SARS-CoV-2 antibody seroprevalence in the general population and high-risk occupational groups across 18 cities in Iran: a population-based cross-sectional study

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Summary

Background Rapid increases in cases of COVID-19 were observed in multiple cities in Iran towards the start of the pandemic. However, the true infection rate remains unknown. We aimed to assess the seroprevalence of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 18 cities of Iran as an indicator of the infection rate.

Methods In this population-based cross-sectional study, we randomly selected and invited study participants from the general population (from lists of people registered with the Iranian electronic health record system or health-care centres) and a high-risk population of individuals likely to have close social contact with SARS-CoV-2-infected individuals through their occupation (from employee lists provided by relevant agencies or companies, such as supermarket chains) across 18 cities in 17 Iranian provinces. Participants were asked questions on their demographic characteristics, medical history, recent COVID-19-related symptoms, and COVID-19-related exposures. Iran Food and Drug Administration-approved Pishtaz Teb SARS-CoV-2 ELISA kits were used to detect SARS-CoV-2-specific IgG and IgM antibodies in blood samples from participants. Seroprevalence was estimated on the basis of ELISA test results and adjusted for population weighting (by age, sex, and city population size) and test performance (according to our independent validation of sensitivity and specificity).

Findings From 9181 individuals who were initially contacted between April 17 and June 2, 2020, 243 individuals refused to provide blood samples and 36 did not provide demographic information and were excluded from the analysis. Among the 8902 individuals included in the analysis, 5372 had occupations with a high risk of exposure to SARS-CoV-2 and 3530 were recruited from the general population. The overall population weight-adjusted and test performance-adjusted prevalence of antibody seropositivity in the general population was 17·1% (95% CI 14·6–19·5), implying that 4 265 542 (95% CI 3 659 043–4 887 078) individuals from the 18 cities included were infected by the end of April, 2020. The adjusted seroprevalence of SARS-CoV-2-specific antibodies varied greatly by city, with the highest estimates found in Rasht (72·6% [53·9–92·8]) and Qom (58·5% [37·2–83·9]). The overall population weight-adjusted and test performance-adjusted seroprevalence in the high-risk population was 20·0% (18·5–21·7) and showed little variation between the occupations included.

Interpretations Seroprevalence is likely to be much higher than the reported prevalence of COVID-19 based on confirmed COVID-19 cases in Iran. Despite high seroprevalence in a few cities, a large proportion of the population is still uninfected. The potential shortcomings of current public health policies should therefore be identified to prevent future epidemic waves in Iran.

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Introduction

COVID-19, the disease first reported in Wuhan in the Chinese province of Hubei in late 2019, has spread and caused high morbidity and mortality worldwide.[1] The spectrum of COVID-19 severity varies widely, from asymptomatic infection to severe outcomes including organ failure and death.[2,3] So far, the main body of evidence on population-level infection and fatality rates in Iran has been solely based on the severe end of the disease spectrum. Thus, in the absence of seroprevalence surveys and an unknown proportion of asymptomatic cases in the country, the true infection rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, remains unclear.[1,4,5]

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For the Farsi translation of the abstract see **Online** for appendix 1

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Research in context

Evidence before this study

Iran was one of the first countries to report an epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and saw a rapid increase in cases nationwide. However, in the absence of seroprevalence studies, the true infection rate in Iran has remained unknown. We searched MEDLINE, PubMed, Embase, medRxiv, and the WHO Global Research Database for publications on the seroprevalence of SARS-CoV-2-specific antibodies, published in English, using the search terms "severe acute respiratory syndrome coronavirus 2", "COVID-19", "seroprevalence", "IgG/IgM antibodies", to August 30, 2020. To date, most seroprevalence studies have not been peer reviewed and estimation of the seroprevalence of SARS-CoV-2-specific antibodies in individuals employed in occupations with a high risk of SARS-CoV-2 exposure has been inadequate. Furthermore, in most studies, the overall prevalence estimates were not further stratified by geographical areas (eg, cities within a county or country) and did not take the potential variation of infection rate in different regions into account.

