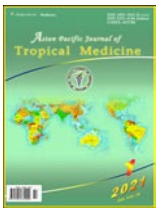

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.304296

Impact Factor: 1.94

Seroprevalence of SARS-CoV-2 in Mazandaran province, Iran

Seyed Abbas Mousavi[1], Hamed Rouhanizadeh[2], Majid Saeedi[3], Mahmood Moosazadeh[4], Abolqasem Ajami[5], Afsaneh Fendereski[6,7], Jamshid Yazdani-Charati[7]✉, Seyed Mohsen Soleimani[8], Nader Ahangar[8], Seyed Hossein Seyedpour[9], Zahra Bandalizaeh[9]

[1]*Psychiatry and Behavioral Sciences Research Center, Institute of Addiction, Department of Psychiatry, Mazandaran University of Medical Sciences, Sari, Iran*

[2]*Department of Pediatrics, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[3]*Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*

[4]*Health Sciences Research Center, Institute of Addiction, Mazandaran University of Medical Sciences, Sari, Iran*

[5]*Deptartment of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[6]*Department of Biostatistics, Mazandaran University of Medical Sciences, Sari, Iran*

[7]*Department of Biostatistics, Health Sciences Research Center, Institute of Addiction, Mazandaran University of Medical Sciences, Sari, Iran*

[8]*General Physician, Deputy of Health, Mazandaran University of Medical Sciences, Sari, Iran*

[9]*Deputy of Health, Mazandaran University of Medical Sciences, Sari, Iran*

ABSTRACT

Objective: To determine the seroprevalance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in the general population of Mazandaran province in Iran and to estimate the percentage of asymptomatic, mild, and severe infections.

Methods: We chose 1 588 inhabitants of Mazandaran province with cluster sampling. We measured their SARS-CoV-2 immunoglobulin M (IgM) and immunoglobulin G (IgG) serum levels. Demographics, risk factors, and symptoms were collected. The seroprevalence of SARS-CoV-2 was calculated by age and city and the World Health Organization (WHO) protocol and further stratified by demographic variables and risk factors. Finally, we identified the symptoms and factors related to COVID-19 with logistic regression.

Results: Two hundred subjects (12.59%) were tested positive for either IgG or IgM. Until May 23, 2020, the prevalence of COVID-19 was 15.26% (95% *CI*: 12.97%-17.79%) based on direct standardization and WHO's standardized age groups. Based on multivariate logistic regression, the incidence of getting an infection increased by an average of 11.6% for every 10-year increase in age (*OR*=1.116, 95% *CI*: 1.008-1.236, *P*=0.035). Furthermore, those in contact with COVID-19 patients had a 66.1% higher risk of developing the disease (*OR*=1.661, 95% *CI*: 1.104-2.497, *P*=0.015). In addition, the chance of getting SARS-CoV-2 infection was almost four times higher in people who had consulted a doctor during the pandemic than those who had not (*OR*=3.942, 95% *CI*: 2.813-5.524, *P*<0.001).

Conclusions: The prevalence of COVID-19 in Mazandaran province could be higher than the officially reported statistics based on diagnostic tests and clinical cases. There seems to be more asymptomatic or mild symptom cases than what was previously reported.

KEYWORDS: SARS-CoV-2; COVID-19; Prevalance; Iran; Serology; IgM; IgG

1. Introduction

Coronarvirus disease 2019 (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a virus from the coronavirus family that has spread in recent years. This new generation of virus attacks the respiratory system[1]. The first case of COVID-19 was officially reported in December 2019 in Wuhan, China, where it was presumed to be of animal origin[2,3]. Its latency period is between 2-14 days. Afterward, it presents with cold-like symptoms such as fever, cough, fatigue, shortness of breath, and gastrointestinal and loss of olfactory symptoms[4,5].

✉To whom correspondence may be addressed. E-mail: Jamshid.Charati@gmail.com

How to cite this article: Mousavi SA, Rouhanizadeh H, Saeedi M, Moosazadeh M, Ajami A, Fendereski A, et al. Seroprevalence of SARS-CoV-2 in Mazandaran province, Iran. Asian Pac J Trop Med 2021; 14(1): 10-16.

Article history: Received 14 August 2020 Revision 20 October 2020
Accepted 21 November 2020 Available online 5 January 2021

Most people who have the disease are asymptomatic or show mild symptoms and recover after a period of rest at home, but some require intensive care[6]. The mortality rate of this disease (about 2% to 3%) seems to be lower than other viral diseases in this family such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). However, because of its widespread prevalence due to its high transmission ability and long latency period, it has become the most important pandemic disease of the past century in the world[7].

