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Original Research Article

Epidemiological feature, viral shedding, and antibody seroconversion among asymptomatic SARS-CoV-2 carriers and symptomatic/presymptomatic COVID-19 patients

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ABSTRACT

Background: Novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is pandemic. However, data concerning the epidemiological features, viral shedding, and antibody dynamics between asymptomatic SARS-CoV-2 carriers and COVID-19 patients remain controversial.

Methods: We enrolled 193 SARS-CoV-2 infected subjects in Ningbo and Zhoushan, Zhejiang, China, from January 21 to March 6, 2020. All subjects were followed up to monitor the dynamics of serum antibody immunoglobulin M (IgM) and IgG against SARS-CoV-2 using colloidal gold-labeled and enzyme-linked immunosorbent assays.

Results: Of those, 31 were asymptomatic SARS-CoV-2 carriers, 148 symptomatic COVID-19 patients, and 14 presymptomatic COVID-19 patients. Compared to symptomatic COVID-19 patients, asymptomatic carriers were younger and had higher levels of white blood cell and lymphocyte, lower level of C-reactive protein, and shorter viral shedding duration. Conversion of IgM from positive to negative was shorter in asymptomatic carriers than in COVID-19 patients (7.5 vs. 25.5 days, $P = 0.030$). The proportion of those persistently seropositive for IgG against SARS-CoV-2 was higher in COVID-19 patients than in asymptomatic carriers (66.1% vs. 33.3%, $P = 0.037$). Viral load was higher in symptomatic patients than presymptomatic patients ($P = 0.003$) and asymptomatic carriers ($P = 0.004$). Viral shedding duration was longer in presymptomatic COVID-19 patients than in asymptomatic carriers (48.0 vs. 24.0 days, $P = 0.002$). Asymptomatic carriers acquired infection more from intra-familial transmission than did COVID-19 patients (89.0% vs. 61.0%, $P = 0.028$). In 4 familial clusters of SARS-CoV-2 infection, asymptomatic carriers were mainly children and young adults while severe COVID-19 was mainly found in family members older than 60 years with comorbidities.

Conclusion: Asymptomatic carriers might have a higher antiviral immunity to clear SARS-CoV-2 than symptomatic COVID-19 patients and this antiviral immunity should be contributable to innate and adaptive cellular immunity rather than humoral immunity. The severity of COVID-19 is associated with older age and comorbidities in familial clustering cases.

Introduction

Novel coronavirus disease in 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a pandemic since early 2020 [1]. The case number keeps increasing. Globally, as of 5:56 pm CET, February 17, 2021, there have been 109,217,366 confirmed cases of COVID-19, includ-

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ing 2,413,912 deaths, reported to World Health Organization [2]. Hospital-based transmission, family clustering transmission, and transmission via public service such as food delivery were the three major epidemiological features of this outbreak at the early stage, and continued to be an important cause of community-based SARS-CoV-2 transmission in a low prevalence region [3–5]. COVID-19 patients and asymptomatic carriers are the main sources of SARS-CoV-2 infection but might have differences in some features [6]. However, information concerning SARS-CoV-2 transmission and viral shedding duration between COVID-19 patients and asymptomatic SARS-CoV-2 carriers remain controversial. It has been summarized from early studies that viral load of asymptomatic carriers is comparable to symptomatic patients [7]. In another study, it has been demonstrated that a considerably higher viral load is present in samples from fatal cases compared to asymptomatic carriers [8]. Difference in the dynamics of antibody against SARS-CoV-2 between asymptomatic carriers and COVID-19 patients remains to be clarified. To understand the differences between asymptomatic and symptomatic SARS-CoV-2 infected subjects, we conducted a study to investigate the epidemiological features, laboratory findings, viral shedding, and antibody conversion of SARS-CoV-2 infected cases among asymptomatic SARS-CoV-2 carriers and symptomatic/presymptomatic COVID patients in two nearby cities, a low prevalence region in Zhejiang, China.