Added value of this study

In this population-based study we assessed the seroprevalence of SARS-CoV-2-specific antibodies in 18 cities in Iran. This is the first seroprevalence study in the Middle East to report the prevalence of anti-SARS-CoV-2 antibodies in the general population as well as in individuals employed in occupations with a high risk of exposure to SARS-CoV-2. Our findings imply that, in the general population, 4 265 542 individuals from the included cities were infected by the end of April, 2020, and that 1 522 798 (35·7%) infected individuals in this population were asymptomatic. Seroprevalence estimates of SARS-CoV-2-specific antibodies showed heterogeneity across the general populations in different cities, ranging from 1·7% to 72·6%. Compared with other seroprevalence studies from around the world, the seroprevalence estimates in the general population of Iran in this study were high.

Implications of all the available evidence

The overall 17·1% seroprevalence rate of SARS-CoV-2-specific antibodies across the cities in this study confirms that a large proportion of the population in Iran is still susceptible to the virus. Public health policies and adequate personal protective equipment among front-line workers are therefore needed to prevent the potential increase in patient load in hospitals across the country and to reduce COVID-19-related morbidity and mortality, especially during the second and third waves of infection. The similar seroprevalence estimates between general and high-risk populations has important public health implications, possibly indicating inadequate or low adherence to infection control measures, which requires further investigation.

As recommended by WHO, measuring the extent of seropositivity could inform the proportion of individuals positive for anti-SARS-CoV-2 antibodies in the population and could further indicate the rate of disease transmission over time.[6,7] Moreover, as the extent of infection in a population depends heavily on social interactions and population density, assessing the proportion of potentially protected individuals in populations with different levels of exposure is crucial.[8,9] In most seroprevalence surveys to date, the cumulative incidence of infection has been solely reported by age group and ethnicity.[4,10] However, antibody testing of individuals with occupations at high-risk of exposure to SARS-CoV-2 because of frequent or high-risk social interactions (eg, supermarket employees) might be necessary for public health decision making on emergency lockdowns or return-to-work policies.[9]

Iran was one of the first countries to report a SARS-CoV-2 epidemic with a rapid case increase nationwide.[7] As of Nov 18, 2020, more than 800 000 confirmed cases and 42 000 COVID-19-related deaths have been reported in the country.[11] The first two COVID-19-related deaths were reported in Qom province early in the epidemic.[7] Because of the high number of cases and increased numbers of patients in hospitals, restrictions on mass gatherings (eg, restaurant closures) were initiated in February, 2020. However, the easing of initial lockdown restrictions in early April potentially contributed to the second wave of SARS-CoV-2 reported in multiple cities of Iran in June, 2020. Therefore, national-level seroprevalence studies are urgently needed to provide an indication of the proportion of the population who have not yet been infected and to plan for future health-care needs.[7]

In this study, we assessed the seroprevalence of anti-SARS-CoV-2 antibodies among the general population in 18 cities of Iran during the first wave of the epidemic. Furthermore, we estimated the prevalence rates of antibody seropositivity among individuals with a high risk of occupational SARS-CoV-2 exposure.

Methods

Study design, population, and sampling

In this population-based cross-sectional study, we used serological testing for anti-SARS-CoV-2 antibodies to assess the prevalence of SARS-CoV-2 infection in 18 cities across 17 provinces in Iran (appendix 2 p 1). The selected cities were Gorgan, Babol, Sari, Rasht, Ardabil, Tabriz, and Urmia in the northern provinces; Tehran, Mashhad, Qom, Esfahan, Hamedan, Sanandaj, and Kermanshah in the central provinces; and Ahvaz, Kerman, Shiraz, and Zahedan in the southern provinces (figure 1).

We randomly selected and invited study participants from the general population and from occupations with a

high risk of SARS-CoV-2 exposure (eg, front-line health and pharmacy workers, taxi drivers, and cashiers or other customer-facing staff) from the cities included in the study, without specific inclusion or exclusion criteria (full details in appendix 2 p 1). Random selection was achieved through a systematic sampling approach, by using a random number generator to select the first name on the list of possible participants in each sub-population, then systematically selecting the next participants (appendix 2 p 1). The general population sample was selected on the basis of individuals' national identification numbers from those registered in the Iranian electronic health record system (SIB) in 11 cities and from those registered with health-care centres in seven cities. Potential participants were contacted by telephone using their telephone number(s) recorded in those systems. SIB includes demographic characteristics and administrative health data for around 72 million of 81 million Iranians (coverage >88%), and a similar level of coverage is reported for health-care centres. The high-risk population sample included individuals who were at an increased risk of exposure to SARS-CoV-2 because of close social contact with infected individuals through their working environment. High-risk individuals were selected from lists of employees provided by the relevant companies or agencies in each city (appendix 2 p 1) and were contacted by phone. High-risk occupations comprised front-line physicians and nurses, non-front-line health-care workers, pharmacy staff, taxi drivers, bank employees, and cashiers of supermarket chains. To increase the rate of participation, testing was done at the place of work (eg, bank or supermarket) from which individuals were selected. Written informed consent was sought from all individuals before enrolment in the study.

