Disease and Diagnosis

→ **Original Article**

Humoral Immune Durability of Anti-SARS-CoV-2 Antibodies in Southern Iran: A Population-Based Survey Study

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Abstract
Background: To promote mitigation strategies and public health response, this study aimed to evaluate the population-based seroprevalence of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in Hormozgan province.
Materials and Methods: The study was conducted within 10 districts of Hormozgan province with 1325 participants for three months by considering three-month successive follow-ups to evaluate the durability of humoral immunity. The participants completed the questionnaire, and blood samples were taken followed by immunoassay SARS-CoV-2 ELISA testing.
Results: In general, 717 (54.1%) males and 596 (45.9%) females participated in this study. In phase one, 147 (11.1%) and 182 (14.7%) tested positive for immunoglobulin M (IgM) and IgG, respectively. Upon three months, 13.8% and 17.8% tested positive for IgG and at least for one of the antibodies. Based on the results, 606 (45.7%) cases reported no symptoms while 673 (50.8%) of them reflected at least one. Among 798 (60.2%) participants, the most common symptoms were headache (n=244, 18.4%), sore throat (n=186, 14.0%), weakness (n=150, 11.3%), muscular pain (n=139, 10.5%), and sputum cough (n=134, 10.1%). The odds of the antibodies in females was 1.37 (95% CI: 1.03, 1.82, $P=0.03$). In phase 2, 43 (5.3%) participants persisted positive for IgG while 559 (73%) tested negative for IgG. Finally, 20% of the suffered participants tested positive for IgG until nine months.
Conclusion: Although IgG antibodies decreased in the first six months, their titers persisted stable for nine months. It seems our population has not reached a desirable level of protection. It is stressed that mass vaccination is needed to prevent future epidemic waves.
Keywords: COVID-19 pandemic, Humoral immune response, Seroepidemiologic study, Antibodies

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Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus in December 2019 in Wuhan, China rapidly prompted a tremendous epidemic resulting in a novel coronavirus disease 2019 (COVID-19) ongoing pandemic across the world and thus becoming a great threat to our societies. Subsequently, the World Health Organization (WHO) acknowledged a public health emergency of worldwide concern on January 30, 2020 (1), and then launched a couple of measures including mitigation, limited travel, lockdown, quarantine, social distancing, and contact tracing (2). Coronavirus is a zoonotic RNA virus of the family Coronaviridae, leading to respiratory infections. Of the known human coronaviruses, three types are extremely infective including SARS-CoV, SARS-CoV-2,

and Middle East respiratory syndrome (MERS)-CoV. Bats were identified as the likely source of SARS-CoV-2, and the virus uses the cell surface receptor, angiotensin-converting enzyme 2 (ACE2) to enter host cells (3). It was realized that the virus was quickly developing regardless of our inadequate knowledge of the disease. There is a consensus on figuring out various aspects of COVID-19 disease, including molecular and clinical characteristics, epidemiology, morbidity, mortality, case fatality rates, prevalence, reproduction rates, transmissibility, actual preventive, therapeutic procedures, and the burden of disease (4, 5). The main query regarding actions against COVID-19 is the impact and enduring immunity against the disease in earlier infected individuals. The current evidence on immune responses regarding their dynamics over time and their clinical implication is systematically inadequate. It seems that the immune system of individuals infected with SARS-CoV-2 confers detectable humoral and cell-mediated immune responses. Several diagnostic approaches are currently directed, including serology tests which can detect antibodies to SARS-CoV-2, suggesting the presence or absence of infection, recognizing individuals with immune response, or assessing previous infections. It is noteworthy that accurate detection of individuals, who formerly had COVID-19, is essential in gauging disease spread, evaluating the achievement of public health interventions (e.g., curfew and quarantine), and identifying individuals with potential immunity (6). Molecular diagnostics, as the gold standard for detecting infected persons, are not perfect for determining accurately affected individuals and the rate of asymptomatic infections. Serological assays commonly possess the potential role in determining the epidemiology all-inclusive, disease burden, asymptomatic infections in a fast turnaround time, early stages of infection, and those with the negative real-time polymerase chain reaction (RT-PCR) test, early detection of infected patients, clinical utility, determination of infection and mortality rates in a population, detection of the infected cases, and likely immunized people (7). Notably, immunoglobulin M (IgM) and IgG antibodies are mainly generated against spike (S), nucleocapsid (N), and receptor binding domain of the SARS CoV-2 proteins. Therefore, ELISA assays and other serological techniques have been developed to detect antibodies based on the afore-mentioned antigens. Remarkably, a wide range of suspected people could be quickly screened by means of modest equipment and reasonable sensitivity and specificity rates while with slight false-negative outcomes. The seroprevalence of anti-SARS-CoV-2 antibodies is warranted to mitigate the spread of the virus and help the authorities to organize the up-to-date social interventions and to estimate the clinical impact of COVID-19. Subsequently, the detection of the specific IgG antibody, as a large scale seroepidemiological study,

