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Prevalence of COVID-19 Virus Infection in Semnan Province

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing a human pandemic disease named COVID-19 has become a major global health concern. Iran as one of the most affected countries needs unprecedented effort for monitoring and evaluation of COVID-19. To determine the seroprevalancer of COVID-19 in Semnan province North-East of Iran.

Methods: Six hundred people were randomly selected using the "SIB database". From 1 to 30 June 2020, 153 participants of Semnan population were enrolled. Blood, nasopharyngeal and oropharyngeal samples were obtained. Prevalence of IgM and IgG antibodies were ascertained using ELISA and Real-time PCR was conducted to evaluate viral load. Estimates of prevalence were standardized by age and sex, based on the 2015 national census of Semnan province.

Results: Seroprevalence showed no difference between females and males and no significant association between age and seropositivity. Among total participants, the age and sex-adjusted prevalence of SARS-CoV2 infection was 19.3% (95% CI, 14.0-26.7 per 100 persons). Approximately 10% of participants had detectable antibodies but showed a negative-PCR result. However, approximately 80% of participants did not show evidence of infection.

Conclusion: The majority of the population in Semnan province has no detectable levels of antibodies to SARS-CoV-2. Therefore, Semnan is considered a SARS-CoV-2 susceptible area. These results emphasize the need for maintaining public health measures to tackle the new epidemic wave.

Keywords: Serology, SARS-CoV-2, Antibody Response, Immunoglobulin, Prevalence

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INTRODUCTION

Since more than 100 years of the 1918 influenza pandemic, in December 2019, the world experienced a pandemic outbreak of unexplained pneumonia by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2, a new member of the beta-coronavirus genus, is responsible for a human- to- human transmissible disease designated coronavirus disease 2019 (COVID-19) that has garnered global attention due to its rapid spread (1). As of 12thAugust 2020, more than 20 million confirmed cases of SARS-CoV-2 have been reported with more than 745,981 attributable deaths worldwide. Iran with more than 331,189 confirmed cases and approximately 18,800 fatalities, is one of the ten most-affected countries in the world (2). SARS-CoV-2 as a single-stranded RNA-enveloped virus (+ssRNA) binds to the human receptor angiotensin-converting enzyme 2 (ACE2) that is expressed in a wide variety of human tissues; including the small intestine, kidneys, heart, and at a lower level in lungs, brain, muscle, and also mucosa of the oral cavity (3, 4). Based on the wide distribution of ACE2 throughout the human body, clinical manifestations of COVID-19 range from an asymptomatic state to fever, cough, headache, and shortness of breath, to more serious form of disease features, including severe pneumonia which leads to acute respiratory distress syndrome and respiratory failure, neurological complications, acute kidney injury, or multiple organ dysfunction, eventually leading to death (5-7).

The rapid outbreak of COVID-19 continues to emerge from many countries, requires the necessity of early detection of SARS-CoV-2 infection. Despite the global efforts, due to the lack of knowledge in several important aspects of SARS-CoV-2 infection, ranging from the pathogenesis and host response, overcome the virus transmission is hampered (8). Currently, reverse transcriptase-polymerase chain reaction (RT–PCR) is the only standard assay for detection of SARS-CoV-2 RNA in nasopharyngeal (NP) and oropharyngeal (OP) samples, sputum or bronchoalveolar lavage samples, especially to screen symptomatic cases (9, 10). Recently, detection of Immunoglobulin M (IgM) and IgG specific to SARS-CoV-2 has also been used for the screening of asymptomatic cases and undetectable viral results of RT-PCR (6). It seems that evaluation of humoral immune responses in concomitant with viral detection is essential to determine SARS-CoV-2 prevalence and provide precise estimates of total or partial immunity in the general population (11).

However, the specific humoral response to SARS-CoV-2 currently remains poorly understood in COVID-19 patients. Conducting serological studies is a practical approach to determine the status of the COVID-19 at provincial and national levels (12). Moreover, these studies quantify the proportion of the population that faced the infection and also have specific antibodies, particularly in asymptomatic cases. Besides, determination of seroincidence is useful to make socio-economic decisions (13). Therefore, this study is aimed at determining the prevalence of COVID-19 in Semnan province, as a major epidemic region of Iran.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional study was conducted from 1 to 30 June 2020 in Semnan University of Medical Sciences affiliated population in Semnan province, Iran. Semnan province has 6 counties covered by Semnan University of Medical Sciences in terms of health services including Semnan, Mahdishahr, Sorkheh, Damghan, Garmsar, and Aradan; and according to the latest national population census performed in 2015, they have a population of 445,014. Considering the formula $n=Z_{1-\alpha/2}p(1-p)/d^2$, the requested sample size was estimated to be 164 taking into account the prevalence of non-infected

