Seroprevalence of COVID-19 Antibodies and Factors Associated with Seropositivity: A Population-based Study

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Abstract

Background: Currently, COVID-19 is a global public health problem. This study aimed to estimate the seroprevalence of antibodies related to Covid-19 in the general population in southern Iran.

Methods: This cross-sectional population-based study of the seroepidemiological type investigated the serological prevalence of COVID-19 from October to December 2020 in Jahrom, Fars province, Iran. A total of 612 participants were selected using multistage cluster random sampling regardless of age or gender. The dataset in the study included the participants' demographic information, the history of exposure to COVID-19 patients, the history of PCR tests, and the history of COVID-19 symptoms in previous months. In addition, this study examined the raw and survey-weight adjusted estimates with Stata version 14. Finally, logistic regression was performed to identify risk factors for serum prevalence.

Results: The participants' mean age was 38.88±13.91 and the majority were 30 to 49 years (51.4%), with a female preponderance (58.7%). The estimated adjusted seroprevalence was 32.66 (95%CI: 28.93-36.63), with 207 positive cases for either IgG or IgM. The results of multivariable logistic regression showed that seropositivity in the participants was 4.95 times more likely associated with a history of positive PCR test (OR: 4.95, 95%CI: 2.46-10.90) and 2.14 times in patients with a history of muscle pain in previous months (OR: 2.14, 95%CI: 1.03-4.47).

Conclusion: The actual number of patients with COVID-19 is significantly higher than the number of cases confirmed by the disease monitoring system based on PCR tests. Therefore, tracking individuals' contact with confirmed patients using extensive testing and segregation of asymptomatic patients can help control the epidemic.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is one of the most important phenomena affecting human society in recent decades. SARS-CoV-2, also called as coronavirus disease 2019 (COVID-19), was first identified in December 2019 in Wuhan, China.[1] Then, it quickly spread worldwide and became a pandemic.[2] To date (August 18, 2021), more than 209,568,892 cases have been confirmed worldwide,

and the cumulative deaths have exceeded 4,398,480.[3] Besides, the first cases in Iran were reported on Feb 2020 in Qom, a holy city in central Iran.[4] Recent data from WorldoMeters on August 18, 2021, reflected 4,556,417 confirmed cases of SARS-Cov-2 and 99,691 deaths in Iran.[5]

Symptoms of COVID-19 are highly variable, ranging from asymptomaticinfections to mild upper respiratory infections to severe cases with acute respiratory syndrome and death.[2]

Asymptomatic cases refer to the positive result of reverse transcriptase-polymerase chain reaction (RT-PCR). Still, they have no typical clinical symptoms or signs and no apparent abnormalities in computed tomography (CT scan).[6, 7]

In recent estimates, 15 to 45% of all COVID-19 infections were asymptomatic.[8, 9] Asymptomatic carriers of COVID-19 can infect their close contacts and are effective in infecting family clusters.[10, 11]

The viral load detected in asymptomatic patients is similar to that in symptomatic patients.[12] Therefore, the spreadability of asymptomatic infections is not low, and these patients are likely to cause a new outbreak.[13-15]

In general, it appears that there are a large number of asymptomatic carriers who have never been tested. Without screening in healthy populations, these asymptomatic carriers will spread COVID-19 infections back into the community. This issue poses a major challenge to the prevention and control of COVID-19 and demonstrates the need for serological testing. Especially in subclinical and asymptomatic infections, serological tests are vital for determining the true prevalence of the infection.[16]

On the other hand, it is necessary to know what percent of the population is infected to properly establish the infection fatality rate (IFR).[17, 18] In addition, serological tests can be used to determine the prevalence of antibodies against COVID-19 in the population and also determine the level of immunity developed in the population (herd immunity).[19, 20]

This information can be used to measure the impact of interventions and implement appropriate policies to control the spread of the epidemic better. Therefore, this study aimed to provide a population-based seropositivity estimate of COVID-19 virus infection in the south of Iran.