Its spread rate is such that in just six months since its emergence, more than 16 000 000 people in 202 countries have been infected and nearly 650 000 people have died of it[8]. It has different death rates in different populations. Its mortality is higher in the older and people with underlying diseases such as cardiovascular disease, diabetes, high blood pressure, acute respiratory illness, and cancer[9].

However, the global statistics of patients with this disease are not accurate. The actual number of patients is probably much higher, because in some parts of the world, due to lack of facilities, there are limited diagnostic tests. Also, a large percentage of infected people are completely asymptomatic and therefore do not seek diagnostic tests[10]. According to the World Health Organization (WHO), we can estimate the prevalence of this disease by studying antibodies such as immunoglobulin G (IgG) and immunoglobulin M (IgM)[11].

The first officially confirmed case of COVID-19 disease in Iran was reported on February 19, 2020. By May 23, 2020, with more than 50 000 cases and more than 3 160 deaths due to the disease, Iran ranked 7th among the countries with the highest number of patients worldwide[12]. According to the WHO, COVID-19's transmission rate can be different in each region of the world. This has also been observed in Iran[13]. Mazandaran province, a forest-abundant region located in the north of Iran and beside the Caspian sea, has always been a tourist destination. Thus, it had the highest incidence of COVID-19 cases after Tehran and Qom provinces in Iran[14,15].

Given the current global situation due to SARS-CoV-2 outbreak and according to WHO's emphasis on establishing rapid and serious preventive measures in all regions, it is necessary to take prompt and effective measures to reduce the prevalence and damage caused by the disease. Epidemiological information can provide a clear picture of its situation in terms of how the disease is spreading. Hence, we did this study for two purposes: (1) to estimate the seroprevalence of SARS-CoV-2 antibodies in the general population of Mazandaran province based on sex, age, and location and (2) to determine the percentage of asymptomatic, mild, and severe infections. We also investigated its risk factors of the infection as secondary outcomes.

2. Materials and methods

2.1. Study design

This cross-sectional sero-epidemiological investigation was conducted between March and May 2020 in Mazandaran province, located in the north of Iran. Considering 30% infection rate, 2.5% accuracy, 95% confidence level, and 24% design effect size, the primary sample size was determined as 1 110 people. To cover for the possible exclusions, we enrolled 1 588 people. According to the WHO's protocol, we did the sampling with 10-household clusters[16].

We expected to have at least two people older than seven years old in each household so that at least one of them would be willing to voluntarily take the test and enter the research. The numbers of clusters were assigned to each city according to the available national census of the city's population and the percentage of its urban and rural population. Mazandaran province has 22 cities. Babol city was excluded because it has its own healthcare authority. Also, the data of two cities listed below were aggregated because they were too close to each other and their populations were somehow homogenous: (1) Chalous and Kelardasht as Chalous and (2) North and South Savadkouh cities as Savadkouh. Thus, 19 cities were included in this research: Galougah, Behshahr, Neka, Miandoroud, Sari, Qaemshahr, Simorgh, Savadkouh, Jouybar, Babolsar, Fereydunkenar, Amol, Mahmoudabad, Chalous, Noor, Noshahr, Abbas Abad, Tonekabon, and Ramsar.

We chose our samples with an average of one person per household. Since each cluster had 10 households, there were 10 participants in each cluster. A total of 159 clusters, including 69 rural and 90 urban clusters, were randomly selected using the landline telephone numbers of the households registered in the national integrated healthcare system. First, the head of the household was invited to participate in the study by telephone. Thus, all the household members older than seven years old were the population of our study. All participants signed informed consent before entering the study.

The exclusion criteria were: (1) living in retirement homes, prisons, or boarding schools, (2) being intolerant for disinfectants, (3) being in the treatment process of COVID-19, and (4) not cooperating until the end of the study.

2.2. Data collection

After selecting the households for each cluster, a researcher explained the research protocol to the head of the household. They were asked to refer to a chosen healthcare center in their city with all of their eligible and willing family members for blood sampling. After signing a consent form, a blood sample was taken from the participants intravenously. We did all the steps considering maximum hygiene. Afterward, a standard checklist was filled in for each participant by experts in each center including medical history, demographic information, risk factors, and common symptoms. We entered the data into the Excel software version 2013 and after three refinements, we transferred them to statistical software.