population equal to 70% (P=0.7), d equal 0.1p (d=0.07), and α=0.05. Sample selection was performed in two steps. First- due to the possibility of non-participation of people in epidemic condition - a preliminary list of 600 participants (conservatively four times the estimated sample size) was developed by randomly selecting the university-affiliated population applying systematic sampling method, using the "SIB database" –the main national database used in Iran to record households' information to provide health services. The three criteria for entering the study were age over 5 years, consent to give biological samples (a blood sample for serological evaluation and a nasopharyngeal and oropharyngeal swab sample to run RT-PCR test), and not be members of thesame household. Then, the selected individuals were invited by telephone to participate in the study. If they agreed to participate in the research, they would be registered in the final list and according to a pre-designed time-table, they were asked to attend the nearest determined laboratory to his/her place of residence for biological sampling. Personal information, including age, gender, and the history of getting COVID-19 virus infection was asked when attending the laboratory.

Detection of SARS-CoV-2 Specific Antibodies

Blood samples were obtained from 153 participants. Anindirect enzyme-linked immunosorbent assay (ELISA) was used to qualitatively detect IgM and IgG antibodies against nucleocapsid antigen (N) of the SARS-CoV-2 virus (Pishtaz Teb Diagnostics Company, Iran). Assays were performed according to the manufacturer's detailed instructions. Briefly, specific antibodies in serum collected from participants react with solid-phase antigens. Subsequently, the conjugated antibody, which contains anti-human IgM/IgG horseradish peroxidase (HRP) conjugate was added into the wells. Following another incubation and addition of chromogen-substrate, development of a yellow color measured spectrophotometrically at 450 nm. A Cut-off Index (COI)<0.9 indicating a negative result and a COI of >1.1 is considered aa appositive result. Based on the clinical findings, lung RT-CT positive results, and a positive RT-PCR test, the manufacturer reported sensitivity of 79.4% for IgM and 94.1% for IgG, and specificity of 97.3% and 98.3%, respectively.

Detection of SARS-CoV-2 Nucleic Acid

Detection of SARS-CoV-2 nucleic acid from the upper respiratory tract through collecting nasopharyngeal (NP) and oropharyngeal (OP) swabs of all participants was performed. Total RNA was extracted using MagCore® 202 Low PCR Inhibition kit (MagCore® Viral Nucleic Acid Extraction Kit), in the automated Viral Nucleic Acid Extractor manner based on the magnetic-particle technology (MagCore®, Taiwan). Total-RNA eluted into 60 µl of elution buffer to obtain high concentration viral RNA and was used as the template for RT- PCR, as recommended by the manufacturer. The quality of extracted-RNAs was measured using NanoDrop OneC Spectrophotometer (Thermo Scientific NanoDrop, DE, USA). Extracted-RNA was maintained at −70°C. Subsequently, reverse Real-Time PCR (rRT-PCR) was performed on SARS-Cov-2, N- and ORF1ab-gene, using Detection kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) (DaAn Gene Co., Ltd. Sun Yat-Sen University, China), according to the manufacturer's protocol. Twenty-five µl PCR reaction mixture for each sample, containing 17 µl of solution A (contains specific primers, probes, Tris (hydroxymethyl) aminomethane-hydrochloric acid buffer), 3 µl of solution B (contains hot start Taq DNA polymerase and c-MMLV reverse transcriptase), and 5 µl of extracted-RNA sample, in addition to positive/negative control was incubated in the 8-tube strips. The reaction was carried out on StepOne™ Real-Time PCR System (Applied Biosystems, USA). Both N- and ORF1ab-genes of each

sample were measured in the FAM and VIC detecting channels; respectively, and Cycle Threshold (CT) values ≤≤40 were considered positive.

Statistical Analysis

Based on the results of the serological and viral tests, the participants were divided into two main groups: probably infected, and probably not-infected, which was set as the criterion for estimating the prevalence of COVID-19 virus infection. Table 1 shows the diagnostic classification based on the concurrent serological and viral-detection test results.