Methods

Study Design

In this cross-sectional population-based study, serological testing for anti-SARS-CoV-2 antibodies was used to assess the seroprevalence of COVID-19 infection in Jahrom, a city located in southern Iran, from October to December 2020.

The sample size included 528 persons given the previous seroprevalence of 22% for Coronavirus infection in Guilan,[21] a p-value of 0.05 was considered statistically significant, 95% confidence level, and design effect of 2.

$$n \geq \frac{\left(Z1-\frac{\alpha}{2}\right)^2 P\,(1-p)}{d^2}$$

The population of Jahrom is approximately 147,780 persons. During the study period, a total of 1522 individuals were visited,1092 met the inclusion criteria, and a total of 612 individuals completed the study (56.04% response rate). The participants were selected using a multistage cluster random sampling approach. Given the number of comprehensive urban health centers, Jahrom was divided into three clusters, and three health centers were randomly selected from each cluster.

In each health center, individuals from different age groups were selected based on the data in the SIB system (An online integrated health system that records and keeps all information about households and the type of health services and programs required and implemented in health centers) by simple random sampling.

Inclusion and Exclusion Criteria

The study was open to all Jahrom residents, regardless of age or gender. Besides, people with the following conditions were excluded from the study: Dissatisfaction with participating in the study; patients with COVID-19 who were in the active phase of the disease or during treatment at the time of the study; and people who were barred from intravenous blood sampling.

Data Collection

The WHO questionnaire was used to interview the participants.[22] The questionnaire includes demographic information such as National Identity Number, birth date, sex, job, nationality, residency, history of exposure to COVID-19 patients, history of PCR tests, and history of COVID-19 symptoms in the previous nine months (from starting date of the COVID-19 epidemic in Iran to the onset of the study). Afterward, an EDTA-coated micro trainer was used to collect 5 mL of venous blood from each participant. ELISA kits (Pishtaz Teb, Tehran, Iran; catalog numbers PT-SARS-COV-2.IgM-96 and PT-SARS-COV-2.IgG-96) approved by the Iranian Food and Drug Administration (FDA) were used to detect SARS-CoV-2-specific IgG and IgM antibodies in the serum samples.

Statistical Analysis

The participants' demographic characteristics were expressed in frequency and percent. The association

between serum prevalence and the study variables was assessed using the chi-square test. Moreover, raw and survey-weight adjusted were estimated to adjust survey weights for nonresponse or coverage errors and reduce variances with Stata version 14 (Stata Corp LP, College Station, TX). Adjusted rates were estimated using the Jahrom population age standard, updated to the 2020 standard population age structure based on the integrated health system (SIB). Next, univariate logistic regression analyses were performed to identify risk factors of serum prevalence. In the next stage, variables with P≤0.2 were enrolled in the multivariate logistic backward technique to identify the effective variable. The results were expressed by odds ratios at the 95% confidence interval. A p-value less than 0.05 was considered statistically significant.

Results

The participants' mean age was 38.88±13.91. 315 (51.4%) participants aged 30 to 49 years, with 359 (58.7%) female preponderance. In total, 166 (27.1%) participants were housekeepers, and 130 (21.2%) had non-governmental jobs. Moreover, 118 (19.3%) participants reported a history of contact with COVID-19 confirmed cases, including close relatives, family members, friends, or colleagues. A total of 195 (31.9%) and 109 (17.8%) participants reported a history of PCR and PCR positive in the past, respectively. Besides, 207 (33.8%) persons tested positive for either IgG or IgM. There were significant differences in seropositivity rates between the participants in terms of age groups, COVID-19 exposure history, a history of PCR, PCR results, and visiting a doctor (P<0.05) (Table 1).

As displayed in Figure 1, the seropositive patients reported a history of symptoms in the previous nine months (from starting date of the COVID-19 epidemic in Iran to the onset of the study), 107 (50.7%) fatigue, 91 (44%) fever, 75 (36.2%) chills and 74 (35.3%) cough, respectively (Figure 1).