would benefit the right proportion of infected individuals and can be considered for finding the exact scale of the disease spread and pandemic (8). Moreover, serological markers serve as the key means for estimating the extent of COVID-19 in the public and recognizing those who are immune or with mild to modest disease that would appear late. The IgM antibody has been detected as early as the fourth day after the onset of COVID-19 symptoms followed by IgG within the next few weeks which the latter, in turn, would indicate the former, intermediate, or late infection. Therefore, it is quite reasonable to organize large-scale ELISA tests to detect anti SARS CoV2 antibodies in suspected cases in the community with a low rate of false negative results. Additionally, inappropriate disease identification due to false-positive results would bring about useless testing, treatment, and lockdown (9). Although the precise durability of the generated immunity is elusive, high levels of neutralizing antibodies targeting SARS-CoV-2 Spike protein seem to offer significant safety against SARS CoV 2 reinfection for several months (10, 11). It is argued that the duration, power, and the extent of the generated immunity could be worth and reliable during the pandemic. With reference to yielded immunity, it is worth detecting robust biomarkers, building efficient components of protection, and revisiting the concepts of hospitalization, disease morbidity and mortality, and virus spread (12). Although ELISA-based IgM and IgG antibody assays yield 95% specificity for the diagnosis of COVID-19, the immune durability and protection conferred by the neutralizing antibodies remain unidentified (13). A reasonable number of studies are running in this regard, and two studies in the United Kingdom declared six-month immune protection against SARS-CoV-2 (14). A seroprevalence survey enrolling 61 000 individuals in Spain showed that 5% of the public had generated antibodies against the spike and nucleoproteins (15). Other studies conducted in California and New York reported the seroprevalence rate based on ethnicity and age group (16, 17). The prevalence rate of the corresponding antibodies varies and is likely higher in Iran, however, our knowledge on immune responses and respective durability in asymptomatic individuals is limited. Based on extensive evidence, asymptomatic people would spread the virus fast, thus more prospective seroepidemiological population-based studies on symptomatic and asymptomatic persons are warranted to address the immune durability and true infection rate (18, 19). Therefore, the current longitudinal, population-level, and cross-sectional study sought to determine SARS-CoV-2 seroprevalence correlating with demographic features and humoral immune durability of anti-SARS-CoV-2 antibodies in Hormozgan Province including 12 cities with diverse geography and known public transmission.

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Materials and Methods

Study Design and Participants

In terms of the cross-sectional study, a longitudinal population-based study was designed to perform the serological tests for anti-SARS-CoV-2 antibodies to determine the prevalence of SARS-CoV-2 infection within 10 districts of Hormozgan province. The overall sample size was calculated at 1325 with reference to the cumulative estimated prevalence of 20% for COVID-19 disease and taking into account 5% precision with a design effect of 5.39. As a prospective investigation, the samples were followed for 10 months (300 days) approximately every three months to evaluate the durability of IgM and IgG humoral antibodies. The assigned districts were Bandar Abbas, Rodan, Minab, Bastak, Bandar Lengeh, Khamir, Kish, Qeshm, HajiAbad, and Sirik. The eligible participants were selected from local residents who were at least stayed for two weeks before the study get started via the probability-proportionate to-size sampling method from the general population, particularly high-risk occupations in terms of exposure to SARS-CoV-2 (e.g., health workers, nurses, public transportation drivers, bank employees, and cashiers). They were acknowledged and then listed by local health care centers without any inclusive or exclusive criteria except for those who suffered from serious underlying chronic diseases and intellectual disability and shown an unwillingness to take part in the study.

The accidental selection was used to get a systematic sampling approach. Through a random selector application, one name on the registry was chosen and this process was repeated until completing our list. The public sampling was based on "national identification number", which is specific for each Iranian person and was registered in the Iranian electronic health record system (SIB) from all cities of Hormozgan province. The individuals were informed through the call phones since their telephone numbers were available in their profiles. SIB provides demographic and administrative health information data for all Iran populations. Samples were mainly focused on people who were more exposed to the SARS-CoV-2 virus because of further exposure to close social face-to-face contact with infected individuals through their job environment. High-risk individuals were extracted from the registration lists provided by the relevant employer in each district and were informed by phone. The assigned participants completed a standardized questionnaire containing demographic, social variable, and comorbidity sections. The first and second features included age, body mass index (BMI), job, blood group, residence area, room for quarantine, face mask, contact status, presence in crowded areas, and use of public transport. The last section consisted of the underlying diseases such as heart diseases, hypertension, diabetes, asthma, chronic kidney diseases, malnutrition, thalassemia, and other chronic diseases. Then, high-risk jobs (including medical staff, housewives, public transportation drivers, shopping malls staff, training units' staff, kitchens and restaurants workers, and office clerks) were compared with other careers. To augment the rate of participation, sampling was implemented at workplace (e.g., bank or supermarket and the like). The presence of infection based on antibody detection was merely identified in this study.

Procedure

At Molecular Medicine Research Center, the demographic characteristics of the samples were completed by an expert via questioning whether they take any medication, have COVID-19-related symptoms, and any of their close relatives have been infected with COVID-19. After completion of the questionnaire, 5 mL of intravenous blood was collected in a gel clot activator tube. The serum was then separated and stored at -20℃. In this study, SARS-CoV-2 ELISA kits (Pishtaz Teb, Tehran, Iran) with the approval of Iran's Food and Drug Administration (Lot numbers PT-SARS-COV-2) were used to assess the amount and maintenance of SARS-CoV-2-specific IgG and IgM antibodies in the serum samples.