Materials and methods

Study design and patients

It is an ambispective cohort study. The study enrolled all diagnosed cases with SARS-CoV-2 infection on Ningbo city and a familial clustering infection with SARS-CoV-2 in Putuo district of Zhoushan city from January 21 to March 6, 2020. Study participants did not receive any vaccination against SARS-CoV-2 during the study period. Epidemiological, clinical characteristics, pathogen and serological test results were collected by Ningbo Municipal Center for Disease Control and Prevention (Ningbo CDC) and CDC of Putuo district, Zhoushan (Putuo CDC). Some baseline information including demographic and pathogenic data of those patients was reported [9,10]. After that, we continued to investigate viral load and viral shedding of SARS-CoV-2, laboratory tests, and the dynamics of serum immunoglobulin M (IgM) and immunoglobulin G (IgG) against SARS-CoV-2 between asymptomatic carriers and COVID-19 patients. All patients were followed up to monitor the dynamics of IgM and IgG against SARS-CoV-2. Those with IgM and IgG serological tests for two times or more within 160 days were included in antibody seroconversion analysis. Diagnoses and disease staging of COVID-19 were carried out according to the Protocol for the Diagnosis and Treatment of COVID-19 (Version 7th), National Health Commission of the People's Republic of China [11]. Specifically, COVID-19 was diagnosed if the patient was tested positive for SARS-CoV-2 genomic RNA and accompanied by clinical symptoms including fever and cough. Asymptomatic carrier was identified in close COVID-19 patient contactors who did not have any symptoms during the course from the date first tested positive to the date tested negative for SARS-CoV-2 genomic RNA. We also classified COVID-19 patients into symptomatic ones with onset of disease and presymptomatic ones. Presymptomatic patients were referred to those tested positive for SARS-CoV-2 genomic RNA but did not have any clinical symptoms until a period of incubation. All diagnosed COVID-19 patients were classified as mild, common, severe, and extremely severe types, according to the criteria [11]. This study was approved by the Ethics Commissions of Ningbo CDC and Putuo CDC. A written informed consent was waived for emerging infectious diseases.

Epidemiological survey

A semi-structured questionnaire was applied to obtain demographic information, exposure information of the familiar clustering cases via face-to-face interview and telephone calls by well-trained professionals. The data regarding any travel history to high risk areas with COVID-19 epidemic, contact with confirmed cases or asymptomatic carriers tested positive for SARS-CoV-2 genomic RNA, contact with patients with some symptoms like fever, dry cough, and expectoration in the past 2–3 weeks before illness onset. Any chance and duration of attending any kinds of population gatherings were recorded as well.

Clinical information included the date of symptom onset and admission to hospitals designated by local governments, clinical manifestation, routine laboratory examinations, and radiographic examinations. The clinical manifestations, chest computed tomography images, and laboratory results of patients in Ningbo were collected from the electronic medical record systems in the two designated hospitals: Ningbo First Hospital and Huamei Hospital. The information of patients from Zhoushan was collected from Zhoushan Maternal and Child Health Care Hospital and Putuo Hospital. Two researchers (PL and YD) independently reviewed all of the data to doubly check the accuracy of data collected.

Examination of SARS-CoV-2 genomic RNA

Quantitative reverse transcription-PCR (qRT-PCR) assay was applied to detect SARS-CoV-2 genomic RNA in nasal and throat swabs, sputum, and feces of patients. Patients in Ningbo were examined using the test kits manufactured by Shanghai BioGerm Medical Technology (Shanghai, China) and Daan Gene Co., Ltd. (Guangzhou, China) [12]. Patients in Zhoushan were examined using the test kits manufactured by Shanghai GeneoDx Biotech Company (Shanghai, China) [13]. Sample was positive if the cycling threshold (CT) values of reverse-transcription polymerase chain reaction (RT-PCR) for the ORF1ab and the N genes were less than 37. Sample was negative if no CT value, or CT value of greater than 40, or unrepeatable CT value in the range of 37–40.

Detection of antibodies against SARS-COV-2

IgM and IgG against SARS-CoV-2 in the frozen reserved serum samples of fasting blood in Ningbo and Zhoushan were tested using colloidal gold-labeled kits supplied by Innovita Biological Technology (Tangshan, China) [14]. Testing results by Innovita ELISA kits were determined by the color reaction. If color intensity of test band was lower than control band, it represented a weak positive result; if the intensity of test band was equal or greater than that of control band, it corresponded to a positive result. Absence of a test line was considered negative. Serum IgM and IgG in the study participants from Zhoushan were also quantified using the enzyme-linked immunosorbent assay (Wending Biotech Co., Nanjing, China). The presence and levels of serum IgM and IgG were presented as optical density (OD)/cut-off value (CO). Sample was positive if the ratio of OD to CO was equal or greater than 1 and negative if the ratio was less than 1. A higher OD/CO value indicated a higher level of antibody concentration.

