

Antibody responses to SARS-CoV-2 in healthy individuals returning to Shenzhen

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Abstract

To verify reliability of antibody detection and investigate population immunity to severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in the local Chinese population. A cross-sectional study was conducted in Shenzhen to detect anti-coronavirus antibodies including, immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA). In the COVID-19 group, nine patients were enrolled after diagnosis. In the control group, 1589 individuals without clinical symptoms (cough, fever, and fatigue) and returning from outside Shenzhen were enrolled. The first study enrollment occurred at the end of February 2020; the final study visit was 18 March 2020. In the COVID-19 group, the seven of nine patients were positive for IgM, IgG, and IgA. Meanwhile, six of the 1589 healthy individuals were found to be weakly positive for IgG. According to SARS-CoV-2 nucleic acid tests, the six individuals were all negative. Strong supplemental support for clinical information can be provided by antibody detection, especially for IgA. According to comparison with overseas reports, the infection rate of the Chinese population outside Shenzhen, China, is significantly low, so most of the population in China is still susceptible. Hence, social distancing measures are still inevitable until a vaccine is developed successfully.

KEYWORDS

COVID-19, IgA, IgG, IgM, SARS-CoV-2

Abbreviations: COVID-19, coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; SAR, severe acute respiratory; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2.

Cantong Zhang and Liancheng Lin contributed equally to this work.

1 | INTRODUCTION

The epidemic of pneumonia caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) has spread rapidly, posing significant threats to international health and the economy, with over 600 000 deaths and more than 14 500 000 confirmed cases as of 21st July 2020.¹ Moreover, Chinese health authorities received reports of 14 newly confirmed coronavirus disease 2019 (COVID-19) cases on Chinese mainland, including five imported cases and nine locally transmitted ones.² As the spread of the COVID-19 pandemic has been relatively contained in China, a serological test for the antibodies is now as important as SARS-CoV-2 nucleic acid detection, as dynamic investigation of the epidemic and the immune status of the virus infection in the population is needed to formulate further prevention and control strategies.

Enzyme-linked immunosorbent assay (ELISA), based on antibody-antigen reaction, is used to detect and measure certain target molecules in a mixture solution.³ The four main types of ELISAs are indirect, direct, sandwich, and competitive. To measure endogenous antibodies, an indirect ELISA is usually applied due to its high sensitivity, massive operation, and flexibility.³ Serological tests are available to detect antibodies to SARS-CoV-2, given the report that endogenous antibody against severe acute respiratory syndrome related coronaviruses (anti-SARSr-CoV) immunoglobulin M (IgM) and immunoglobulin G (IgG) ELISA kits were developed using SARS-CoV Rp3 nucleoprotein as an antigen,⁴ which shares over 90% amino acid identity with all SARSr-CoVs.⁵ In addition, a study declared that IgM and IgG performed well in serodiagnosis for COVID-19 with great specificity, as detected by ELISA.⁶

Here we attempted to detect antibodies against SARS-CoV-2 in COVID-19 patients and healthy individuals by ELISA kits. Thus, we evaluated the effectiveness of the kits, assessed the stage of the SARS-CoV-2 epidemic and analyzed the amount of herd immunity in a local area of China.

2 | MATERIALS AND METHODS

2.1 | Samples

In the disease group, a total of nine patients with COVID-19 from Shenzhen Second People's Hospital were enrolled at the end of February (Table 1). The male-to-female ratio was 5:3, with a mean age of 49.4 (aged 30-72). In the control group, a total of 1589 healthy individuals who worked as builders returning from outside Shenzhen without any clinical symptoms (cough, fever, and fatigue) were enrolled in this cross-sectional study in March. All blood samples from the two groups were tested in Shenzhen. The mean age of these people is 36.4 (aged 11-89). The male-to-female ratio is 15:1.

2.2 | Antibody measurement

Serum samples were tested using ELISA kits supplied by Shenzhen Sciarray Biotech Co, Ltd, according to the manufacturer's instructions. For the IgG test, designed by the indirect ELISA principle, 96-well microplates were coated with the nucleocapsid of SARS-CoV-2, and diluted samples were added (100 µL/well), followed by incubation for 30 minutes at 37°C. Then, the cells were washed three times with phosphate-buffered saline with Tween 20, and an horseradish peroxidase-conjugated anti-human IgG monoclonal antibody was added (100 µL/well). Following incubation (20 minutes, 37°C) and chromogenic reaction, the optical density value at 450 nm was measured by a microplate reader. The cutoff value of this test was defined by receiver operating characteristic curves. IgM and IgA ELISA assays were applied in similar operating steps.