Test Validation

As mentioned earlier, ELISA Kit SARS-COV-2 was applied to measure the titer of antibodies in this study. According to the manufacturer's announcement, the sensitivity and specificity were 85.84% and 99.40%, as well as 82.2% and 91.28% for the IgM and IgG kits, respectively. IgM kits have been designed based on the antibody capture method. Plate wells were coated with the anti-human IgM antibody. The samples were diluted inside the wells while processing. All IgM in the serum sample (including anti-SARS-COV-2 IGM) bound to antibodies at the well floor. The unattached antibodies were removed after the initial wash. Then, it was added to the IgM and formed a complex. After washing, the dye solution was poured into the wells. The intensity of the blue color illustrated the amount of the formed immune complex in the wells. Adding the solvent turned the blue color to yellow, which has the best light absorption at a wavelength of 450 nm. With regard to IgG assay, the plate wells were coated with SARS-COV-2 virus N antigens (core coating). Similar to the previous process, diluted samples were poured into the wells. In the presence of antibodies against SARS-COV-2 antigens, these antibodies bound to the well antigens. Then, after washing, by adding anti-IgG antibodies binding to the HRP enzyme, if any anti-SARS-COV-2 anti-IgG antibody was present, IgG-anti-human labels were also attached to them. Upon washing, the dye solution was poured into the wells, and the intensity of the blue color was proportional to the formed immune complex. The addition of a solvent solution turned the blue color yellow, which has the best light absorption at a

wavelength of 450 nm.

Statistical Analysis

Continuous and categorical variables were reported by the mean, standard deviation (SD), as well as the number and percentage, respectively. The chi-square test was applied to examine the association between two categorical variables. Twelve variables including age, BMI, gender, smoking status, job, history of diabetes, hypertension, chronic kidney disease, cardiac disease, chronic lung disease, asthma, and thalassemia were used to detect their association with the binary dependent variable (the presence or absence of antibodies. Simple logistic regression was applied to estimate the crude odds ratio (OR), where the binary variables of the presence or absence of antibodies (yes/no) were considered as the response, and independent variables with a P value of less than 0.25 were included in the final model (multiple logistic regression) to estimate the adjusted ORs. A $P < 0.05$ was considered statistically significant, and data were analyzed using IBM SPSS software, version 26.

Results

Table 1 provides participants' demographic and clinical characteristics. Of 1325 participants, 717 (54.1%) cases were males with the mean (SD) age of 38.75 (11.99) years. In phase one of the study, 20.5% of participants tested positive at least for IgM or IgG antibodies while 147 (11.1%) and 182 (14.7%) of them tested positive for IgM and IgG, respectively. Phase 2 of the study was started after three months, and the results revealed that 5.6%, 13.8%, and 17.8% of the individuals tested positive for IgM, IgG, and at least for one of the antibodies, respectively. At least one of the specified comorbid conditions including diabetes, hypertension, chronic kidney disease, and cardiac disease was present in 192 (14.5%) individuals. According to self-reports, 798 (60.2%) cases were present in crowded areas irrespective of the duration and quality of the exposure. As shown in Table 2, some of the most common clinical manifestations, which have been positive for the past three months, were headache (n = 244, 18.4%), sore throat (n = 186, 14.0%), weakness (n = 150, 11.3%), muscular pain (n = 139, 10.5%), and sputum cough (n = 134, 10.1%). Overall, 606 (45.7%) participants had no symptoms while 673 (50.8%) of them reported at least one manifestation. Further, 52 (3.9%), 32 (2.8%), and 97 (7.3%) cases experienced a decreased level of smell, taste, and runny nose.

Based on data (Table 3), the odds of having antibody was 1.37 times higher for females compared to their counterparts by controlling other factors (OR = 1.37, 95% CI: 1.03, 1.82, $P = 0.03$). Moreover, the odds of having antibody was 2.17 times higher for the patients with thalassemia in comparison with other patients (OR = 2.17, 95% CI: 1.27, 3.70, $P = 0.004$). The likelihood of the raised

antibodies in hypertensives upon exposure was 1.21 (0.74, 1.94) compared with unexposed ones. Furthermore, other confounding variables were controlled, and the odds of antibody presence significantly decreased by 0.020 by 1 unit rise in the BMI ($P < 0.05$). At first glance, however, the latest finding may seem strange since the people having recovered from COVID-19 usually wish to refer to blood transfusion centers to donate blood. Therefore, patients with thalassemia regularly coming to get blood would receive antibodies from the donor's blood. Our findings (Table 4 and Figures 1 and 2) represented that 43 (5.3%) of individuals remained positive for IgG in phase 2 although 632 (82.5%) and 559 (73%) of them tested negative for IgM and IgG, respectively. The results further indicated that 10 out of 43 participants, who tested positive for IgG in both phases, remained positive until nine months (Figure 3). By the age stratification of IgG seroprevalence in phases one and two, the most humoral durability was among the age groups of 30-39 and 40-49 years old, respectively (Figures 4a and 4b).