The study proposal and protocol were approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1399.308).

Procedures

At each collaborating centre, an interviewer asked participants a series of questions about their demographic characteristics, medical history, recent COVID-19-related symptoms, and COVID-19-related exposures. After completion of the questionnaire, a laboratory technician collected 5 mL of venous blood into an EDTA-coated microtainer. Iran's Food and Drug Administration-approved SARS-CoV-2 ELISA kits (Pishtaz Teb, Tehran, Iran; catalogue numbers PT-SARS-COV-2.IgM-96 and PT-SARS-COV-2.IgG-96) were used to assess the presence of SARS-CoV-2-specific IgG and IgM antibodies in serum samples. Detailed information on sample collection and ELISA kits is provided in the appendix 2 (p 3).

Test validation

The manufacturer-reported sensitivity and specificity of the ELISA kits were, respectively, 94·1% and 98·3% for the SARS-CoV-2 IgG ELISA kit and 79·4% and 97·3% for the SARS-CoV-2 IgM ELISA kit. These values were based on samples from 34 patients who had COVID-19 clinical symptoms and positive RT-PCR results, and 111 serum samples collected and stored at –20°C before the SARS-CoV-2 pandemic.

We independently validated the accuracy of the ELISA kits using serum samples (collected within 2–4 weeks of symptom onset) from 154 patients with RT-PCR-confirmed COVID-19 and 110 serum samples collected 2 years before the pandemic that were stored in the Digestive Diseases Research Institute biobank (scenario 1; appendix 2 p 5). Among the 154 samples positive for SARS-CoV-2 on RT-PCR, 103 samples tested positive for either IgG (94 [61%]) or IgM (79 [51%]) with the ELISA kits, corresponding to a collective sensitivity of 66·9% (95% CI 58·9–74·2%). 108 of 110 pre-COVID-19 samples tested negative for both IgG and IgM SARS-CoV-2-specific antibodies, corresponding to a collective specificity of 98·2% (95% CI 93·6–99·8). However, as a sensitivity analysis, we combined manufacturer's data with our data (188 positive samples and 221 negative samples in total; scenario 2; appendix 2 p 5). Combining the manufacturer's