Statistical analysis

Categorical variables were presented as count (%) and compared using the χ^2 test or the Fisher exact test. Continuous variables were described using median and interquartile range (IQR) values and then compared using Mann–Whitney U test or Kruskal Wallis test. These statistical analyses were two-sided and performed using R, version 3.6.2 (R Foundation for Statistical Computing, Can-

berra, Austria). Scatter diagram to demonstrate the distribution of IgM and IgG against SARS-CoV-2 among asymptomatic carriers and symptomatic and presymptomatic COVID-19 patients were generated by R. A *P* value of <0.05 was considered significant for two independent groups. An adjusted *P* value of <0.017 was considered significant by Bonferroni-Dunn test for pairwise comparison among three groups.

Results

Epidemiological characteristics of asymptomatic SARS-CoV-2 carriers, symptomatic COVID-19 patients, and presymptomatic COVID-19 patients

A total of 193 SARS-CoV-2 infected subjects were enrolled in this study. Of those, 31 were asymptomatic carriers, 148 symptomatic COVID-19 patients, and 14 presymptomatic COVID-19 patients. Medium temporal interval of presymptomatic patients developing symptoms were 2.79 days (IQR, 1.00–5.22). Of the 187 patients from Ningbo, 3 family clusters were included. Those patients were close contacts of diagnosed COVID-19 patients and then tested positive for SARS-CoV-2 genomic RNA, from January 21 to March 6, 2020. A family cluster was from Putuo, Zhoushan. A 41-year-old man, who once contacted with a COVID-19 relative from Hubei, China, was the first COVID-19 case who transmitted SARS-CoV-2 to other five family members from January 31 to February 3, 2020 during celebrating Spring Festival in Putuo. All family members were tested positive for SARS-CoV-2 genomic RNA, with 1 asymptomatic SARS-CoV-2 carrier and 5 symptomatic COVID-19 cases. Our epidemiological survey indicated the family members did not have opportunity to get the infection from other sources.

Asymptomatic SARS-CoV-2 carriers were significantly younger than symptomatic COVID-19 patients and presymptomatic COVID-19 patients. Compared to symptomatic COVID-19 patients, asymptomatic SARS-CoV-2 carriers had higher levels of circulating white blood cell (WBC) and lymphocyte, lower levels of C-reactive protein (CRP) and viral load, and shorter viral shedding time. Interestingly, viral load was significantly lower in presymptomatic COVID-19 patients than in symptomatic ones. The viral shedding duration was significantly longer in presymptomatic COVID-19 patients than in asymptomatic carriers. The first-time serological tests showed that nearly one-third of asymptomatic carriers and symptomatic COVID-19 patients were seronegative for IgM against SARS-CoV-2 while the seronegative rates for IgG to SARS-CoV-2 were around 7% in the two populations, respectively (Table 1).

Dynamics in seroconversion of IgM and IgG against SARS-CoV-2 between asymptomatic carriers and COVID-19 patients

Of the 193 study subjects, 74 (15 asymptomatic carriers and 59 COVID-19 patients) had two consecutive test results of IgM and IgG against SARS-CoV-2 within 160 days. SARS-CoV-2-specific IgM seroconversion from positive to negative or weak positive occurred in 9 (60.0%) asymptomatic carriers and in 28 (47.5%) COVID-19 patients (*P* = 0.647). However, the median time interval of IgM seroconversion from positive to negative was 7.50 (IQR, 4.75−11.50) days in asymptomatic carriers, which was significantly shorter than 25.50 (IQR, 6.75−56.75) days in COVID-19 patients (*P* = 0.030). SARS-CoV-2-specific IgG seroconversion from positive to negative or weak positive occurred in 8 (53.4%) asymptomatic carriers, while this occurred in 15 (25.5%) COVID-19 patients (*P* = 0.059). Importantly, 33.3% (n = 5) of asymptomatic carriers were consistently seropositive for IgG against SARS-CoV-2, this percentage was 66.1% (n = 39) in COVID-19 patients (*P* = 0.037). Furthermore, there was