Out of 766 individuals from 1325 participants attending the second follow-up visit, 76 individuals attended the third phase, of whom 32 men were equivalent to 4.3% of the whole study population. Out of 629 individuals who tested negative for IgG in the first follow-up visit, 16 cases were positive in the next visits. In fact, 10 individuals remained positive through the three follow-up visits.

Discussion

The seroprevalence of SARS-CoV-2-specific antibodies was estimated in 10 districts with diverse population densities in southern Iran, Hormozgan province. In the present study, the proportion of individuals with IgG as neutralizing antibodies against SARS-CoV-2 remained relatively stable across the study for nine months, irrespective of whether the participants were symptomatic or not.

The review of the literature revealed conflicting reports that human coronaviruses may offer approximately two-year immunity as detectable neutralizing antibodies against SARS-CoV. An investigation demonstrated that IgG antibodies against MERS-CoV remained stable for more than one year upon the disease onset (20-22). Additional longitudinal studies evaluating the titers of neutralizing antibodies and the kinetic of immunity against SARS-CoV-2 were warranted for prolonged periods. Immunity against SARS-CoV-2 was stated to persist stable over 4 months (23). Unprecedently, the SARS-CoV-2 virus has so far turned out as a pandemic and posed an extreme hazard to public health. Various clinical manifestations due to the SARS-CoV-2 virus pointed out that most aspects of COVID-19 status are still indefinite. Deciphering the features leading to publicized COVID-19 disease will possibly aid to manage the level of the consequences and design mitigation procedures.

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Table 1. Demographics and Clinical Characteristics of Male and Female Participants

Characteristic			Total (N=1325) No. (%)	Females (n=596) No. (%)	Males (n=717) No. (%)	P Value
Phase 1	Antibody	Positive IgM	147 (11.1)	84 (6.4)	62 (4.7)	0.002
		Positive IgG	182 (14.7)	82 (6.2)	98 (7.5)	0.962
		At least one of them	272 (20.5)	137 (10.4)	132 (10.1)	0.041
Phase 2 (n=766)	Antibody	Positive IgM	43 (5.6)	13 (1.7)	28 (3.7)	0.010
		Positive IgG	113 (13.8)	44 (5.9)	66 (8.8)	0.010
		At least one of them	136 (17.8)	52 (7.0)	80 (10.7)	0.002
Age (y)		<25	112 (8.5)	75 (6.3)	34 (2.8)	<0.001
		25-35	432 (32.6)	209 (17.4)	223 (18.6)	
		35-45	370 (27.9)	146 (12.2)	221 (18.5)	
		≥45	291 (22.0)	117 (9.8)	173 (14.4)	
		Missing data	120 (9.1)			
BMI (kg/m²)		<18.5 (underweight)	87 (6.6)	53 (4.3)	34 (2.8)	0.001
		18.5-24.9 (Normal)	635 (47.9)	287 (23.4)	344 (28.1)	
		25.0–29.9 (Overweight)	412 (31.1)	161 (13.1)	247 (20.1)	
		30.0-39.9 (Obese)	90 (6.8)	50 (4.1)	39 (3.2)	
		≥40 (Severely obese)	11 (0.8)	6 (0.5)	5 (0.4)	
		Missing data	90 (6.8)			
Smoking status		Cigarette	33 (2.5)	1 (0.1)	32 (2.5)	<0.001
		Drug	17 (1.3)	3 (0.2)	14 (1.1)	0.019
		Alcohol	9 (0.7)	1 (0.1)	8 (0.6)	0.037
		At least one of them	45 (3.4)	5 (0.4)	40 (3.1)	<0.001
Job		Low risk	733 (55.3)	323 (26.7)	409 (33.8)	0.850
		High risk	488 (36.8)	214 (17.7)	265 (21.8)	
		Missing data	104 (7.8)			
Blood group		A	179 (13.5)	75 (6.6)	104 (9.1)	0.064
		B	192 (14.5)	75 (6.6)	113 (9.9)	
		AB	65 (4.9)	31 (2.7)	33 (2.9)	
		O	363 (27.4)	153 (13.4)	209 (18.3)	
		Unaware	526 (39.7)	177 (15.5)	171 (15.0)	
Pregnancy		No	541 (40.8)	541 (40.8)	-	
		Yes	60 (4.5)	60 (4.5)	-	
		Missing data	7 (0.5)			
Contact[a]		No	947 (71.5)	428 (33.8)	518 (40.9)	0.755
		Yes	327 (24.7)	148 (11.7)	172 (13.6)	
		Missing data	51 (3.8)			
Presence in crowded centers[b]		No	475 (35.8)	246 (19.5)	229 (18.1)	0.001
		Yes	798 (60.2)	334 (26.4)	455 (36.0)	
		Missing data	52 (3.9)			
Cardiac disease		No	1228 (92.7)	555 (43.5)	664 (52.1)	0.693
		Yes	56 (4.2)	27 (2.1)	29 (2.3)	
		Missing data	41 (3.1)			
Hypertension		No	1180 (89.1)	533 (41.8)	638 (50.0)	0.900
		Yes	104 (7.8)	48 (3.8)	56 (4.4)	
		Missing data	41 (3.1)			
Diabetes		No	1230 (92.8)	552 (43.2)	669 (52.3)	0.271
		Yes	57 (4.3)	30 (2.3)	27 (2.1)	
		Missing data	38 (2.9)			
Chronic lung disease		No	1274 (96.2)	576 (45.1)	689 (53.9)	0.549
		Yes	13 (1.0)	7 (0.5)	6 (0.5)	
		Missing data	38 (2.9)			
Asthma		No	1242 (93.7)	557 (43.6)	676 (52.9)	0.128
		Yes	44 (3.3)	25 (2.0)	19 (1.5)	
		Missing data	39 (2.9)			
Chronic kidney disease		No	1242 (93.7)	569 (44.5)	664 (51.9)	0.070
		Yes	46 (3.5)	15 (1.2)	31 (2.4)	
		Missing data	37 (2.8)			
Malnutrition		No	1279 (96.5)	581 (45.5)	689 (53.9)	
		Yes	8 (0.6)	2 (0.2)	6 (0.4)	
		Missing data	38 (2.9)			
Thalassemia		No	1207 (91.1)	553 (43.3)	646 (50.5)	0.159
		Yes	80 (6.0)	30 (2.3)	49 (3.8)	
		Missing data	38 (2.9)			