Case	Sex	Age	SARS-CoV-2 nucleic detection			Antibodies detection			Date	
			ORF1ab	N	E	IgM	IgG	IgA	Onset of symptoms	Antibodies detection
#1	F	38	+	+	+	+	±	+	01-24	01-28
#2	M	47	+	-	+	+	±	±	01-23	01-28
#3	F	30	+	+	+	-	-	+	01-29	01-29
#4	M	49	+	+	+	+	-	+	01-27	01-29
#5	M	71	+	+	-	+	+	+	01-26	02-01
#6	M	63	+	-	+	+	+	+	01-26	02-01
#7	M	52	+	+	-	-	-	±	01-28	02-01
#8	M	43	+	+	-	-	-	-	02-02	02-05
#9	F	72	+	+	-	-	-	-	02-01	02-01

Note: N, N genes; E, E genes; -, negative; ±, weakly positive; +, positive.

Abbreviations: COVID-19, coronavirus disease 2019; F, female; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; M, male; ORF1ab, open reading frame; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2.

TABLE 1 The testing results of patients with COVID-19

TABLE 2 Clinical and laboratory findings of nine patients with COVID-19 in Shenzhen, Guangdong province, China

Case	#1	#2	#3	#4	#5	#6	#7	#8	#9
Age and sex	38 (F)	47 (M)	30 (F)	49 (M)	71 (M)	63 (M)	52 (M)	43 (M)	72 (F)
Clinical findings									
Highest temperature (°C)	37.5	40	37.9	37.5	38.5	37.7	37.8	36.1	37.8
Fever	+	+	+	+	+	+	+	-	+
Cough	+	-	-	-	-	+	-	+	-
Myalgia or fatigue	+	-	-	-	-	+	-	-	-
Sore throat	-	-	-	-	+	-	-	+	-
Sputum production	-	-	-	-	-	-	-	+	-
Headache	-	-	+	-	-	-	-	-	-
Diarrhea	-	+	-	+	-	-	-	-	-
Laboratory findings									
White blood cell count ($\times 10^9/L$)	3.67	8.16	3.59	6.67	5.54	3.99	6.62	5.54	2.14
Lymphocyte count ($\times 10^9/L$)	0.74	1.23	0.55	1.34	0.92	1.11	0.58	2.14	0.86
Antibodies findings									
IgM	+	+	-	+	+	+	-	-	-
IgG	±	±	-	-	+	+	-	-	-
IgA	+	±	+	+	+	+	±	-	-

Note: Normal range of white blood cell count and lymphocyte count are 3.5 to 9.5 ($\times 10^9/L$) and 1.1 to 3.2 ($\times 10^9/L$), respectively.

Abbreviations: COVID-19, coronavirus disease 2019; F: female; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; M: male.

3 | RESULTS

Among the nine patients with COVID-19, two male patients (aged 63 and 71) were found to be positive for antibodies (IgG, IgM, and IgA), while a male patient and a female patient (aged 43 and 72, respectively) were found to be negative in the same tests. For the rest of the female patients in the group, one (aged 38) was positive by IgM and IgA ELISAs and was weakly positive by IgG ELISA, while another (aged 30) was positive by IgA ELISA and negative by IgM and IgG ELISAs. Male patient results differed: one (aged 47) was tested weakly positive by IgG and IgA ELISAs and positive for IgM antibody; another (aged 49) tested positive for IgM and IgA antibodies and negative in IgG antibody; and yet another tested negative for IgM and IgG antibodies, and weakly positive for IgA antibody. As shown in Table 1, the results supported that IgM, IgG, and IgA performed well in serodiagnosis for COVID-19 with great specificity, as detected by ELISA. And we listed Table 1 and Table 2 to record their clinical findings and laboratory findings.

Furthermore, ELISA was performed for 1,589 builders, who came from Guangdong (approximately 25%), Sichuan (approximately 16%), Hunan (approximately 16%), Jiangsu (approximately 7%), Guangxi (approximately 7%), and Guizhou (approximately 4%), which showed that six were weakly positive for IgG antibodies, with a sensitivity of 0.38% (6 out of 1589). In detail, the OD value of these six samples was between 0.210 and 0.600. Our results showed no individuals with IgM and IgA antibodies. Specifically, among the six individuals with positive results, four came from Hunan Province and the rest were from Guangdong Province. These six samples all came from male subjects. In addition, these six individuals were self-isolated under medical observation for 14 days. Their SARS-CoV-2 nucleic acid tests were all negative.