2.3. Laboratory examination method

IgG and IgM antibodies were measured in a laboratory in Mazandaran province with an enzyme-linked immunosorbent assay (ELISA) kit (Danesh Bonyan Pishtaz Teb Company, Iran). The sensitivity and specificity of the used ELISA kit were 59% and 100%, respectively, based on a diagnostic study on 60 participants [30 COVID-19 patients (confirmed with polymerase chain reaction and computed tomography) and 30 healthy individuals].

2.4. Statistical analysis

First, the variables were described using mean, standard deviation, frequency, and percentage. Then, the seroprevalence of IgG and IgM was calculated based on the WHO protocol, totally and for each variable, separately. The relationship between serum prevalence and the variables was examined using *Chi*-square test. The disease-related factors were then identified using a logistic regression model. Hence, we first did a logistic regression on each variable separately.

We classified seropositive participants based on their number of symptoms into four groups: (1) participants without symptoms, (2) participants with two common sympotoms as mild symptoms (fever > 37.8 ℃ and fatigue), (3) participants with two to five symptoms as moderate symptoms (fever > 37.8 ℃, fatigue, headache, cough and body aches) and (4) with six or more symptoms as severe symptoms. The important factors were identified with univariate logistic regression. We used Yates' correction for varriables with low frequency. We added all the factors with P<0.3 (P-to-enter multivariate model) in the multivariate logistic regression. Afterwards, we used multivariate logistic regression model's backwad elimination procedure significance level to detect the effective variables lower than 0.05. We did all the analyses with the statistical package for social sciences (SPSS) software version 25.0, and also R 4.0.0 and geographic information systems (GIS) softwares. P<0.05 is considered statistically significant difference.

3. Results

Mazandarn province has approximately 2 670 000 inhabitants. Among them, 1 588 people entered the study with cluster sampling. Totally, 45.7% of the participants were male and 65.87% of the participants were between 30 and 60 years old (Table 1). There were significant differences in sero-positivity rates between people in contact with COVID-19 patients and those without contact, and the difference was also significant among people from different cities (χ^2=59.87, df=19, P<0.001) (Tables 1 and 2).

Considering each test separately, 11.02% had a positive IgG test result and 4.84% had a positive IgM test result (Table 3). Considering both tests together, the results were positive in 3.27% of the participants. However, 12.59% of the participants had a positive result for only one of the tests (either IgG or IgM). Considering that a person who had either a positive IgG or IgM test result could be regarded as seropositive, there was 15.26% (12.97%-17.79%) prevalence of COVID-19 in Mazandaran province (Table 3). Important factors were identified with univariate logistic regression. We added all factors with P<0.3 (P-to-enter multivariate model) in the multivariate logistic regression. After that, we used multivariate logistic regression model backwad elimination procedure signifnat level for detecting effective variable P<0.05 (Table 4).

According to the results of multivariate logistic regression, the incidence of getting an infection increased by an average of 11.6% for every 10-year increase in age (OR=1.116, 95% CI: 1.008-1.236, P=0.035) (Table 5). In addition, those in contact with COVID-19 patients were 66.1% more likely to develop the disease (P=0.015). Also, the incidence of having a positive serology was almost four times higher in people who had been to a doctor in less than one month before the study for reasons other than COVID-19 compared to those who had not (P<0.001). According to *Chi*-square test, the prevalence of this disease was significantly related to the condition of contact with an infected person and the city of residence (P<0.05) (Tables 1 and 2).

We asked the participants whether they had any symptoms within four weeks before entering the study. Based on their responses from

Table 1. Demographic characteristics and seroprevalence according to the variables.