200 (32.67%) and 50 (8.16%) participants had positive test results for IgG and IgM, respectively (Table 2). Considering both tests, the results were positive in 43 (7.02%) participants. Nevertheless, 207 (33.82%) participants were positive for either IgG or IgM. Given that a person who tested positive for IgG or IgM could be considered positive, the COVID-19 prevalence in Jahrom was 32.66 (28.93-36.63) (Table 2).

The result of the univariate analysis showed that the history of COVID-19 exposure, history of PCR, positive PCR results, visiting a doctor, fatigue,

Table 1: Seroprevalence according to demographic characteristics of study participants

Variables	Categories	Overall participants (%)	No. seropositive	Unadjusted seroprevalence% (95% CI)	Population-weight adjusted% (95% CI)	P value
Gender	Men	253 (41.3)	92	36.36 (30.64-42.49)	36.53 (30.55-42.95)	0.265
	Women	359 (58.7)	115	32.03 (27.39-37.05)	29.84 (25.23-34.90)	
Age, year	7-9	8 (1.3)	3	37.50 (11.46-73.54)	37.40 (12.44-71.52)	0.025
	10-19	31 (5.1)	9	29.03 (15.66-47.40)	28.75 (15.62-46.80)	
	20-29	126 (20.6)	38	30.15 (22.75-38.76)	30.67 (23.19-39.33)	
	30-39	160 (26.1)	43	26.87 (20.54-34.30)	26.97 (20.64-34.40)	
	40-49	155 (25.3)	70	45.16 (37.46-53.09)	45.14 (37.46-53.05)	
	50-59	77 (12.6)	23	29.87 (20.65-41.06)	29.87 (20.70-41.01)	
	≥60	55 (9)	21	38.18 (26.27-51.70)	38.09 (26.28-51.49)	
Occupation	Non-governmental employee	130 (21.2)	43	28.46 (21.33-36.85)	27.99 (20.69-36.68)	0.438
	Government employee	108 (17.6)	43	32.40 (24.21-41.83)	28.67 (20.93-37.90)	
	Health personnel	101 (16.5)	35	32.67 (24.20-42.45)	32.38 (23.76-42.39)	
	Housekeeper	166 (27.1)	58	39.75 (32.56-47.42)	39.56 (32.04-47.61)	
	Retired	34 (5.6)	11	41.17 (25.89-58.36)	42.70 (27.23-59.73)	
	Academic student	38 (6.2)	10	26.31 (14.64-42.64)	26.26 (14.69-42.43)	
	Student	35 (5.7)	7	34.28 (20.42-51.47)	33.84 (19.96-51.19)	
COVID-19 exposure history	Yes	118 (19.3)	15	40.67 (32.14-49.80)	40.58 (31.77-50.04)	<0.001
	No	263 (43)	117	31.53 (26.20-37.44)	29.41 (24.06-35.40)	
	Unknown	231 (37.7)	75	32.90 (27.12-39.24)	32.13 (26.24-38.66)	
History of PCR	Yes	195 (31.9)	93	28.71 (22.78-35.49)	27.45 (21.44-34.41)	
	No	417 (68.1)	112	36.14 (31.64-40.89)	35.08 (30.48-39.98)	<0.001
History of PCR result	Positive	109 (17.8)	69	34.86 (26.42-44.36)	33.68 (25.07-43.52)	<0.001
	Negative	58 (9.5)	12	27.58 (17.50-40.61)	24.90 (15.20-38.01)	
Visiting a doctor	Yes	202 (33)	100	34.65 (28.38-41.50)	33.49 (27.07-40.59)	<0.001
	No	410 (67)	107	33.41 (29.00-38.13)	32.26 (27.77-37.11)	