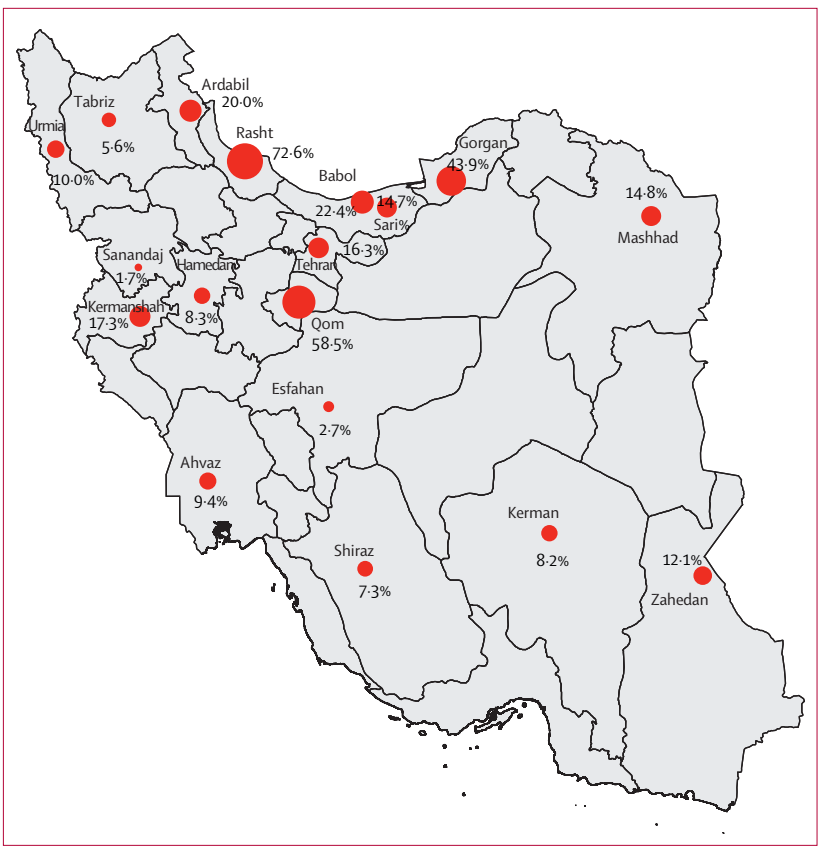

Figure 1: **Seroprevalence of severe acute respiratory syndrome coronavirus 2 in the general population in each city included in the study**

The area of each spot on the map is proportional to the seroprevalence in that city. Full data are provided in figure 3 and appendix 2 (p 7). Northern provinces: Gorgan, Babol, Sari, Rasht, Ardabil, Tabriz, Urmia. Central provinces: Tehran, Mashhad, Qom, Esfahan, Hamedan, Sanandaj, Kermanshah. Southern provinces: Ahvaz, Kerman, Shiraz, Zahedan.

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See Online for appendix 2

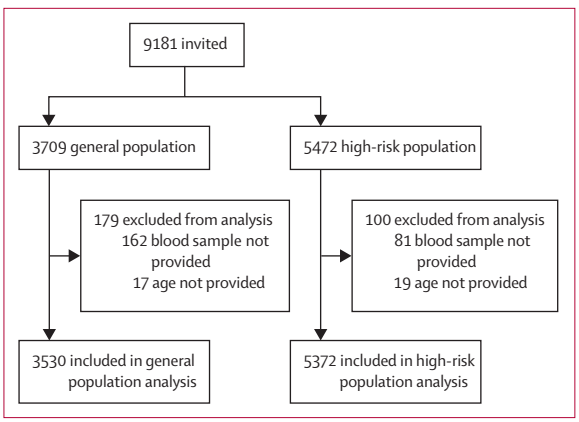

Figure 2: **Study profile**

data with our data yielded a sensitivity of 71·8% (64·8–78·1) and a specificity of 98·2% (95·4–99·5). The estimated performance of the kits in scenario 1 was used as the primary test characteristic in this study. The scenario 2 test performance was then used to adjust the prevalence rates, which were later compared with the scenario 1 test-adjusted estimates.

Covariates

Demographic variables included age, sex, and city of residence. A comorbid state was defined as presence of at least one of the following self-reported medical conditions: heart disease, hypertension, lung disease, asthma, diabetes, fatty liver disease, cirrhosis, hepatitis B, hepatitis C, autoimmune hepatitis, HIV, kidney disease, thalassaemia, haemophilia, dementia, multiple sclerosis, malignancy, inflammatory bowel disease, and history of organ transplantation. COVID-19-related symptoms included cough, fever, chills, anosmia, sore throat, headache, shortness of breath, diarrhoea, conjunctivitis, weakness, myalgia, arthralgia, altered level of consciousness, and chest pain, experienced during the 12 weeks preceding questionnaire completion. On the basis of self-reported symptoms, participants were further categorised as asymptomatic, paucisymptomatic (one to three symptoms), or symptomatic (four or more symptoms).

Statistical analysis

The sample size needed to estimate prevalence in the study was calculated to be 9057 on the basis of a 1% margin of error, a seroprevalence rate of 15%, a type I error rate of 0·05, and a design effect of 1·85. The required sample size in each city was proportional to each city's population relative to the total population of all cities included. Detailed information on sample size calculation is provided in appendix 2 (p 1).

Baseline characteristics were described separately for each city. The population seroprevalence of SARS-CoV-2-specific antibodies was estimated for the overall general population, the general population by city, the overall and high-risk populations by occupation type, the high-risk population by city, and all individuals (ie, irrespective of antibody positivity) who completed the symptom questionnaire.

To assess the seroprevalence of SARS-CoV-2-specific antibodies in the general population, we first estimated the overall crude frequencies of positive tests, stratified by age and sex, as a proportion of the total sample size for the general population. This crude prevalence was then weighted for age, sex, and the population size of each city using the 2016 population and household census in Iran. Finally, the weighted estimate of prevalence was adjusted for test performance as reported in scenario 1 and scenario 2.