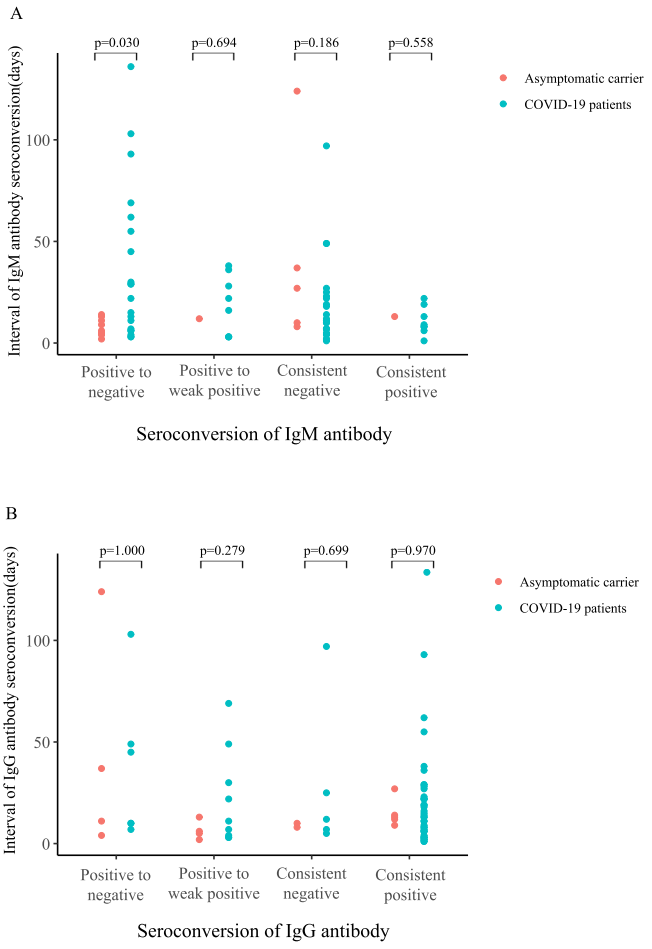

Fig. 1. Temporal intervals of IgM and IgG seroconversion in asymptomatic SARS-CoV-2 carriers and in COVID-19 patients during the follow-up.
IgM, immunoglobin M; IgG, immunoglobin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, novel coronavirus disease in 2019.

no significant difference in the time interval of IgG seroconversion between the two groups (Table 2, Fig. 1).

Intrafamilial transmission of SARS-CoV-2

Four family clusters were included in the study, 3 from Ningbo and 1 from Zhoushan. There are 24 family members in the 4 family clusters, 15 (62.5%) acquired SARS-CoV-2 infection and 9 (37.5%) did not get the infection. Of the 15 patients, 7 were asymptomatic carriers and 8 were COVID-19 patients. Of the 7 asymptomatic carriers, 3 were children at the age of 12 years or younger, 3 adults aged from 18 to 60 years, and a 75-year-old woman. Of the 8 COVID-19 cases, 4 were older than 60 years and diagnosed as severe cases, 3 of the 4 severe cases had comorbidities. The remaining 4 were mild patients at the age between 18 and 60 years. Only one of the 4 mild cases had an underlying disease. Although intrafamilial transmission was the major cause of acquiring SARS-CoV-2 infection, the proportion of those acquiring SARS-CoV-2 infection via intra-familial transmission was significantly higher in asymptomatic carriers than in COVID-19 patients (89% vs. 61%, *P* = 0.028) (Fig. 2). In the familial cluster in Putuo, the index case's wife who acquired the infection from his husband at the very beginning had typical dynamic features in antibodies. The titers of IgM and IgG started to decrease after nearly a month's increase after the exposure, and then increased again (Fig. 3). The second increase in IgM

Table 1
Baseline information of COVID-19 patients and asymptomatic SARS-CoV-2 carriers in Ningbo and Zhoushan cities of Zhejiang province, China.