Characteristics	Overall sample *n* (%)	Sero-positive (200 patients) *n* (%)	Unadjusted seroprevalence % (95% *CI*)	Population-weight adjusted % (95% *CI*)	*Chi*-square	*df*	*P*-value
Gender							
Male	725 (45.7)	88 (44.0)	12.13 (9.87-14.71)	20.57 (16.74-24.94)	0.25	1	0.62
Female	863 (54.3)	112 (56.0)	12.97 (10.84-15.37)	21.99 (18.38-26.05)			
Age (years)							
7-9	11 (0.69)	1 (0.5)	9.09 (0.45-40.10)	15.40 (0.77-67.97)	7.54	7	0.37
10-19	111 (6.99)	10 (5.0)	9.00 (4.61-15.93)	15.26 (7.82-27.00)			
20-29	131 (8.25)	17 (8.5)	12.97 (7.96-19.65)	21.99 (13.49-33.30)			
30-39	340 (21.41)	33 (16.5)	9.70 (6.78-13.29)	16.45 (11.50-22.52)			
40-49	381 (23.99)	49 (24.5)	12.86 (9.74-16.59)	21.79 (16.52-28.12)			
50-59	325 (20.47)	52 (26.0)	16.00 (12.19-20.38)	27.11 (20.67-34.55)			
60-69	206 (12.97)	27 (13.5)	13.10 (8.99-18.31)	22.21 (15.23-31.04)			
⩾70	83 (5.23)	11 (5.5)	13.50 (7.15-22.01)	22.46 (12.13-37.31)			
District							
Rural	688 (43.3)	83 (41.5)	12.06 (9.75-14.71)	20.44 (16.53-24.93)	0.31	1	0.58
Urban	900 (56.7)	117 (58.5)	13.00 (10.90-15.35)	22.03 (18.47-26.02)			
Contact history							
Yes	187 (11.8)	42 (21.0)	22.45 (16.85-29.04)	36.07 (28.56-49.22)	18.74	1	<0.001
No	1 401 (88.2)	158 (79.0)	11.27 (9.67-13.4)	19.11 (16.40-22.10)			
Travel after the beginning of pandemic							
Yes	109 (6.9)	19 (9.5)	17.43 (11.18-25.50)	29.54 (18.95-43.22)	2.47	1	0.12
No	1 479 (93.1)	181 (90.5)	12.17 (10.56-13.93)	20.63 (17.90-23.61)			
Flu vaccine							
Yes	41 (2.6)	3 (1.5)	7.31 (2.01-19.01)	12.40 (3.41-32.22)	1.07	1	0.30
No	1 547 (97.4)	197 (98.5)	12.73 (11.12-14.50)	21.58 (18.85-24.57)			

Table 2. Demographic characteristics and seroprevalence according to city.

City*	Overall sample *n* (%)	Sero-positive (200 patients) *n* (%)	Unadjusted seroprealence % (95% *CI*)	Population-weight adjusted % (95% *CI*)
Ramsar	40 (2.5)	6 (3.0)	15.00 (6.73-29.53)	25.42 (11.40-50.05)
Tonekabon	101 (6.4)	7 (3.5)	6.93 (3.13-13.54)	11.74 (5.30-22.96)
Abbas Abad	50 (3.1)	9 (4.5)	18.00 (9.44-30.68)	30.50 (16.01-52.01)
Chalous	80 (5.0)	5 (2.5)	6.25 (2.49-13.95)	10.59 (4.22-23.65)
Noshahr	70 (4.4)	8 (4.0)	11.42 (5.08-21.06)	19.37 (8.61-35.69)
Noor	70 (4.4)	6 (3.0)	8.57 (3.79-17.44)	14.52 (6.42-29.57)
Mahmoodabad	60 (3.8)	14 (7.0)	23.33 (13.62-35.62)	39.54 (23.09-60.39)
Fereydunkenar	30 (1.9)	3 (1.5)	10.00 (2.77-25.95)	16.94 (4.69-43.99)
Amol	242 (15.2)	24 (12.0)	9.92 (6.58-14.34)	16.80 (11.14-24.29)
Babolsar	80 (5.0)	23 (11.5)	28.75 (19.69-39.88)	48.72 (33.38-67.60)
Savadkouh	50 (3.1)	3 (1.5)	6.00 (1.65-16.37)	10.16 (2.80-27.75)
Qaemshahr	169 (10.6)	11 (5.5)	6.50 (3.37-11.33)	11.03 (5.71-19.19)
Simorgh	20 (1.3)	5 (2.5)	25.00 (10.41-47.40)	42.37 (17.63-80.34)
Jouybar	40 (2.5)	9 (4.5)	22.50 (11.84-37.97)	38.13 (20.07-64.36)
Sari	267 (16.8)	38 (19.0)	14.23 (10.35-18.99)	24.12 (17.55-32.20)
Miandoroud	30 (1.9)	8 (4.0)	26.67 (13.08-44.86)	45.19 (22.16-76.03)
Neka	70 (4.4)	12 (6.0)	17.14 (9.51-27.59)	29.05 (16.12-46.76)
Behshahr	100 (6.3)	7 (3.5)	7.00 (3.16-13.68)	11.86 (5.36-23.19)
Galougah	19 (1.2)	2 (1.0)	10.52 (1.90-31.57)	17.84 (3.22-53.52)

*SARS-CoV-2 sero-positive rate of cities had significant differences (*Chi*-square=59.87, *df*=18, *P*<0.001).