PCR: Polymerase chain reaction, CI: Confidence Interval

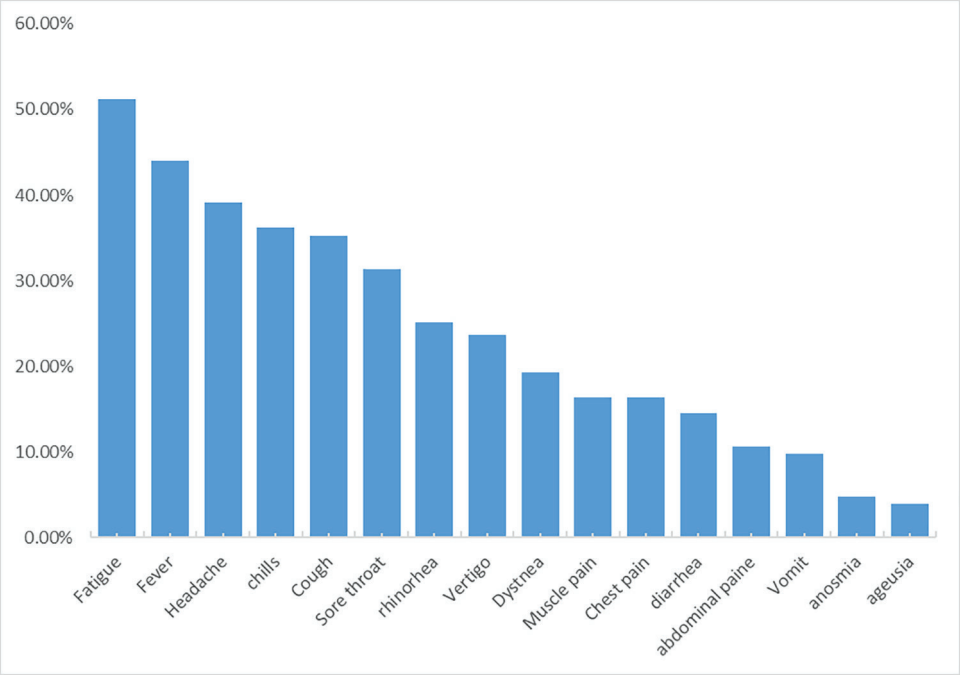

Figure 1: Frequency of COVID-19 symptoms in previous nine months in 207 seropositive cases

Table 2: Seroprevalence estimates of COVID-19 in Jahrom city, Fars province, Iran (%)

Antibody seropositivity	Frequency	Unadjusted seroprevalence % (95% CI)	Population-weight adjusted% (95% CI)
IgG	200	32.67 (29.06-36.50)	31.53 (27.84-35.47)
IgM	50	8.16 (6.24-10.62)	7.03 (5.54-9.07)
IgG and IgM	43	7.02 (5.24-9.34)	6.02 (4.05-8.04)
IgG or IgM	207	33.82 (30.17-37.67)	32.66 (28.93-36.63)

CI: Confidence Interval, IgG: ImmunoglobulinG, IgM: Immunoglobulin M

fever, headache, chills, cough, sore throat, rhinorrhea vertigo, dyspnea, muscle pain, chest pain, diarrhea, and vomiting were associated with seropositivity of COVID-19 in the participants (P<0.05) (Table 3).

The chance of seropositivity of antibodies in the individuals with a previous history of positive PCR test was about five times more likely than those with a history of negative PCR test (OR: 4.95,95% CI: 2.46-10.90). This chance was 2.14 times higher in people with muscle pain than those without muscle pain. (OR: 2.14, 95% CI: 1.03-4.47) (Table 4).

Discussion

The result of this study revealed that the seroprevalence estimate of antibodies of Covid- 19 after adjusting for the population was between 28.93% and 36.63%. Furthermore, Seroprevalence was higher among the participants in the age group of 40-49 years, those with a history of contact with patients with confirmed COVID-19, the participants with a history of PCR test, the people with a history of positive PCR, and the participants who had seen a doctor because of suspected COVID-19 clinical symptoms.