	Asymptomatic carriers (N = 31)	Symptomatic COVID-19 patients (N = 148)	Presymptomatic COVID-19 patients (N = 14)	P^*	$P^{\&}$	$P^{\#}$
Gender				0.453	1.000	0.750
Male	15 (48.4)	50 (33.8)	7 (50.0)			
Female	16 (51.6)	98 (66.2)	7 (50.0)			
Age, years	42.00 (24.00–55.00)	53.00 (38.00–62.75)	55.00 (39.50–71.00)	**<0.001**	**0.002**	1.000§
<30	9 (29.0)	17 (11.5)	1 (7.1)			
30–59	18 (58.1)	89 (60.1)	8 (57.1)	0.076	0.345	1.000
≥60	4 (12.9)	42 (28.4)	5 (35.7)			
Underlying diseases				1.000	1.000	1.000
No	24 (77.4)	104 (71.7)	9 (64.3)			
Yes	7 (22.6)	41 (28.3)	5 (35.7)			
WBC, 10^9/L	5.83 (5.00–7.11)	4.63 (3.80–5.69)	5.83 (4.73–6.75)	**<0.001**	1.000	0.075§
Lymphocyte, 10^9/L	1.53 (1.32–2.11)	1.22 (0.86–1.60)	1.26 (0.89–1.86)	**0.003**	0.420	1.000§
CRP, mg/L	1.00 (0.60–2.99)	6.90 (2.04–18.20)	3.07 (0.97–14.45)	**<0.001**	0.317	0.885§
Viral shedding, day	24.00 (21.00–30.80)	46.50 (35.00–58.00)	48.00 (23.75–51.25)	**<0.001**	**0.002**	1.000§
qRT-PCR-CT values	31.40 (27.50–34.50)	29.00 (24.25–32.00)	33.50 (28.25–36.25)	**0.004**	0.410	**0.003**§
IgM				1.000	1.000	1.000
Negative	12 (38.7)	43 (31.9)	5 (50.0)			
Positive	19 (61.3)	91 (67.4)	5 (50.0)			
Weak positive	0 (0.0)	1 (0.7)	0 (0.0)			
IgG				0.112	1.000	0.504
Negative	2 (6.5)	10 (7.4)	1 (10.0)			
Positive	25 (80.6)	122 (90.4)	8 (80.0)			
Weak positive	4 (12.9)	3 (2.2)	1 (10.0)			

Abbreviations: WBC, white blood cell; CRP, C-reactive protein; CT, cycling threshold; IQR, interquartile range.
P^*, asymptomatic carrier vs. symptomatic COVID-19 patients.
$P^{\&}$, asymptomatic carrier vs. presymptomatic COVID-19 patients.
$P^{\#}$, symptomatic COVID-19 patients vs. presymptomatic COVID-19 patients.
§Continuous variables are expressed as median (IQR). *P* value was calculated using Kruskal Wallis test.
Categorical variables are expressed as n (%). *P* values were calculated using χ^2 test and Fisher's exact test.

Table 2
Seroconversion of IgG and/or IgM antibody against SARS-CoV-2 during follow-up time.

	Overall	Asymptomatic carrier (N = 15)	COVID-19 patients (N = 59)	*P*
Seroconversion of IgM antibody				0.647
From positive to negative	28 (37.8)	8 (53.3)	20 (33.9)	0.234
From positive to weak positive	9 (12.2)	1 (6.7)	8 (13.6)	0.676
Consistent negative	28 (37.8)	5 (33.3)	23 (39.0)	0.772
Consistent positive	9 (12.2)	1 (6.7)	8 (13.6)	0.676
Time interval of IgM antibody seroconversion, days				
From positive to negative	13.00 (6.00–33.75)	7.50 (4.75–11.50)	25.50 (6.75–56.75)	**0.030**
From positive to weak positive	16.00 (3.00–28.00)	12.00 (12.00–12.00)	19.00 (3.00–30.00)	0.694
Consistent negative	11.50 (7.00–25.50)	27.00 (10.00–37.00)	11.00 (7.00–22.50)	0.186
Consistent positive	9.00 (8.00–13.00)	13.00 (13.00–13.00)	8.50 (7.50–14.50)	0.558
Seroconversion of IgG antibody				0.059
From positive to negative	10 (13.5)	4 (26.7)	6 (10.2)	0.110
From positive to weak positive	13 (17.6)	4 (26.7)	9 (15.3)	0.446
Consistent negative	7 (9.5)	2 (13.3)	5 (8.5)	0.624
Consistent positive	44 (59.5)	5 (33.3)	39 (66.1)	**0.037**
Time interval of IgG antibody seroconversion, days				
From positive to negative	24.00 (10.00–48.00)	24.00 (9.25–58.75)	27.50 (10.00–48.00)	1.000
From positive to weak positive	7.00 (4.00–22.00)	5.50 (4.25–7.75)	11.00 (4.00–30.00)	0.279
Consistent negative	10.00 (7.50–18.50)	9.00 (8.50–9.50)	12.00 (7.00–25.00)	0.699
Consistent positive	13.50 (6.75–24.00)	13.00 (12.00–14.00)	14.00 (6.00–25.00)	0.970

Data are median (IQR), n (%), *P* values compare using χ^2 test, Fisher's exact test, or Kruskal Wallis test. IQR, interquartile range.

and IgG against SARS-CoV-2 was correlated to the time that she took care of her parents who had severe COVID-19 in the hospital.