Note. BMI: Body mass index; IgM: Immunoglobulin M; IgG: Immunoglobulin G; COVID: Coronavirus disease.
[a] Contact with a person with COVID-19, [b] Presence in crowded centers (Medical centers, doctor's office, school, university, elderly care centers, workplace, theater, concert, conference, and presence in a lab and contact with the samples of the coronavirus).

Table 2. Clinical Manifestations Related to SARS-CoV-2 Virus Exposure in the Past 3 Months

Characteristic		Total (N=766) No. (%)	Females (n=387) No. (%)	Males (n=360) No. (%)	P Value
Cough	No	1189 (89.7)	541 (42.4)	639 (50.1)	0.553
	Yes	97 (7.3)	41 (3.2)	55 (4.3)	
	Missing data	39 (2.9)			
Cough sputum	No	1105 (83.4)	505 (41.1)	590 (48.0)	0.342
	Yes	134 (10.1)	56 (4.6)	78 (6.3)	
	Missing data	86 (6.5)			
Fever	No	1211 (91.4)	547 (42.9)	654 (51.3)	0.689
	Yes	73 (5.5)	35 (2.7)	38 (3.0)	
	Missing data	41 (3.1)			
Chills	No	1233 (93.1)	562 (44.1)	661 (51.8)	0.807
	Yes	52 (3.9)	23 (1.8)	29 (2.3)	
	Missing data	40 (3.0)			
Headache	No	1049 (79.2)	458 (35.7)	581 (45.3)	0.009
	Yes	244 (18.4)	130 (10.1)	114 (8.9)	
	Missing data	32 (2.4)			
Sore throat	No	1105 (83.4)	494 (38.6)	601 (46.9)	0.216
	Yes	186 (14.0)	93 (7.3)	93 (7.3)	
	Missing data	34 (2.6)			
Shortness breath	No	1221 (92.2)	553 (43.1)	658 (51.3)	0.626
	Yes	72 (5.4)	35 (2.7)	37 (2.9)	
	Missing data	32 (2.4)			
Diarrhea	No	1235 (93.2)	563 (43.9)	662 (51.6)	0.756
	Yes	57 (4.3)	25 (2.0)	32 (2.5)	
	Missing data	33 (2.5)			
Decreased smell	No	1241 (93.7)	568 (44.3)	663 (51.7)	0.276
	Yes	52 (3.9)	20 (1.6)	32 (2.5)	
	Missing data	32 (2.4)			
Decreased taste	No	1256 (94.8)	574 (44.7)	672 (52.4)	0.322
	Yes	37 (2.8)	14 (1.1)	23 (1.8)	
	Missing data	32 (2.4)			
Gastrointestinal bleeding	No	1192 (90.0)	539 (42.1)	646 (50.4)	0.142
	Yes	98 (7.4)	47 (3.7)	48 (3.8)	
	Missing data	35 (2.6)			
Nausea	No	1219 (92.0)	541 (42.3)	668 (52.2)	0.003
	Yes	70 (5.3)	44 (3.4)	26 (2.0)	
	Missing data	36 (2.7)			
Vomiting	No	1257 (94.9)	564 (44.1)	683 (53.4)	0.005
	Yes	33 (2.5)	23 (1.8)	10 (0.8)	
	Missing data	35 (2.6)			
Weakness, lethargy	No	1143 (86.3)	522 (40.7)	612 (47.7)	0.579
	Yes	150 (11.3)	65 (5.1)	84 (6.5)	
	Missing data	32 (2.4)			
Muscular pain	No	1152 (86.9)	529 (41.3)	613 (47.9)	0.305
	Yes	139 (10.5)	58 (4.5)	81 (6.3)	
	Missing data	34 (2.6)			
Arthralgia	No	1261 (95.2)	577 (45.4)	676 (53.2)	0.377
	Yes	19 (1.4)	6 (0.5)	11 (0.9)	
	Missing data	45 (3.4)			
Consciousness	No	1066 (80.5)	474 (37.2)	585 (45.9)	0.171
	Yes	114 (8.6)	54 (4.2)	57 (4.5)	
	Missing data	145 (10.9)			
Convulsions	No	1278 (96.5)	585 (45.9)	683 (53.6)	0.092
	Yes	7 (0.5)	1 (0.1)	6 (0.5)	
	Missing data	40 (3.0)			
Runny nose	No	1191 (89.9)	537 (42.0)	645 (50.5)	0.208
	Yes	97 (7.3)	50 (3.9)	46 (3.6)	
	Missing data	37 (2.8)			
Chest pain	No	1231 (92.9)	563 (44.0)	658 (51.4)	0.413
	Yes	59 (4.5)	24 (1.9)	35 (2.7)	
	Missing data	35 (2.6)			
Total	No symptom	606 (45.7)	260 (20.5)	342 (27.0)	0.062
	At least one symptom	673 (50.8)	323 (25.5)	344 (27.1)	
	Missing data	46 (3.5)			