To describe the results, the mean and standard deviation(±SD) of numerical variables and the number and percentage of qualitative ones were used. To estimate the age and sex standardized prevalence of infection, the distribution of age and sex groups of the province's population in the 2015 national census was used (Table 2). To increase the precision of estimates, prevalence estimates were calculated for defined districts defined by aggregating the data of nearby counties (distinct 1: Semnan, Mahdishahr, and Sorkheh counties; distinct 2: Damghan county; and distinct 3: Garmsar and Aradan counties). The Agresti-Coull method was used to calculate the 95% confidence intervals for point estimates of prevalences. The software used was Stata-11(StataCorp LP; College Station, USA).

RESULTS

One hundred and fifty-three people participated in the study. The mean (±SD) age of the participants was 38.4±18.5 years. The youngest participant was 6 and the oldest was 87 years old. According to their statements, 3(2.0%)reported the history of

Table 1. Diagnostic classification based on the concurrent tests results.

Descriptions	Tests			Infection
	RT-PCR	IgM	IgG	
May be in the window period	+	-	-	Yes
May be in the early stage	+	+	-	
May be in the active phase	+	+	+	
May be in the late or recurrent stage	+	-	+	
May be in the early stage, PCR result may be FN	-	+	-	
May have had a past infection, and has recovered	-	-	+	
May be in the recovering stage, PCR result may be FN	-	+	+	
May be susceptible	-	-	-	No

FN: false negative

Table 2. The age and sex distribution of the population in Semnan province based on the 2015 national census (set as reference population for standardization).

Sex	Age group	%
Male	<20	14.56
	20-39	20.14
	40-59	11.16
	≥60	4.92
Female	<20	13.89
	20-39	19.49
	40-59	10.79
	≥60	5.05
All	-	100

infection according to the diagnosis of the treating physician clinically and based on chest radiography. None of them had a history of the previous test and only one person (a 60 years old man) was hospitalized due to disease. Based on the results of tests performed on all participants, the infection was reported in 33 people. Table 3 demonstrates the characteristics of participants in the study by probably infection diagnosis for COVID-19.

The frequency distribution of the participants in terms of the diagnostic classification based on the results of concurrent serological and RT-PCR tests can be found in Table 4. As we see in Table 4, at the time of the study, about 10% of all positive individuals were diagnosed at the window stage, and nearly 80% did not show evidence of infection. Sex and age-standardized prevalence/100 of the possible infection of COVID-19 in Semnan University of Medical Sciences population in June 2020,

Table 3. Characteristics of participants in the study by probably infection diagnosis for COVID-19 in Semnan University of Medical Sciences (June, 2020).

Characteristics		Infection (% row)				P*	Total (% column)	
		No		Yes				
		Count	%	Count	%		Count	%
Gender	Male	55	82.1	12	17.9	0.332	67	43.8
	Female	65	75.6	21	24.4		86	56.2
County	Semnan	26	81.3	6	18.7	0.199	32	20.9
	Damghan	31	75.6	10	24.4		41	26.8
	Garmsar	32	86.5	5	13.5		37	24.2
	Mahdishahr	13	61.9	8	38.1		21	13.7
	Aradan	7	100	0	0.0		7	4.6
	Sorkheh	11	73.3	4	26.7		15	9.8
Age group	<20	26	89.7	3	10.3	0.015	29	19.0
	20-39	49	84.5	9	15.5		58	37.9
	40-59	34	73.9	12	26.1		46	30.1
	60 and more	11	55.0	9	45.0		20	13.1
Diabetes	Yes	11	84.6	2	15.4	0.571	13	8.5
	No	109	77.9	31	22.1		140	91.5
Hypertension	Yes	14	77.8	4	22.2	0.943	18	11.8
	No	106	78.5	29	21.5		135	88.2
Smoking	Yes	17	85.0	3	15.0	0.444	20	13.1
	No	103	77.4	30	22.6		133	86.9
Total		120	78.4	33	21.6		153	100

*Chi-square test

Table 4. Infection status of COVID-19 in study participants by gender in Semnan University of Medical Sciences (June, 2020).

Descriptions	Male		Female		Total	
	Count	%	Count	%	Count	%
Window Period	7	10.4	8	9.3	15	9.8
Active phase	1	1.5	2	2.3	3	2.0
Late or recurrent stage	1	1.5	0	0.0	1	0.7
Early stage, PCR result may be FN	1	1.5	5	5.8	6	3.9
Past infection, and has recovered	1	1.5	2	2.3	3	2.0
Recovering stage, PCR result may be FN	1	1.5	4	4.7	5	3.3
Susceptible	55	82.1	65	75.6	120	78.4

FN: False Negative

Table 5. Sex and age standardized prevalence of the possible infection of COVID-19 in Semnan University of Medical Sciences population (June, 2020).