Table 3. Seroprevalence estimates in Mazandaran province (%).

Antibody seropositivity	Seroprevalence (95% *CI*)		
	Unadjusted	Age and gender WHO adjusted	Age and county WHO adjusted
IgG	11.02 (9.54-12.64)	15.36 (13.08-17.94)	13.55 (11.37-15.98)
IgM	4.84 (3.84-6.02)	5.55 (4.16-7.22)	4.69 (3.45-6.25)
IgG and IgM	3.27 (2.45-4.26)	3.41 (2.34-4.76)	2.88 (1.92-4.17)
IgG or IgM	12.59 (11.02-14.30)	17.29 (14.84-19.97)	15.26 (12.97-17.79)

Immunoglobulin M (IgM); immunoglobulin G (IgG).

Table 4. Univariate logistic regression analysis of risk factors of SARS-CoV-2 seropositivity in the study population.

Variable	β	Std. error	Exp (β)	95% *CI* for EXP (β)		*P* value
				Lower	Upper	
Gender	0.077	0.152	1.080	0.801	1.455	0.615
Medical related job	0.315	0.179	1.370	0.964	1.947	0.079
Age (Binned)	0.099	0.055	1.105	0.992	1.230	0.070
Location	0.085	0.153	1.089	0.806	1.471	0.578
Contact history	0.824	0.195	2.279	1.556	3.336	<0.001
Travel	0.414	0.265	1.513	0.901	2.541	0.118
Flu vaccine	-0.614	0.605	0.541	0.165	1.769	0.310
Visiting a doctor	1.447	0.167	4.248	3.065	5.889	<0.001
Absence from school	1.335	0.245	3.801	2.354	6.139	<0.001
Heart disease	0.415	0.265	1.514	0.901	2.543	0.117
Diabetes mellitus	0.236	0.236	1.266	0.797	2.009	0.318
Dialysis and kidney disease	0.742	0.410	2.100	0.941	4.690	0.070
Thalassemia*	0.830	1.635	2.307	0.094	56.820	0.609
Immunodeficiency	0.066	0.763	1.068	0.239	4.770	0.931
Liver disease	-0.078	0.757	0.925	0.210	4.073	0.917
High blood pressure	0.095	0.213	1.099	0.724	1.668	0.656
Brain disease	-0.348	0.531	0.706	0.250	1.997	0.512
Body mass index > 40	-0.530	0.606	0.589	0.179	1.932	0.382
Smoking*	-6.180	6.320	0.020	0.000	0.000	502.000
Pregnancy*	0.830	1.635	2.307	0.094	56.820	0.609
Fainting	0.558	1.121	1.747	0.194	15.714	0.618
Cancer	0.442	0.785	1.556	0.334	7.252	0.574
Chemotherapy	-0.138	1.063	0.871	0.108	7.003	0.897

-: The model could not be fit becauase of ferquncy of this factor is very low. β is regression coefficients in this model. This provides information about the relationships of predictors (such as age, contact patients and visiting a doctor) in the model to the dichotomous dependent variables (status of serology, IgG or IgM were positive). Exp (β) is odds ratio. It is a measure of the effect of a predictor variable on the ratio of pobabilty of sreopositivity divided by the probability of negative serology. *The model was fitted using Yates corretion becauase of these variables' low ferquency.

Table 5. Multivariate logistic regression analysis of risk factors of SARS-CoV-2 seropositivity in the study population.

Variable	β *	Std. error	Exp (β)**	95% *CI* for EXP (β)		*P* value
				Lower	Upper	
Age (Binned)	0.110	0.052	1.116	1.008	1.236	0.035
Contact patients	0.507	0.208	1.661	1.104	2.497	0.015
Visiting a doctor	1.372	0.172	3.942	2.813	5.524	<0.001
Constant	-2.896	0.291	0.055			<0.001

β * is regression coefficients in this model. This provided information about the relationships of predictors (such as age, contact patients and visiting a doctor) in the model to the dichotomous dependent variables (status of serology, IgG or IgM were positive). Exp (β)** is odds ratio. It is a measure of the effect of a predictor variable on the ratio of pobabilty of seropositivity divided by the probability of negative serology.