Note. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

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Table 3. Effective Predictors in the Presence or Absence of Antibodies (Yes/No)

Predictor	Antibodies (Yes/No)			
	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P* Value
Age (y)	1.007 (0.995, 1.019)	0.261	-	-
BMI	0.987 (0.962, 1.011)	0.290	-	-
Gender (Ref: females)	1.390 (1.069, 1.811)	0.014	1.253 (0.958, 1.638)	0.098
Using any drug (Ref: nonsmokers)	0.834 (0.412, 1.690)	0.616	-	-
High risk job (Ref: Low risk job)	0.980 (0.735, 1.306)	0.891	-	-
Cardiac disease (Ref: No)	1.423 (0.817, 2.476)	0.213	-	-
Hypertension (Ref: No)	1.335 (0.866, 2.054)	0.190	1.303 (0.843, 2.013)	0.232
Chronic lung disease (Ref: No)	0.457 (0.114, 1.822)	0.267	-	-
Asthma (Ref: No)	0.470 (0.158, 1.403)	0.177	0.463 (0.158, 1.349)	0.158
Kidney disease (Ref: No)	1.031 (0.478, 2.227)	0.937	-	-
Thalassemia (Ref: No)	1.885 (1.229, 2.889)	0.004	1.962 (1.259, 3.055)	0.003
Diabetes (Ref: No)	1.395 (0.822, 2.370)	0.217	-	-

Note. OR: odds ratio; CI: confidence interval; BMI: body mass index.

Table 4. Antibody Seroprevalence of Participants in Both Phases

		Phase 2	
		IgM	
		Positive	Negative
Phase 1	**IgM** Positive	6 (0.8%)	91 (11.9%)
	IgM Negative	37 (4.8%)	632 (82.5%)
		IgG	
		Positive	Negative
	IgG Positive	43 (5.6%)	94 (12.3)
	IgG Negative	70 (9.1%)	559 (73.0%)

Note. IgM: Immunoglobulin M; IgG: Immunoglobulin G.

Diagnosis by serological assays could be decisive in the case of mild to moderate illness. In other words, these tests at population levels play an efficient role as seroprevalence surveillance for detecting and screening immune individuals from the affected patients (24). These are advantageous evidence while PCR tests, as the gold standard, are inaccessible or overdue. Even extensive interest points to a diverse group of antibodies which address whether an individual could be protected from COVID-19 relapse or reinfection. There is a consensus that SARS-CoV-2 infections are underreported, and many seroprevalence studies have been performed in diverse populations without sufficient specificity. A large heterogeneity was observed among countries in the seroprevalence-derived estimates partly due to the type and diversity of performance of serological assays (25-28).

The overall population of SARS-CoV-2-specific antibody seroprevalence in 18 cities of the high-risk population of Iran was 17.1% (95% CI 14.6-19.5) by the end of April 2020, which was higher than the earlier report (29). However, the results of our longitudinal study by the end of May 2020, represented that 13.7% of the participants were positive for IgG. One study in Guilan province in the north of Iran showed that the test-adjusted seroprevalence was 33% (30). The observed difference among the seroprevalence estimates across Iran could be related to various regional impacts of a large number of influential factors including strict lockdown policies, social interactions, population density, mitigation measures, protected individuals, different levels of exposure, prevention strategies (e.g., physical distancing), and the use of face masks to further protect from SARS-CoV-2 public transmission (31). There is a huge gap of knowledge on SARS-CoV-2 infection and the elicited immune response among the exposed public. Moreover, the seroconversion of neutralizing antibodies remained more in affected people having mild COVID-19 perhaps than in those with severe disease.