Discussion

To characterize the epidemiological features including immune response, viral transmission, and antibody seroconversion in asymptomatic SARS-CoV-2 carriers, we made a comprehensive comparison between asymptomatic carriers and COVID-19 patients in this study. Compared to symptomatic COVID-19 patients, asymptomatic SARS-CoV-2 carriers were younger and had higher levels of circulating WBC and lymphocyte and a lower level of CRP. These data indicate that asymptomatic carriers have a stronger antiviral immunity and a lower level of systemic inflammation. It has been proven that innate and adaptive lymphocytes and inflammatory factors were closely related to disease progression of COVID-19, from mild to severe [15,16]. In a previous prospective study, we have demonstrated that lower circulating counts of T lymphocytes, $CD4^+$ T cells, and $CD8^+$ T cells as well as higher circulating levels of neutrophil proportion, neutrophil/lymphocyte ratio, interleukin-6, CRP, and procalcitonin facilitate the progression of COVID-19. Of those, $CD8^+$ T cell exhaustion plays an important role in the pathogenesis of COVID-19 [17]. Disease severity is negatively associated with NK cells and $CD3^+$,

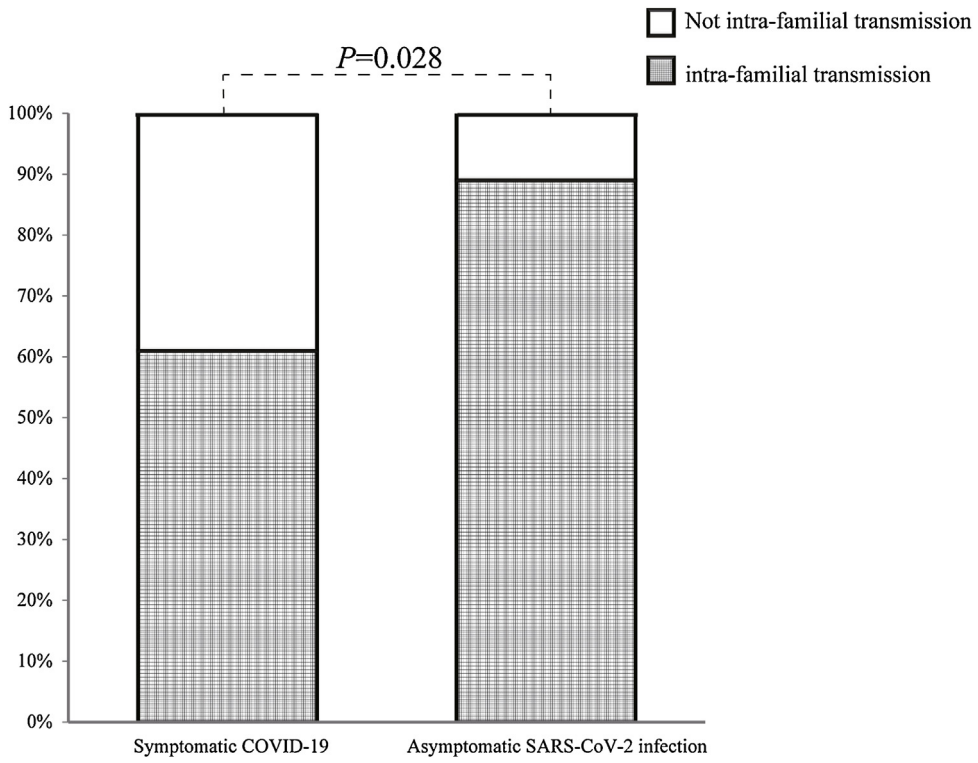

Fig. 2. Proportion of patients acquiring SARS-CoV-2 via intra-familial transmission in asymptomatic carriers and in COVID-19 patients.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, novel coronavirus disease in 2019.