To assess the seroprevalence of SARS-CoV-2-specific antibodies in the high-risk population, we reported the crude and test performance-adjusted prevalence of SARS-CoV-2 antibody positivity in the high-risk groups separately for each city. The overall crude prevalence, weighted by city population, and test performance-adjusted prevalence of SARS-CoV-2-specific antibodies were then estimated for the high-risk population by occupation type.

The overall crude prevalence and test performance-adjusted prevalence of SARS-CoV-2 antibody positivity for those with self-reported COVID-19-related symptoms were assessed.

95% CIs for unweighted seroprevalence were estimated using exact binomial models and a bootstrap method was used to construct the 95% CIs for weighted and adjusted estimates.[4,12] All statistical analyses were done with STATA software (version 12). The statistical approach used for population weighting, test performance adjustment, and the bootstrap method is detailed in appendix 2 (pp 1, 3).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Among 9181 individuals who were contacted across 18 cities (total population 25 061 939), all individuals initially agreed to participate in the study (telephone response rate 100%). However, 243 (2·6%) individuals refused to provide blood samples at the participation centres and were excluded. 36 individuals did not provide their demographic information, including age, and were also excluded. Of the 8902 individuals included in the analysis (figure 2), 1157 (13·0%) participants did not complete the questions on COVID-19-related symptoms and 1095 (12·3%) did not complete the comorbidity questionnaire. Among the 8902 individuals included in the analysis, 5372 (60·3%) had occupations with a high