As stated by the WHO, the risk of SARS-CoV-2 transmission in certain occupations might be increased, however, this report was not consistent across all population-level surveys. Although the findings of our study in Hormozgan province revealed non-significant differences among high-risk versus low-risk jobs in Iran (1.02, 1.01-1.37, *P*:0.89), the adjusted seroprevalence of SARS-CoV-2-specific antibodies varied significantly across the country, and the highest estimates were found in Rasht (72.6%, 53.9-92.8) and Qom (58.5%, 37·2-83.9). According to reports (20, 32), the total seroprevalence in the high-risk community of Iran (particularly Rasht, Qom, Gorgan, and Babol) was 20% (18.5-21.7). The health professionals and academics argue that due to regular or close interactions and the likelihood of asymptomatic transmission, the risk of the virus spread in specific workplaces possibly increases through the aforementioned circumstances. Hence, there is a global consensus that the nature of the jobs may directly contribute to the elevated risk of SARS-CoV-2 exposure.

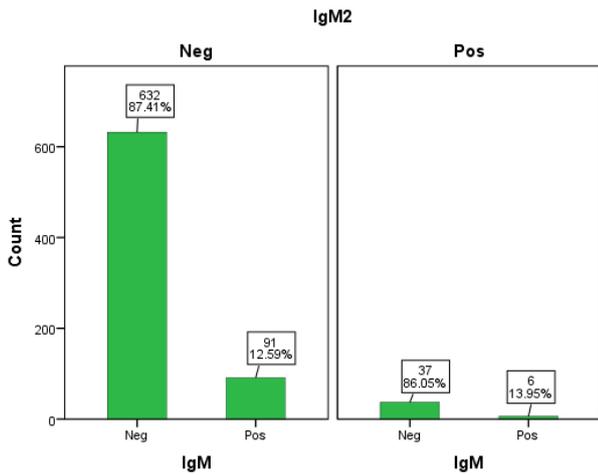Mortality and fatality rates due to COVID-19 represent

Figure 1. IgM in Two Time Points. *Note*. IgM: Immunoglobulin M; IgG: Immunoglobulin G. IgM and IgM2 are for the first second stages, respectively. Neg: Negative; Pos: Positive.
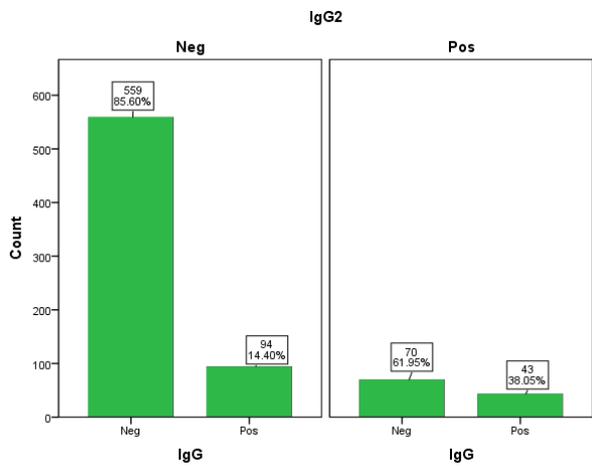

Figure 2. IgG in Two Time Points. *Note*. IgM: Immunoglobulin M; IgG: Immunoglobulin G. IgG and IgG2 are for the first second stages, respectively. Neg: Negative; Pos: Positive.
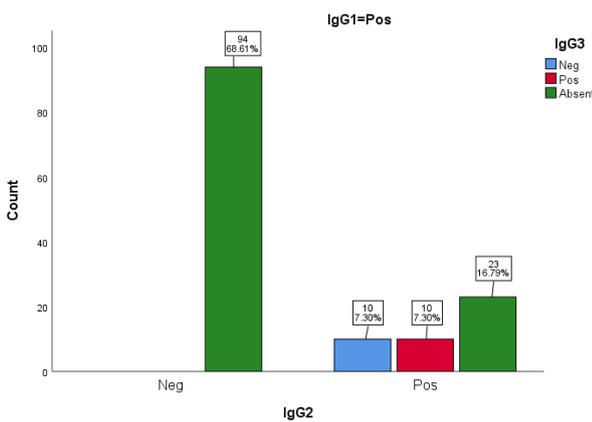

Figure 3. IgG Durability Through Nine Months Including Three Phases. *Note*. IgG: Immunoglobulin G; Neg: Negative; Pos: Positive.