200 seropositive individuals, 89 (44.5%, 89/200) had no symptoms and 49 (24.5%, 49/200) had mild symptoms. A total of 25 (12.5%) seropositive from 200 participants had severe symptoms. The most common symptoms of seropositive individuals included fever and chills, which were reported in 51 (25.5%) and 49 (24.5%) seropositive participants. Afterward, it was dry cough reported 41 from 200 (20.5%) of the participants and then headache and body aches with in 38 (19.0%) and 37 (18.5%) patients (Table 6).

Table 6. Frequency of symptoms of COVID-19 in 200 seropositive cases.

Symptom	*n* (%)
Redness of eyes	4 (2.0)
Fever	51 (25.5)
Chills	49 (24.5)
wheezing	8 (4.0)
Headache	38 (19.0)
Diarrhea	25 (12.5)
fatigue	28 (14.0)
Body ache	37 (18.5)
Sore throat	24 (12.0)
Chest pain	15 (7.5)
Nausea and vomiting	18 (9.0)
Loss of smell	14 (7.0)
Dry cough	41 (20.5)
Running nose	18 (9.0)
Shortness of breath	21 (10.5)
Loss of appetite	19 (9.5)
Stomachache	15 (7.5)
Loss of taste	20 (10.0)
No symptoms	89 (44.5)
Mild symptoms	49 (24.5)
Moderate symptoms	37 (18.5)
Severe symptoms	25 (12.5)

From 200 seropositive participants, 38.0% (76/200) had a history of visiting a physician in less than one month before entering the study. Only 13.5% (27/200) had a definitive diagnosis of SARS-CoV-2 infection. A total of 90 (45.0%) seropositive participants diagnosed with the disease were at least 50 years old However, considering all the participants, 614 (38.7%) of them were older than 50 years old (Table 1). Therefore, the crude rate of SARS-CoV-2 infection among those younger than 50 years old was 110 from 974 (11.3%). But this index in participants older than 50 years old was 90 from 614 (14.7%). So, the chance of SARS-CoV-2 infection in participants older than 50 years old was 30% higher than others (*P*=0.049). All of them had a history of high blood pressure and 18.5% had a history of diabetes. The highest prevalences were in Joybar, Miandrood, Babolsar, Mahmoudabad, and Simorgh cities (Figure 1).

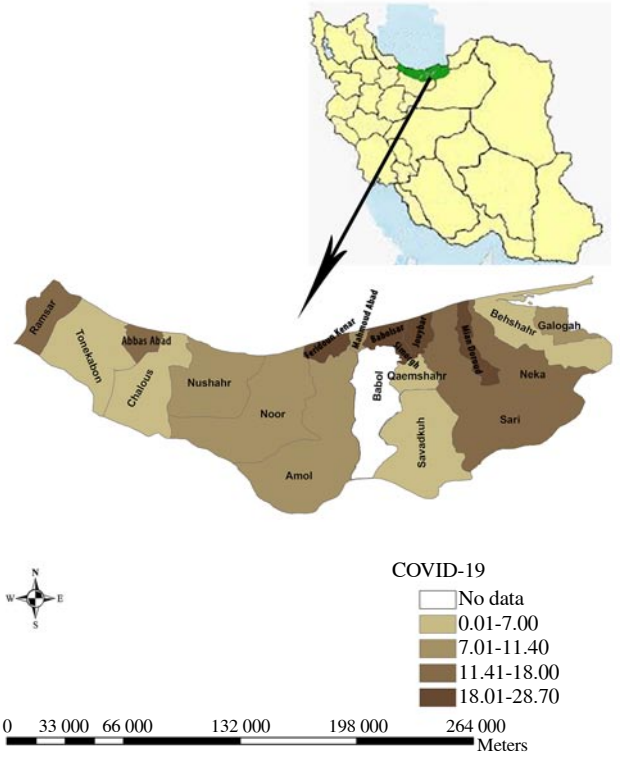

Figure 1. The distribution of IgM or IgG seropositive cases based on city (county) in Mazandaran province.

4. Discussion

Mazadaran province has a high population density with 137 people per square kilometer and is ranked 4th in terms of population density among the 31 provinces of Iran. Also, it has one of the highest number of tourists because of its various attraction sites, forests, and beaches. Thus, according to the official reports of Iran's Statistics Center, it is the second touristic province of Iran[17]. Hence, it is a high-risk region for COVID-19.