4 | CONCLUSION AND DISCUSSION

Currently, there are several reasons why many SARS-CoV-2 nucleic acid test kit results are false negative, including incorrect sample collection; improper sampling time; incorrect approach to preservation; transportation and disposal of the samples; and mutations of the virus.⁶ Additionally, our findings for nine patients with COVID-19 supported that the detection of serum antibodies provides strong supplemental support for clinical information, which is consistent with the study of Zhao et al.⁷ Moreover, in addition to saving time, antibody testing can also detect previous exposure, which may be useful for epidemiological research. Meanwhile, it can verify that vaccines are working as intended during clinical trials. As seven patients were found to be positive for IgA antibodies, we speculated that the detection of serum IgA antibody could help with the diagnosis of COVID-19, while researchers focus more on IgM and IgG antibodies.⁸ This finding also meant that the IgA-based ELISA kit was more sensitive than the IgG-based ELISA kit and IgM-based ELISA kit, but a larger sample is needed to assess the sensitivities of these kits.

As shown in Table 2, patients (#1, #2, #5, #6) who were serologically positive for IgM, IgG, and IgA were inclined to cause over two kinds of symptoms, especially for fever. Hence, we speculated that the greater the postinfection response, the greater the likelihood of antibodies produced in the person with immune sound system, but a larger sample is needed to verify this hypothesis.

In addition, the results of antibody tests (IgG, IgM, and IgA) were negative in two patients with COVID-19 (#8 and #9), which may be caused by the low titer of the antibody due to the testing time. People need to be infected for several days before the body can produce antibodies for COVID-19 diagnosis.⁹ The negative IgG, IgM, and IgA results in

two patients (#8 and #9) were possibly caused by the phase of antibody production as their period from onset to detection were less than 3 days (Table 1). Therefore, the results of ELISA can be used for clinical reference, which could greatly relieve the huge clinical diagnosis pressure.

It is worth noting that the kit is against the severe acute respiratory syndrome (SARS)-like coronavirus, not only against SARS-CoV-2, but the IgG originally infected with SARS may also be positive. Here are another two ways to detect the antibody, such as colloidal gold-immunochromatographic assay (GICA) and ELISA kits based on spike protein. In detail, GICA based on mouse anti-human IgM monoclonal antibody (microchain), showed a sensitivity of 81.3% (74 out of 91) and specificity of 100% (35 out of 35) specificity.¹⁰ Another study demonstrated a good performance evaluation of the SARS-CoV-2 specific IgG/IgM detection assay.¹¹ In our study, ELISA kits based on spike protein did not use the six seropositive (weak) control samples at that time, and we could not trace back the six suspected infections due to privacy protection and design defeat of study.

A sampling survey from Heinsberg showed that 14% of individuals possessed anti-SARS-CoV-2 IgG antibodies,¹² and a test of 200 people in Chelsea, reported by The Boston Globe, found that 64 of those had antibodies for the virus.¹³ Combined with our findings of 0.38% positivity for anti-SARS-CoV-2 IgG antibody, the infection rate of these individuals from local areas is significantly lower than that of overseas reports, which hinders herd immunity, a pattern of immunity that could protect a population from the emergence of a new infection,¹⁴ in the short term in China. Besides, a study finished in Guangzhou, Guangdong between 9 March and 10 April 2020, showing 4 of 442 (0.9%) factory workers with IgG antibody,¹⁵ whose cumulative seroprevalence of IgG is bigger than that of our research done in Shenzhen, Guangdong which is further south to Wuhan. Hence, until a vaccine is developed successfully, social distancing measures and wearing masks are still inevitable.

The study had several limitations to note. First, our study population was not drawn by random sampling, so the estimation of the seroprevalence was subject to potential sampling bias. Second, due to the cross-sectional design of this study, the dynamic changes of antibody titer in nine confirmed cases and six suspected cases were not tested, as long-term follow-up will be important to define the value of these serology markers. Third, the observed seroprevalence in our study could potentially underestimate the true prevalence rate of the disease, owing to the window phase of antibody production. Fourth, lacking verification of spike detection ELISA kits, the data missed some comparison.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

CZ and LL were responsible for the literature search and writing of the manuscript. LL, FL, ML, QL, ZD, and GC performed the serological testing. YD and DG were responsible for the study design, data

interpretation. DG were responsible for the patient recruitment, and data collection. YD, DT, and GC collected and analyzed the data.

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