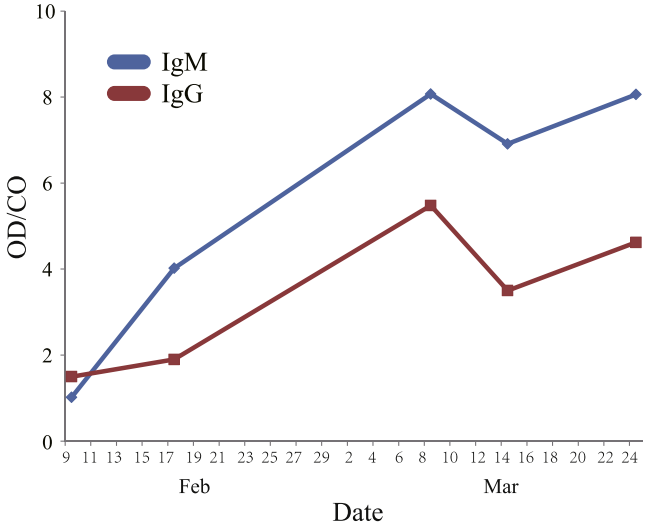

Fig. 3. The dynamics of IgM and IgG levels in a given COVID-19 patient in Putuo district of Zhoushan, Zhejiang, China, from February 9 to March 24, 2020.
OD, optical density; CO, cut-off value; IgM, immunoglobin M; IgG, immunoglobin G; COVID-19, novel coronavirus disease in 2019.

$CD4^+$, and $CD8^+$ T lymphocyte levels, while intensive expansion of highly cytotoxic effector T cell subsets, such as $CD4^+$ effector-granulysin, $CD8^+$ effector-granulysin, and NKT CD160, is associated with convalescence of COVID-19 patients [18,19]. These evidences strongly indicate that damage of innate immunity and T cell-mediated immunity, which might be caused by proinflammatory factor-induced inflammation, play key roles in the development of COVID-19.

In this study, IgG and IgM against SARS-CoV-2 were tested using the kits from Innovita Biological Technology. Innovita Kits have been proven to be reliable for this purpose [20]. We found that 7.4% of COVID-19 patients were seronegative for IgG against SARS-CoV-2 at the first-time serological test, indicating SARS-CoV-2 might not induce sufficient humoral immunity. In this cohort study, IgM seroconversion from positive to negative was much faster in asymptomatic carriers than in COVID-19 patients (P = 0.030). The overall rate of IgG seroconversion from positive to negative or weak positive was around 30% within 160 days after the diagnosis, indicating that IgG against SARS-CoV-2 is not stable. Virus-specific IgG decayed substantially in most individuals [21]. Importantly, seroconversion of IgG against SARS-CoV-2 from positive to negative or weak positive occurred 53.4% in asymptomatic carriers and 25.5% in COVID-19 patients, while consistently seropositive rate of IgG against SARS-CoV-2 was significantly higher in COVID-19 patients than in asymptomatic carriers (P = 0.037). The similar observations concerning rapid seroconversion of the antibody against SARS-CoV-2 or short-lived immune response after mild infection were also reported in the frontline health care personnel in the US and active workers in France [22,23]. These data indicate that humoral immunity against SARS-CoV-2 was not efficiently aroused in asymptomatic carriers or in those with a stronger innate and cell immunity. The presence of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 patients, indicates the feasibility of vaccination program [24,25]. However, there are no evidences showing that other antibodies have decreased IgG over time. The mechanism by which IgG against SARS-CoV-2 rapidly declined remains unknown. Besides, repeat exposure to the same virus may arouse a higher humoral immunity. A family cluster in this study should be a suitable example to address this issue. The index patient's wife should be once more infected by the same SARS-CoV-2 from her patients, because both antibodies increased again after declined. Interestingly, we observed that the profile of IgM and IgG against SARS-CoV-2 was quite similar during the course, in contrast to most viral infections. Our observation is quite consistent with previous reports and the Interim Guidelines for COVID-19

Antibody Testing [26–28]. Our data imply that boosting vaccination with SARS-CoV-2 might be important.