The prevalence of COVID-19 disease was 15.26% (12.97%-17.79%) in Mazandaran province based on direct standardization and WHO's standard age groups while considering the sensitivity

and characteristics of the available kits. Thus, according to the population of the province (excluding Babol city), the number of patients has been between 346 299 to 473 993 people until May 23, 2020. More than 70% of people had a maximum of two symptoms, indicating that they had mild illness. This condition was most common in people younger than 40 years old.

The prevalence of COVID-19 in Mazandadran province was lower than its prevalence in Gilan province (its neighboring province) which has been 22% to 33% until May 23, 2020[18]. But it was higher than the reported prevalence in some countries and regions, such as Japan with 2.7%[19] and California in the United States with 2.8% prevlance[20].

An important reason for estimating the prevalence is to have the possible mortality rate from the disease. The number of officially unpublished reported deaths from the disease in the province until July 27, 2020 was 1 432 people. Thus, the disease mortality rate has been 0.054%. Considering the estimated prevalence of COVID-19 in this study, its mortality rate in the province can be adjusted to 0.329%. In the study conducted in Gilan province, the estimated mortality rate was 0.08% to 0.12%, which was much lower than the mortality rate of the disease worldwide[18].

About 12.5% of the participants had severe symptoms (Table 6). Therefore, it is estimated that the actual number of cases of COVID-19 in society is much higher than what has been officially reported. However, since only a small percentage of the patients have had severe symptoms, only a small percentage of the infected cases have referred to medical centers. Based on the age and city adjustments in this study, it is estimated that between 407 000 and 461 643 people have been infected with the disease. Nearly 70% of the participants had less than two symptoms and had not visited a medical center.

In many studies worldwide, the estimated number of infected people has been higher than the official reports. For example, in the city of Santa Clara, California, United States, the prevalence was 2.8%, which was 55 times higher than the official reports[20]. Also, the number of IgG-positive individuals was 50 123 in Kobe city of Japan, which was 706 times higher than the officially reported number of cases for that city[19]. The main reason for this inconsistency can be due to the asymptomatic nature of the disease in many infected people and that many people with mild symptoms never refer to medical centers for a test. Also, the accuracy of diagnostic kits might also have a small effect in this regard.

Given that more than 89.5% of seropositive individuals had asymptomatic or mild symptoms, it is estimated that between 364 000 and 413 000 people living in Mazandaran province had a mild or asymptomatic COVID-19. They were the asymptomatic carriers. Therefore, undertaking the principles of self-care and hygiene, especially in washing hands and social distancing, can be useful in controlling this pandemic disease.

Our results showed that the prevalence of the disease varies at different ages. The prevalence was higher at older ages. The highest prevalence was in the 40 to 50 years old age group. The high incidence of the disease in older adults, who usually have underlying diseases, is not unexpected. However, because the death rate is higher in people older than 60 years old, this can be a reason for the decrease in the number of people with positive serum results. Regarding the cities, the highest prevalences were in Miandrood, Babolsar, Simorgh, and Mahmoudabad, which are coastal cities and touristic locations. This increases the prevalence rate could be due to overcrowding and the influx of travelers from high-risk cities.

The most important symptoms associated with COVID-19 included general symptoms such as fever, chills, fatigue, body aches, headaches and chest pain, gastrointestinal symptoms such as abdominal pain, diarrhea, and dry cough, and loss of sense of smell and taste. Thus, having these symptoms can be signs of having COVID-19 and can help to diagnose it faster. Symptoms such as red eyes, loss of appetite, nausea and vomiting, runny nose, and abdominal pain were not significantly associated with the disease.

Residents of organizational centers with high population density, such as prisons and nursing homes, did not enter the study, which is a limitation for the present study.

To conclude, the prevalence of COVID-19 in Mazandaran province could be higher than the officially reported statistics based on diagnostic tests and clinical cases. There seems to be more asymptomatic people than what was previously reported. To control the pandemic, it is necessary to carry out the basic principles of healthcare and social distancing. Using the mass media to promote healthy behaviors during the pandemic can be effective.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Authors' contributions

S.A.M, J.Y.C, H.R,M.S, M.M and A.A designed the study. S.M.S, N.A and A.F carried out the data collection. IgG and IgM antibodies were measured by S.H.S and Z.B. Data analysis and interpretation were done by J.Y.C and A.F. S.A.M, J.Y.C, H.R, M.S, M.M, A.A and A.F wrote the manuscript.