	Population size		Sex	Age, years						Comorbid conditions		Contact with COVID-19 patients	
	Total	Sample	Male	≤19	20–29	30–39	40–49	50–59	≥60	No	Yes (≥1)	No	Yes
Overall	25 061 939	8902	5059 (56·8%)	151 (1·7%)	1240 (13·9%)	2995 (33·6%)	2612 (29·3%)	1339 (15·0%)	565 (6·3%)	5623/7843 (71·7%)	2220/7843 (28·3%)	5993/8635 (69·4%)	2642/8635 (30·6%)
Ahvaz	1 184 788	367	212 (57·8%)	1 (0·3%)	43 (11·7%)	153 (41·7%)	111 (30·2%)	44 (12·0%)	15 (4·1%)	256/366 (69·9%)	110/366 (30·1%)	272/367 (74·1%)	95/367 (25·9%)
Ardabil	529 374	210	140 (66·7%)	2 (1·0%)	28 (13·3%)	59 (28·1%)	67 (31·9%)	36 (17·1%)	18 (8·6%)	137/208 (65·9%)	71/208 (34·1%)	146/210 (69·5%)	64/210 (30·5%)
Babol	250 217	177	91 (51·4%)	2 (1·1%)	24 (13·6%)	42 (23·7%)	56 (31·6%)	36 (20·3%)	17 (9·6%)	122/177 (68·9%)	55/177 (31·1%)	120/177 (67·8%)	57/177 (32·2%)
Esfahan	1 961 260	542	392 (72·3%)	1 (0·2%)	65 (12·0%)	210 (38·7%)	202 (37·3%)	51 (9·4%)	13 (2·4%)	467/521 (89·6%)	54/521 (10·4%)	482/526 (91·6%)	44/526 (8·4%)
Gorgan	350 676	302	175 (57·9%)	1 (0·3%)	38 (12·6%)	71 (23·5%)	121 (40·1%)	50 (16·6%)	21 (7·0%)	206/301 (68·4%)	95/301 (31·6%)	179/301 (59·5%)	122/301 (40·5%)
Hamedan	554 406	226	122 (54·0%)	4 (1·8%)	40 (17·7%)	72 (31·9%)	62 (27·4%)	39 (17·3%)	9 (4·0%)	87/137 (63·5%)	50/137 (36·5%)	53/134 (39·6%)	81/134 (60·4%)
Kerman	537 718	234	111 (47·4%)	3 (1·3%)	44 (18·8%)	63 (26·9%)	81 (34·6%)	32 (13·7%)	11 (4·7%)	100/151 (66·2%)	51/151 (33·8%)	194/231 (84·0%)	37/231 (16·0%)
Kermanshah	946 651	534	344 (64·4%)	10 (1·9%)	88 (16·5%)	144 (27·0%)	172 (32·2%)	100 (18·7%)	20 (3·7%)	381/525 (72·6%)	144/525 (27·4%)	342/523 (65·4%)	181/523 (34·6%)
Mashhad	3 001 184	903	459 (50·8%)	14 (1·6%)	160 (17·7%)	318 (35·2%)	233 (25·8%)	145 (16·1%)	33 (3·7%)	299/426 (70·2%)	127/426 (29·8%)	534/868 (61·5%)	334/868 (38·5%)
Qom	1 201 158	349	250 (71·6%)	3 (0·9%)	49 (14·0%)	114 (32·7%)	116 (33·2%)	52 (14·9%)	15 (4·3%)	203/344 (59·0%)	141/344 (41·0%)	161/341 (47·2%)	180/341 (52·8%)
Rasht	679 995	244	128 (52·5%)	1 (0·4%)	40 (16·4%)	72 (29·5%)	62 (25·4%)	48 (19·7%)	21 (8·6%)	170/243 (70·0%)	73/243 (30·0%)	144/244 (59·0%)	100/244 (41·0%)
Sanandaj	412 767	193	97 (50·3%)	17 (8·8%)	28 (14·5%)	60 (31·1%)	54 (28·0%)	27 (14·0%)	7 (3·6%)	135/189 (71·4%)	54/189 (28·6%)	143/191 (74·9%)	48/191 (25·1%)
Sari	309 820	323	192 (59·4%)	13 (4·0%)	40 (12·4%)	81 (25·1%)	106 (32·8%)	51 (15·8%)	32 (9·9%)	249/320 (77·8%)	71/320 (22·2%)	214/314 (68·2%)	100/314 (31·8%)
Shiraz	1 565 572	416	256 (61·5%)	2 (0·5%)	51 (12·3%)	191 (45·9%)	103 (24·8%)	37 (8·9%)	32 (7·7%)	292/413 (70·7%)	121/413 (29·3%)	370/413 (89·6%)	43/413 (10·4%)
Tabriz	1 558 693	451	280 (62·1%)	1 (0·2%)	59 (13·1%)	153 (33·9%)	162 (35·9%)	66 (14·6%)	10 (2·2%)	340/444 (76·6%)	104/444 (23·4%)	331/450 (73·6%)	119/450 (26·4%)
Tehran	8 693 706	2793	1390 (49·8%)	59 (2·1%)	318 (11·4%)	994 (35·6%)	731 (26·2%)	422 (15·1%)	269 (9·6%)	1719/2445 (70·3%)	726/2445 (29·7%)	1857/2713 (68·4%)	856/2713 (31·6%)
Urmia	736 224	299	216 (72·2%)	1 (0·3%)	45 (15·1%)	99 (33·1%)	98 (32·8%)	47 (15·7%)	9 (3·0%)	227/294 (77·2%)	67/294 (22·8%)	216/293 (73·7%)	77/293 (26·3%)
Zahedan	587 730	339	204 (60·2%)	16 (4·7%)	80 (23·6%)	99 (29·2%)	75 (22·1%)	56 (16·5%)	13 (3·8%)	233/339 (68·7%)	106/339 (31·3%)	235/339 (69·3%)	104/339 (30·7%)

Data are n, n (%), or n/N (%).

Table 1: **Baseline characteristics of study participants by city**

risk of exposure to COVID-19 and 3530 (39·7%) were recruited from the general population. The first date of data collection was April 17, 2020, in Tehran, and the last date was June 2, 2020, in Zahedan. In 14 cities the data collection was finalised by April 30, 2020.

Table 1 shows the demographic characteristics of participants by city. Overall, 5059 (56·8%) participants were men, most were aged 30–39 years (2995 [33·6%]) or 40–49 years (2612 [29·3%]), 2220 (28·3%) of 7843 with available data had at least one comorbid condition, and 2642 (30·6%) of 8635 reported recent contact with a person confirmed to have COVID-19.

494 individuals tested positive for SARS-CoV-2-specific IgG or IgM antibodies with the ELISA kits in the general population, resulting in a crude seroprevalence of 14·0% (exact binomial 95% CI 12·9–15·2; table 2). After weighting the sample by age, sex, and city population size, and adjusting for test performance, the population weight-adjusted and test-adjusted seroprevalence was 17·1% (14·6–19·5). The seroprevalence estimate for the general population implies that 4 265 542 (95% CI 3 659 043–4 887 078) individuals from the 18 cities in the study were infected by the end of April, 2020.