Compared to COVID-19 patients, asymptomatic carriers had a lower level of viral load and shorter viral shedding time (Table 1). Our finding is different from a study carried out in Chongqing that asymptomatic carriers had a significantly longer duration of viral shedding than the symptomatic patients [29]. In early stage of COVID-19 studies, asymptomatic carriers might include presymptomatic patients while the viral shedding duration was significantly longer in presymptomatic COVID-19 patients than in asymptomatic carrier (Table 1). Lower viral load and shorter viral shedding duration in asymptomatic carriers should be unlikely caused by the neutralizing antibody, because the antibody, either IgM or IgG, was declining more rapidly in asymptomatic carriers than in COVID-19 patients. Innate immunity and cell-mediated immunity should play key roles in repressing viral replication in asymptomatic carriers [17,30]. Lower viral load and shorter viral shedding duration should be due to a relative stronger antiviral immunity, as a high viral load often predisposes adverse outcomes of COVID-19 [8,31]. To develop effective vaccine against SARS-CoV-2, it is important to arouse the specific cell immunity, instead of focusing on humoral immunity.

We found that SARS-CoV-2 viral load was significantly lower in presymptomatic COVID-19 patients than in symptomatic ones (Table 1), which is inconsistent with two other studies [32,33]. It has been reported that SARS-CoV-2 viral load in the upper respiratory tract appears to peak in the first week of illness, at the time of symptom onset [34,35]. Thus, SARS-SoV-2 load should keep increasing in the incubation period and cause symptom when it reaches the top. The difference in SARS-SoV-2 load between presymptomatic and symptomatic COVID-19 patients depends on the time points of samplings. Asymptomatic carriers had a lower level of viral load and shorter viral shedding duration, indicating that the transmissibility of asymptomatic carriers was relative weaker. In the 4 familial clusters, asymptomatic carriers were mostly children and young adults, mild patients were young and middle-aged adults between 18 and 60 years, and severe cases were older than 60 years with comorbidities. Family members were exposed to the same source of infection. However, they had diverse clinical manifestations. Our observation is quite in consistent with the previous studies that children acquire SARS-CoV-2 infection mostly have mild respiratory symptoms or are asymptomatic, whereas elderly patients with COVID-19, especially male patients, are more likely to progress into severe-type and even die of this disease [36–39]. Thus, the host immunity and underlying inflammation, which is often affected by ageing, comorbidities, and dysregulated macrophage response [17,37,38,40], should be the major determinants of disease severity of COVID-19. Although asymptomatic carriers often acquire the infection from family members, they can transmit SARS-CoV-2 into family members and hospital centers, and eventually kill aged members. As a considerable percentage of SARS-CoV-2 infections may be asymptomatic or presymptomatic, enhanced testing approaches are needed to detect those who transmit the virus.

Our study has some limitations. First, follow-up should be extended to observe the duration of SARS-CoV-2-specific antibodies. Second, sample size of asymptomatic carriers with SARS-CoV-2 infected was relatively small.

Conclusions

Asymptomatic carriers have a higher level of antiviral immunity and lower level of inflammation than do symptomatic COVID-19 patients. This antiviral immunity should not be contributable to humoral immunity because both IgM and IgG against SARS-CoV-2 are declining more rapidly in asymptomatic carriers than in COVID-19 patients. The severity of COVID-19 is associated with older age and comorbidities in familial clustering cases. Our data also suggest that boosting vaccination with SARS-CoV-2 should be important. This study may help elucidate the mechanisms by which SARS-CoV-2 interacts with host immunity in determining the outcome of SARS-CoV-2 infection, and optimize the strategy of vaccination to terminate SARS-CoV-2 pandemic.

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Conflicts of interest

None declared.

Ethics approval and consent to participate

This study was approved by the Ethics Commission of Ningbo CDC and Putuo CDC.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Miao Liu and Yi Chen: investigation, field survey, data organization. Ping Li and Yibo Ding: quality control, data analysis, and composition of figures and table. Leijie Liu and Bo Yi: investigation, etiological identification, antibody test. Hongjun Dong, Xuying Lao and Ting Wu: project management. Dongliang Zhang and Xiaojie Tan: data entry and analysis. Keqing Ding, Haibo Wang and Zhongfa Wang: laboratory test and etiological identification. Guozhang Xu and Guangwen Cao: conceptualization, investigation, supervision, and funding acquisition. Guangwen Cao wrote this manuscript.

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