The COVID-19 seroprevalence in the studied area was higher than some other areas of Iran, including Gilan province with 22% to 33% COVID-19 seroprevalence until May 2020[21], Mazandaran province with 13%-18% COVID-19 seroprevalence until May 2020,[23] Tehran with 21% until October 2020,[24] and Yazd province with 13% to 17% COVID-19 seroprevalence until June 2020.[25] Furthermore, the COVID-19 seroprevalence rate in this study was higher than previous seroprevalence estimates in some countries and regions, such as Korea with 7.6% until Jun 2020,[26] Washington with 1.2% until April 2020,[27] Bangalore, India with 8.5% until September 2020,[28] Spain with 5% until May 2020,[29] and Geneva, Switzerland with 4.8% until May 2020.[30]

A systematic review and meta-analysis showed that up to August 2020, COVID-19 seroprevalence in the general population varied from 0.37% to 22.1%, with a pooled estimate of 3.05% to 3.15% that varied from 1.45% in South America to 5.27% in Northern Europe.[20]

Seroprevalence differences observed in various regions can be attributed to factors such as time of the study, chance variation, cultural differences, political decision-making, health system and prevention/control policy strategy, or the efficiency of the adopted strategies and policies.[20, 31]

Table 3: The risk factors could predict seropositivity of COVID-19 by uni-variate logistic regression

Variables	Category	OR (95%CI)	P value
Age	yr	0.99 (0.97-1.00)	0.103
Gender	Female	Ref	-
	Male	1.21 (0.86-1.70)	0.265
History of COVID-19 exposure	No	Ref	-
	Yes	1.33 (1.10-1.61)	0.003
History of PCR	No	Ref	-
	Yes	2.46 (1.73-3.51)	<0.001
Result of PCR	Negative	Ref	-
	Positive	6.61 (3.13-13.93)	<0.001
Visiting a doctor	No	Ref	-
	Yes	2.77 (1.95-3.95)	<0.001
Fatigue	No	Ref	-
	Yes	2.44 (1.73-3.45)	<0.001
Fever	No	Ref	-
	Yes	3.50 (2.41-5.09)	<0.001
Headache	No	Ref	-
	Yes	1.62 (1.13-2.30)	0.007
Chills	No	Ref	-
	Yes	3.69 (2.46-5.52)	<0.001
Cough	No	Ref	-
	Yes	2.90 (1.96-4.29)	<0.001
Sore throat	No	Ref	-
	Yes	1.95 (1.32-2.86)	0.01
Rhinorrhea	No	Ref	-
	Yes	1.60 (1.07-2.41)	0.022
Vertigo	No	Ref	-
	Yes	1.81 (1.19-2.77)	0.006
Dyspnea	No	Ref	-
	Yes	1.82 (1.15-2.89)	0.01
Muscle pain	No	Ref	-
	Yes	3.11 (2.19-4.37)	<0.001
Chest pain	No	Ref	-
	Yes	1.79 (1.09-2.93)	0.02
Diarrhea	No	Ref	-
	Yes	1.84 (1.09-3.11)	0.02
Abdominal pain	No	Ref	-
	Yes	1.29 (0.73-2.28)	0.36
Vomit	No	Ref	-
	Yes	1.84 (1.09-3.11)	0.02
Anosmia	No	Ref	-
	Yes	1.66 (0.70-3.91)	0.24
Ageusia	No	Ref	-
	Yes	1.21 (0.49-2.97)	0.67

yr: year, OR: Odds Ratio, CI: Confidence Interval, PCR: polymerase chain reaction, Ref: Reference group,"-"Not applicable

Table 4: The risk factors could predict seropositivity of COVID-19 by multivariable logistic regression

Variables	Category	OR (95%CI)	P value
Result of PCR	Negative	Ref	-
	Positive	4.95 (2.46-10.90)	<0.001
History of Muscle pain in previous months	No	Ref	-
	Yes	2.14 (1.03-4.47)	0.041

OR: Odds Ratio, Ref: Reference group, PCR: polymerase chain reaction "-"Not applicable, CI: Confidence Interval

One of the important reasons for estimating the prevalence is to estimate the possible infection fatality rate of the disease (rather than the case fatality rate).[18, 21] According to the official report of Jahrom Health Center, until February 27, 2020, a total of 13804 patients were diagnosed based on PCR tests, and 228 died due to the disease. Consequently, the case fatality rate was 1.65%. According to the estimated seroprevalence, its infection fatality rate in the study area can be adjusted to 0.488%. In two studies

conducted in Mazandaran and Gilan provinces, the adjusted infection fatality rates were 0.329% and 0.08% to 0.12%, respectively,[21, 23] the infection fatality rates in California ranged from 0.12-0.2%.[32]

The previous estimate of case fatality rate using lag time for fatality in China ranged from 0.25-3.0%,[33] far higher than the currently estimated infection fatality rate.