Distinct	Prevalence/100*	95%CI **	
		Lower	Upper
Semnan-Mahdishahr-Sorkheh	25.7	16.1	36.5
Damghan	18.9	9.9	34.3
Garmsar-Aradan	10.7	4.5	24.4
Total	19.3	14.0	26.7

*Age and sex standardized estimations for prevalence based on population distribution of Semnan province according to the 2015 national census, ** Agresti-Coull method for confidence interval calculation.

was estimated to be 19.3 (95% CI: 14.0, 26.7). Regarding the point estimates, the prevalence was between 10.7 in Garmsar-Aradan distinct, and 25.7 in Semnan-Mahdishahr-Sorkheh distinct. Prevalence in Damghan was estimated to be 18.9 per 100 (Table 5).

DISCUSSION

In this cross-sectional study, we have presented the findings of the prevalence of specific IgM and IgG antibodies against SARS-CoV-2 concurrently with RT-PCR results. The results revealed that approximately 21.6% of participants have a confirmed-COVID-19 disease. Given that none of the participants was in the same household, appropriate physical and social distancing is promptly needed. However, 80% of the participants showed no evidence of infection and considered susceptible cases. This result suggests that a sizeable proportion of the Semnan province population has susceptibility to SARS-CoV-2 infection. There were no differences in seroprevalence between females and males. In harmony with others, seroincidence findings of this study also revealed that there is no clear association between age and seroprevalence (14).

We observed an overall standardized point prevalence of 19.3% in Semnan province. The adjusted age and sex prevalence of COVID-19 infection were highest in Semnan-Mahdishahr-Sorkheh, while Garmsar-Aradan experienced the lowest rate of COVID-19 incidence. At the time of the study, about 10% of all positive individuals were diagnosed at the window stage. Indeed, infected people may not have detectable antibody levels until 6-7 days after symptom onset (15), while according to the Lauer *et al*. study, the median incubation period of SARS-CoV-2 infection is estimated to be 5.1 days (16). Therefore, these asymptomatic carriers may cause larger clusters of infection.

The findings from this provincial study also indicated that the prevalence of SARS-CoV-2 specific IgM is around 4%. Detection of IgM antibodies can be used for screening and finding the new cases, because the disease may still be in the active phase in positive cases. Also, approximately 5% of participants simultaneously had a detectable titer of specific IgM and IgG, it seems that the disease was not in active phase in a significant percentage of the population. Liu *et al*. reported that specific IgM antibodies are not detectable in the very early days of infection (17). It has demonstrated that IgM antibodies emerge first, approximately 5 days with peaking at 28 days post-symptom onset. Also, anti-SARS-CoV-2 specific IgG antibodies were identifiable from day 7 onwards, peaking at approximately day 49 (1, 18, 19). However, it is reported that the less severe forms of the disease are associated with less or delayed antibody responses, and it is a challenge to serological assays (20).

However, an extensive prevalence study on the targeted population can anticipate the status of the epidemic in a community, facilitate the implementation of health interventions, and evaluate the most optimal time to reopen

schools, universities, and social activities (12). Although recent development of highly sensitive techniques for viral detection such as Real-Time PCR has provided important and required information about the incidence rates and clinical manifestations in course of COVID-19 infection, the performance of these techniques depends on the stage of infection (21) and quality of collected samples and often gives false negative results owing to low viral quantity in the upper respiratory specimens (14, 22). On the other hand, disease severity and type of symptoms are due to not only the viral infection but also the host response. Therefore, using RT–PCR and serological tests in conjunction is advantageous with strong support to the diagnosis and prevalence studies.

In conclusion, we observed that less than 20% of participants had COVID-19 infection, during the period of study, and the most of participants are susceptible to infection that requires more health system attention that should be considered to face a future epidemic wave. Moreover, a considerable proportion of the study population had an asymptomatic and undetected active infection. Therefore, social distance and efforts to identify and isolate new cases and their contacts are imperative for predicting the epidemic situation of COVID-19 and future epidemic control. In this way, seroepidemiological studies are necessary to tackle the disease spread, to the screen of the population, to determine the time of antibody production, care, and treatment.

Conflict of interest: None declared.

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