References

[1] Heymann DL, Shindo N. COVID-19: What is next for public health? *Lancet* 2020; **395**(10224): 542-545.

[2] Du Toit A. Outbreak of a novel coronavirus. *Nat Rev Microbiol* 2020;

18(3): 123.

[3] Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. *J Med Virol* 2020; **92**(4): 401-402.

[4] Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: A descriptive study. *Chin Med J* 2020; **133**(9): 1015-1024.

[5] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**(10223): 497-506.

[6] Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, et al. Coronavirus disease 2019 (COVID-19): A perspective from China. *Radiology* 2020; **296**(2): 15-25.

[7] Yang S, Cao P, Du P, Wu Z, Zhuang Z, Yang L, et al. Early estimation of the case fatality rate of COVID-19 in mainland China: A data-driven analysis. *Ann Transl Med* 2020; **8**(4): 1-6.

[8] Worldometers. *Countries where COVID-19 has spread*. 2020. [Online]. Available from: https://www.worldometers.info/coronavirus/countries-where-coronavirus-has-spread/. [Accessed on 20 May 2020].

[9] Tavakoli A, Vahdat K, Keshavarz M. Novel coronavirus disease 2019 (COVID-19): An emerging infectious disease in the 21st century. *ISMJ* 2020; **22**(6): 432-450.

[10] Hoffman T, Nissen K, Krambrich J, Ronnberg B, Akaberi D, Esmaeilzadeh M, et al. Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. *Infect Ecol Epidemiol* 2020; **10**(1): 1-5.

[11] WHO. *Population-based age-stratified seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection, 26 May 2020.* [Online]. Available from: https://apps.who.int/iris/bitstream/handle/10665/332188/WHO-2019-nCoV-Seroepidemiology-2020.2-eng.pdf. [Accessed on 24 June 2020]

[12] Worldometers. *COVID-19 coronavirus pandemic 2020*. [Online]. Available from: https://www.worldometers.info/coronavirus/. [Accessed on 13 June 2020].

[13] WHO. *Coronavirus disease 2019 (COVID-19): Situation report-51*. 2020. [Online]. Available from: https://apps.who.int/iris/bitstream/handle/10665/331475/nCoVsitrep11Mar2020-eng.pdf?sequence=1&isAllowed=y. [Accessed on 23 May 2020].

[14] Akbarian Ronizi SR, Roshan GR, Negahban S. Assessment of tourism climate opportunities and threats for villages located in the northern coasts of Iran. *IJER* 2016; **10**(4): 601-612.

[15] Arab-Mazar Z, Sah R, Rabaan AA, Dhama K, Rodriguez-Morales AJ. Mapping the incidence of the COVID-19 hotspot in Iran–implications for travellers. *Trav Med Infect Dis* 2020; **101630**(34): 1-4.

[16] WHO. *Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection, 17 March 2020.* [Online]. Available from: https://apps.who.int/iris/bitstream/handle/10665/331656/WHO-2019-nCoV-Seroepidemiology-2020.1-eng.pdf. [Accessed on 13 May 2020].

[17] Statistical Center of Iran. *Survey results of national tourists. Spring 2016: Statistical Center of Iran.* [Online]. Available from: https://www.amar.org.ir/Portals/0/News/1396/gardesh%201-95.pdf. [Accessed on 13 May 2020].

[18] Shakiba M, Nazari SS, Mehrabian F, Rezvani SM, Ghasempour Z, Heidarzadeh A. *Seroprevalence of COVID-19 virus infection in Guilan province, Iran.* 2020. [Online]. Available from: https://www.medrxiv.org/content/10.1101/2020.04.26.20079244v1.abstract. [Accessed on 10 May 2020].

[19] Asako Doi M, Kentaro Iwata M, Hirokazu Kuroda M, Toshikazu Hasuike M, Seiko Nasu M, Aya Kanda M, et al. *Seroprevalence of novel coronavirus disease (COVID-19) in Kobe, Japan.* 2020. [Online]. Available from: https://www.medrxiv.org/content/10.1101/2020.04.26.20079822v2.full.pdf. [Accessed on 25 May 2020].

[20] Bendavid E, Mulaney B, Sood N, Shah S, Ling E, Bromley-Dulfano R, et al. *COVID-19 antibody seroprevalence in Santa Clara county, California.* 2020. [Online]. Available from: https://www.medrxiv.org/content/medrxiv/early/2020/04/30/2020.04.14.20062463.full.pdf. [Accessed on 25 May 2020].