected. Whether the virus itself has mutated such that it no more induces severe symptoms of pneumonia or whether the increased RT-PCR coverage has exposed individuals with SARS-CoV-2 who did not have overt symptoms remains unknown.

Apart from RT-PCR, detecting specific antibodies for SARS-CoV-2 (IgM or IgG) is considered reliable. Some assays to detect SARS-CoV-2 IgM/IgG antibodies are enzyme-linked immunosorbent assay [5], quantum dot immunofluorescence assay [6], chemiluminescence immunoassay [7], electrochemiluminescence immunoassay [8] and

immunochromatographic test [9]. IgM antibodies are induced upon recent exposure to SARS-CoV-2, whereas IgG antibodies are produced a few days after exposure; however, the induction period is variable among patients, with differences in symptom severity [6]. The sensitivity and specificity of IgG detection depend on the methods and samples. For instance, the sensitivity and specificity are 96.8% and 99.8% using a serum sample and a chemiluminescence immunoassay [7], 100% and 100% using a serum sample and an electrochemiluminescence immunoassay [10], 86.1-94.4% and 100% using a serum sample or 88.66% and 90.63% using whole blood samples and an immunochromatographic test, respectively [9,11] . In this case, using whole blood examples and the SARS-CoV-2 antibody detection kit (IgG), the sensitivity was 98.4% based on the calculation used [11]. The sensitivity may be relatively higher in this examination because of following reasons: 1) the IgG+ responses were checked at least twice by more than two researchers at 15-min intervals and 2) approximately 78% of cases that were SARS-CoV-2 IgG+ were tested again to avoid false-positive results, as described in the Methods section. The specificity might be 100% because the IgG+ strips of the kits were checked using whole blood samples from two volunteers who had symptoms of COVID-19.

A similar evaluation using the same SARS-CoV-2 antibody detection kit was performed among patients who visited Kobe City Medical Center General Hospital without COVID-19 symptoms [12,13]. Thirty-three of 1,000 serum samples were IgG+ (3.3%; 95% CI: 2.3%–4.6%), and 50,123 people (95% CI: 34,934–69,868) in Kobe city were found to be SCV-IHP. The present study was limited to the volunteers who worked in the hospital and had more chances of close contact with COVID-19 patients. However, the total ratio of IgG+ SCV-IHP was 0.79% (95% CI: 0.34%–1.22%), which was lower than that reported in previous studies. The estimated number of SCV-IHP in Wakayama may be 2,780 (95% CI: 2,677–2,883) out of 354,063, based on the results from Wakayama Medical University. Until the last testing day (November 30th 2020), the total number of COVID-19 positive cases confirmed by RT-PCR in Wakayama city was 146, and all were severe/mild symptomatic or asymptomatic cases. If the current study's results were to be extrapolated to Wakayama city, only 5.25% (146 of 2,780) of the potential COVID-19 positive cases might be figured out based on the estimated IgG+ SCV-IHP. However, the enrolled volunteers were limited to hospital workers and the targets of our survey was different to common people. Therefore, the estimated result does not correctly reflect the situation of community in Wakayama. For example, the total number of nurses in Wakayama prefecture is about 4,897; so far, the estimated IgG+ SCV-IHP nurses may be 47 based on our survey (5 out of 530 nurses). However, it was not clear whether the infection of nurses was caused by SARS-oV-2 patients or community acquired and how many nurses have been taken care of COVID-19 patients. From the viewpoint of personal information protection, the detailed infection routes of the IgG+ volunteers were unknown. Hence, the statistical considerations might seem to be meaningless.

CONCLUSION

The current findings indicate that there was no bias in the data due to differences in the professions of the IgG⁺ volunteers. The estimated number of IgG⁺ volunteers in the branch hospital who had experience caring for COVID-19 patients was 8–9 based on the calculation. In fact, most of the volunteers in the branch hospital checked whether they had been infected with SARS-CoV-2 by RT-PCR approximately 2 months prior to the IgG testing day, and all of them had tested negative. These results suggest that the hospital staff at Wakayama Medical Hospital and its branch had high awareness regarding infection control on September 2020.

The immunochromatographic IgG test may lead to false-positive or false-negative due the cross-reactivity to other viruses depended on the commercial kit, but it is simple, rapid, and convenient method and has a relatively high sensitivity and specificity. In the near future, anyone could test for SARS-CoV-2 infection easily using the immunoglobulin test kit, regardless of them being symptomatic or asymptomatic, leading to prevention of the spread of infection by "super-spreaders". Even if the vaccine is developed and distributed to the general public, the monitoring of IgG for SARS-CoV-2 will be very important and meaningful due to IgG disappearance. Further, SCV-IHP need to be identified as they may have very strong IgG antibodies against SARS-CoV-2, which can serve as an artificial antibody to overcome the worldwide pandemic. The high sensitivity IgG test may be useful as a "rule out" test for COVID-19 cases. The study results emphasize that anyone can be infected with SARS-CoV-2 while still being asymptomatic.

ACKNOWLEDGMENT

REFERENCES

1. Watanabe M. The COVID-19 Pandemic in Japan. Surg Today. 2020;50:787-793.

2. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z, et al. Characteristics of COVID-19 infection in Beijing. J Infect. 2020;80:401-406.

3. Zhang J, Wu S, Xu L. Asymptomatic carriers of COVID-19 as a concern for disease prevention and control: more testing, more follow-up. Biosci Trends. 2020;14:206-208.

4. Day M. Covid-19: identifying and isolating asymptomatic people helped eliminate virus in Italian village. BMJ. 2020;368:11-65.

5. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill. 2020;25:80.

6. Nishiura H, Kobayashi T, Miyama T, Suzuki A, Jung SM, Hayashi K, et al. Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19). Int J Infect Dis. 2020;94:154-155.

7. Sun B, Feng Y, Mo X, Zheng P, Wang Q, Li P, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect. 2020;9:940-948.

8. Suda G, Ogawa K, Kimura M, Maehara O, Kitagataya T, Ohara M, et al. Time-dependent changes in the seroprevalence of COVID-19 in asymptomatic liver disease outpatients in an area in Japan undergoing a second wave of COVID-19. Hepatol Res. 2020;78:89.

9. Kaneko S, Nukui Y, Arashiro T, Aiso Y, Sugii M, Hadano Y, et al. Clinical validation of an immunochromatographic SARS-Cov-2 IgM/IgG antibody assay with Japanese cohort. J Med Virol. 2020.

10. Liu X, Wang J, Xu X, Liao G, Chen Y, Hu CH. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect. 2020;9:1269-1274.

11. Ma H, Zeng W, He H, Zhao D, Jiang D, Zhou P, et al. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol. 2020;17:773-775.

12. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020.

13. Asako KI, Hirokazu K, Toshikazu H, Seiko N, Aya K, Tomomi N, et al. Estimation of seroprevalence of novel coronavirus disease (COVID-19) using preserved serum at an outpatient setting in Kobe, Japan: A cross-sectional study. Med Rxiv. 2020.