The highest age-stratified seroprevalence was observed in individuals aged 60 years or older (population weight-adjusted and test-adjusted prevalence 29·2% [21·4–37·5]; table 2). Among 867 individuals with at least one comorbid condition, the test-adjusted prevalence was 22·4% (18·0–27·7). Finally, the population weight-adjusted and test-adjusted seroprevalence estimate in

	Sample size, n	Seropositive participants, n	Seroprevalence, %			
			Crude	Weighted*	Adjusted for test scenario 1	Adjusted for test scenario 2
Total	3530	494	14·0% (12·9–15·2)	12·9% (11·3–14·5)	17·1% (14·6–19·5)	15·8% (13·6–18·2)
Sex						
Male	1795	244	13·6% (12·0–15·3)	12·5% (10·5–14·7)	16·5% (13·4–19·9)	15·3% (12·5–18·5)
Female	1735	250	14·4% (12·8–16·2)	13·2% (11·1–15·3)	17·5% (14·3–20·8)	16·3% (13·3–19·3)
Age, years						
≤19	140	15	10·7% (6·1–17·0)	11·8% (5·1–19·3)	15·4% (5·1–26·9)	14·3% (4·8–25·0)
20–29	470	44	9·4% (6·9–12·4)	7·4% (4·9–10·4)	8·7% (4·7–13·2)	8·1% (4·4–12·3)
30–39	992	117	11·8% (9·8–14·0)	12·1% (9·4–15·1)	15·8% (11·7–20·4)	14·7% (10·9–19·0)
40–49	937	141	15·0% (12·8–17·5)	14·1% (11·3–16·9)	18·9% (14·6–23·2)	17·6% (13·6–21·6)
50–59	590	87	14·7% (12·0–17·9)	15·7% (12·2–19·5)	21·4% (16·0–27·2)	19·9% (14·9–25·3)
≥60	401	90	22·4% (18·5–26·8)	20·8% (15·8–26·2)	29·2% (21·4–37·5)	27·1% (19·9–34·8)
Comorbidity						
Yes	867	156	18·0% (15·5–20·7)	16·4% (13·5–19·8)	22·4% (18·0–27·7)	20·8% (16·8–25·8)
No	1971	257	13·0% (11·6–14·6)	12·7% (10·7–14·9)	16·8% (13·7–20·1)	15·6% (12·8–18·7)
Contact with confirmed COVID-19 patients						
Yes	601	122	20·3% (17·2–23·7)	21·8% (16·6–27·4)	30·8% (22·7–39·3)	28·6% (21·1–36·5)
No	2717	343	12·6% (11·40–13·9)	10·9% (9·5–12·4)	14·0% (11·8–16·3)	13·0% (11·0–15·2)

Seroprevalence data are % (95% CI). *Weighted by age, sex, and city population. When a variable was stratified it was removed from the weights.

Table 2: **Seroprevalence of severe acute respiratory syndrome coronavirus 2-specific IgG and IgM antibodies in the general population**

individuals who reported a contact with a person with confirmed COVID-19 (30·8% [22·7–39·3]) was considerably higher than that in individuals without such contact (14·0% [11·8–16·3]).

In the general population, in an analysis stratified by city, the highest population weight-adjusted and test-adjusted seroprevalence estimates were in the cities of Rasht (72·6% [53·9–92·8]), Qom (58·5% [37·2–83·9]), Gorgan (43·9% [31·4–58·3]), and Babol (22·4% [11·9–35·1]; figures 1, 3; appendix 2 p 7).

Among the 5372 individuals in the high-risk population, 819 individuals tested positive for SARS-CoV-2-specific IgG or IgM antibodies (figure 3). The overall population weight-adjusted and test-adjusted seroprevalence was 20·0% (18·5–21·7; table 3). Similarly to the general population, the highest test-adjusted prevalence estimates in high-risk populations were in Rasht, Qom, Gorgan, and Babol (figure 3; appendix 2 p 8).

In general, the population weight-adjusted and test-adjusted prevalence in the high-risk population did not show any considerable variation among different occupational groups, ranging from 18·0% (14·6–21·6) in non-front-line health-care workers to 22·0% (17·1–26·8) in cashiers of supermarket chains (table 3).