significant differences in various age groups and genders. A global COVID-19 meta-analysis indicated that the male gender is considered as a risk factor for higher morbidity and mortality. Consistent with the largest study
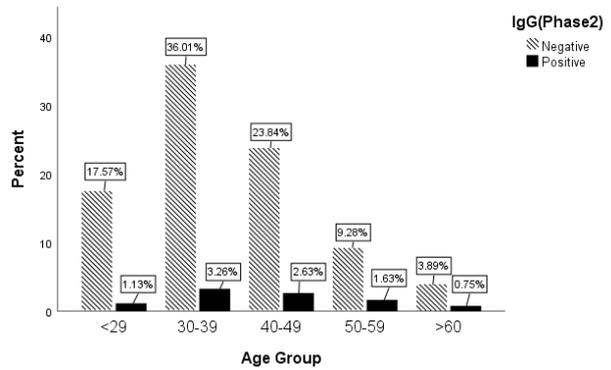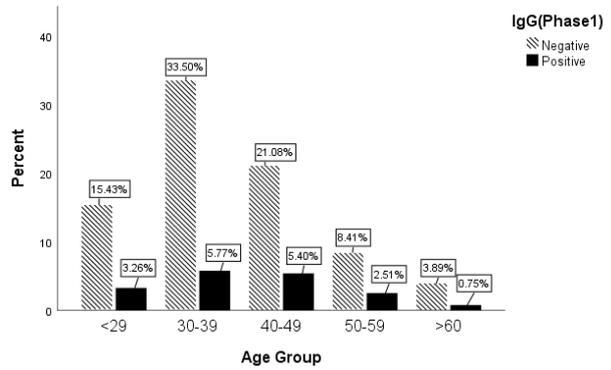
Figure 4. (a) Seroprevalence of IgG in Phase 1 Stratified by the Age Group. **(b).** Seroprevalence of IgG in Phase 2 Stratified by the Age Group. *Note*. IgG: Immunoglobulin G.

on SARS-CoV-2 antibody seroprevalence in the general population across 18 cities in Iran, important differences were observed (1.37, 1.03-1.82, $P = 0.03$) in the immune response amongst males and females (32). It seems that likely the observed gender bias could not be the sole driving factor in the COVID-19 pandemic, however, gender-based socio-cultural and behavioral differences may be influencing some aspects of the pandemic (33). Although no significant difference was found in the immune response among aged individuals, the other research reported an age-linked waning in B cells and immunity, leading to higher death in COVID-19 (34). Given that the participants of our study were selected from general healthy or asymptomatic individuals, none of the effective predictors (including diabetes, obesity, hypertension, and chronic obstructive pulmonary and cardiovascular disorders) could represent an association with an abnormal immune response. In fact, the raised immune response of individuals, particularly those with an underlying disease is not quite realized yet. Understanding the durability of humoral responses spanning a long period, the decline of antibody responses at the community level is vital for vaccination strategy and herd immunity (15, 35).

Based on the findings of the current study, almost the same proportion of asymptomatic individuals (45.7%) was found as the globally reported one (40%-45%) by previous

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research (36), indicating that our findings are unlikely affected by recall bias to overestimate the incidence of asymptomatic infection. In contrast, the seroprevalence estimates in the general population of Iran were reported to be higher (35.7%) than the global ones. Hence, health community policies and mitigation measures should be directed, particularly for the high-risk population to prevent the next waves of infections (37). Irrespective of the fact that whether individuals were asymptomatic, the durability of immune responses was nearly stable, indicating that passive and active immune policies should be carefully administered to prevent people from severe recurrence or reinfection.

The present study has some limitations. First, the lack of clinical manifestations in individuals with raised SARS-CoV-2-specific antibodies was not confirmed, thus it was impossible to estimate when they got infected or to evaluate the right time of antibody production. In addition, self-reported symptoms and recall bias did not overestimate the disease incidence (likely to be modest). Likewise, although antibody testing is a promising approach, the sensitivity of the SARS-CoV-2 ELISA kits was lower than the one reported in other countries. These tests were not completely assessed, and thus need further validation (38). Next, it is prevailing that infected people with modest symptoms may be of more interest to take part in such studies, probably leading to the overestimated prevalence. We did our best to strictly follow up the participants for at least nine months in such a way to minimize the impact of repeated exposures. Notably, the cross-reaction of the SARS-CoV-2 antibodies of other coronavirus strains could result in false-positive detection of non-standard seroprevalence. Moreover, the performance of the variances of these tests in clinical and analytical validity is a matter of concern which is undeniable. Additionally, these surveys are quite qualitative, and the existence of neutralizing antibodies can be established by a plaque reduction neutralization test.

In addition to the humoral response, human immunity gradually increases a cellular response against SARS-CoV-2 infection as well. More studies evaluating the humoral and cellular responses are required to appreciate who might have some protection in spite of a negative antibody effect (39, 40).

In conclusion, the findings of this regional study imply that the prevalence of seropositivity is likely to be extremely higher compared to the reported prevalence rate in confirmed COVID-19 cases in Iran. Despite the high seroprevalence estimates in some cities, the low overall prevalence estimates highlight the fact that a proportion of the population in Iran is still uninfected. These findings advocate that the population of Hormozgan has not yet obtained the required level of herd immunity, indicating that this population is still susceptible to SARS-CoV-2 infection and the occurrence of the next waves. Although these findings are based on less than 0.1% of the total population in Hormozgan province, they have provided significant seroprevalence data for national and local public health policies.

Authors' Contributions
HF, MH, MH, HM, AH, and AG: Conceptualization and study design; AN and HA: Experimental and laboratory bench work, and data collection; SM and SF: Data and statistical analysis; FF and AA: Data collection; HM and FN: Coordination and data collection; AN: Study design, data collection, manuscript drafting. All authors reviewed and approved the final manuscript.

Informed Consent
Written informed consent was obtained from each participant upon the fulfillment of inclusion/exclusion criteria and a willingness to participate in the study.

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