The present study showed that 50.7 % of seropositive patients were symptomatic. Moreover, The univariate analysis showed that fatigue, fever, headache, chills, cough, sore throat, rhinorrhea vertigo, dyspnea, muscle pain, chest pain, diarrhea, and vomiting were associated with COVID-19 seropositivity in the participants ($P<0.05$).

One study of the clinical features of COVID-19 patients in Wuhan, China reported that 98% of patients had a fever and 78% had a body temperature above 38 degrees. Moreover, 76% of the patients reported cough, 44% fatigue and muscle pain, 55% dyspnea, 28% sputum, and 3% diarrhea.[34]

Therefore, these symptoms can be a sign of COVID-19 and help detect it earlier. The multivariable analysis results demonstrated that seropositivity in the participants was 2.14 times more likely associated with a history of muscle pain in previous months.

Given that more than 49.3% of seropositive patients were asymptomatic, it is estimated that 21076 to 26686 individuals living in Jahrom had asymptomatic infection COVID-19 (and were asymptomatic carriers).

The proportion of asymptomatic infections reported in different studies varies greatly, ranging from 4% to 41%.[15, 35] Therefore, adhering to hygienic principles, especially hand washing, social distancing, mask use, and active case finding using massive testing and isolation of asymptomatic patients can help control the COVID-19 epidemic.

This study also showed that seroprevalence was higher in patients aged 30-49 (51.4%), confirming the high prevalence of the disease in middle-aged people who are usually more economically active and less able to stay home, and more likely to be infected.

In the present study, men showed a higher serological prevalence of COVID-19 than women, but this difference was insignificant. Other studies in Yazd[25] and Spain[29] reported no significant difference in the prevalence of serology in men and women. Women and men respond differently to pathogens, which can be an important factor influencing viral load and mortality rate. Studies have shown that the disappearance of RNA in men with COVID-19 is delayed. On the other hand, estrogen in women strengthens the immune system, and testosterone suppresses the immune system. However, the molecular mechanism underlying COVID-19 in men and women is still unclear, and further studies are needed.[36, 37]

Limitation

One of the limitations of this study was the individuals' low response rate (56%) in the studyIn addition, because this study was conducted after the third peak of the epidemic in Iran, many people did not come for tests and blood samples and preferred to stay at home. Therefore, those who entered the study may be different from those who did not participate, which could affect the prevalence estimates. In addition, it is suggested to study residents of organizational centers with high population density, such as prisons and sanatoriums in the future.

Conclusion

The number of patients with COVID-19 is significantly higher than the that of cases confirmed by the disease monitoring system based on PCR tests. Therefore, tracking individuals' contact with confirmed patients using extensive testing and segregation of asymptomatic patients can help control the epidemic. In addition, using social marketing principles can also be useful to improve community health behavior during the epidemic.

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Vice Chancellor for Research at Jahrom University of Medical Sciences, Jahrom, Iran, approved the study The authors would like to appreciate all participants who collaborated in this study sincerely.

Authors' Contribution

The initial idea of this study was by VR and RZ. FH and VR contributed to the design and management of the study, the manuscript's writing, and implementing all reviewers' comments. VR and RZ participated in the statistical analysis. SH and NR contributed to the writing of the manuscript. MCh and ZS contributed to the questionnaire design, edition, and revision. Finally, FH and VR contributed to the translation of the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This research project was approved by the Ethics Committee of the Jahrom University of Medical Science, Fars, Iran (IR.JUMS.REC.1399.076). Written informed consent was obtained from the participants before entering the study. In addition, written consent for the children was obtained from their parents/legal guardians.

Conflict of interest: None declared.

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