7745 (84·7%) individuals responded to the COVID-19-related symptoms questions in the questionnaire (appendix 2 p 9). Overall, the prevalence of antibody seropositivity among individuals with symptoms was considerably higher than that in individuals with no symptoms. The highest test-adjusted prevalence estimates were in the 608 individuals with self-reported anosmia (75·0% [68·8–81·2]) and the 716 individuals with fever (60·8% [55·3–66·5]).

The test-adjusted prevalence increased in proportion to the number of self-reported COVID-19 symptoms: 14·1% (12·5–15·8) in asymptomatic individuals, 20·1% (17·8–22·6) in paucisymptomatic individuals, and 43·3% (39·6–47·1) in symptomatic individuals (appendix 2 p 9).

1164 (15·0%) of the 7745 who responded to the questions on COVID-19-related symptoms tested positive for SARS-CoV-2 (appendix 2 p 10). Among 1164 antibody-positive individuals, 416 (35·7%) did not experience any COVID-19-related symptoms, suggesting that an estimated 1 522 798 (1 306 278–1 744 686) individuals infected before the end of April were asymptomatic in the total populations of the 18 cities in the study.

Because of the higher test sensitivity (71·8%) in the scenario 2 test performance, the scenario 2 test-adjusted prevalence estimates were lower than the scenario 1 estimates. However, the observed trends for estimated prevalence rates were consistent between the two test performance scenarios in all the analyses (appendix 2 pp 3–6).

Discussion

In this population-based cross-sectional study, the seroprevalence of SARS-CoV-2-specific antibodies was estimated in 18 cities with high population densities from the north, centre, and south of Iran, and it was higher than previous reports from Iran and other countries. In a study in Guilan province in Iran, the test-adjusted seroprevalence was 33% among the five included

counties (Rasht, Anzali, Lahijan, Astara, and Roudbar), and the population weight-adjusted seroprevalence in Rasht county was 24%.[7] By comparison, the population weight-adjusted seroprevalence in the general population for Rasht city in our study (49·1%; appendix 2 p 7) was considerably higher. The observed difference between estimates might be related to different study designs and sampling methods (household *vs* individual level) and the fact that, in our study, individuals were solely recruited from Rasht city and not the entire county. Moreover, the observed variation in prevalence estimates among included cities in this study could be explained by the fact that epidemic protocols were initiated far earlier in some cities than others.[7,10] For instance, Qom and Guilan were the first and second provinces to report increased numbers of cases early in the epidemic,[7] which might have been related to the ongoing trading relationships with Wuhan, China, in January, 2020.

Our overall seroprevalence estimate of 17·1% (adjusted for population weighting and test performance) was also higher than reported US estimates from Santa Clara (2·8%),[4] New York state (14·0%),[10] and Los Angeles (4·1% [unweighted proportion of individuals positive for IgG or IgM antibodies]),[13] and also from Spain (5·0%).[14] The higher seroprevalence estimates in our study might be explained by the timing of epidemic initiation in Iran. Because Iran was among the first countries that reported a SARS-CoV-2 epidemic—earlier than the USA and European countries—a greater proportion of the Iranian population might have been exposed to the virus during the same time period. Furthermore, the higher seroprevalence estimates in our study could be partly related to test characteristics and the low sensitivity of our test compared with tests that were used in other countries. However, as the overall crude seroprevalence estimate among the general population (ie, 14·0%) in our study was still considerably higher than seroprevalence estimates in countries such as Spain, the observed difference between countries is more likely to be related to epidemic conditions and the applied health regulations in each region than to test characteristics.[14]

Consistent with previous reports,[4,7,10,13] we found a substantial difference between the officially reported number of confirmed COVID-19 cases and the seroprevalence-based case estimate. Although the total number of reported cases in Iran is currently more than 800 000 individuals, our estimate of about 4 265 542 individuals infected by the end of April, 2020, suggests that the preliminary SARS-CoV-2 ascertainment rate of 0·6% and the number of cases estimated from simulation studies have underestimated the epidemic conditions in Iran.[4,15–18] Furthermore, consistent with a previous meta-analysis, we found that 35·7% of seropositive individuals in our study were asymptomatic, representing about 1 522 798 individuals.[19] These findings emphasise the importance of prevention strategies such as physical distancing (ie, maintaining a distance of >1·5 m from other individuals) and use of face masks to further protect the general population from SARS-CoV-2 community transmission.

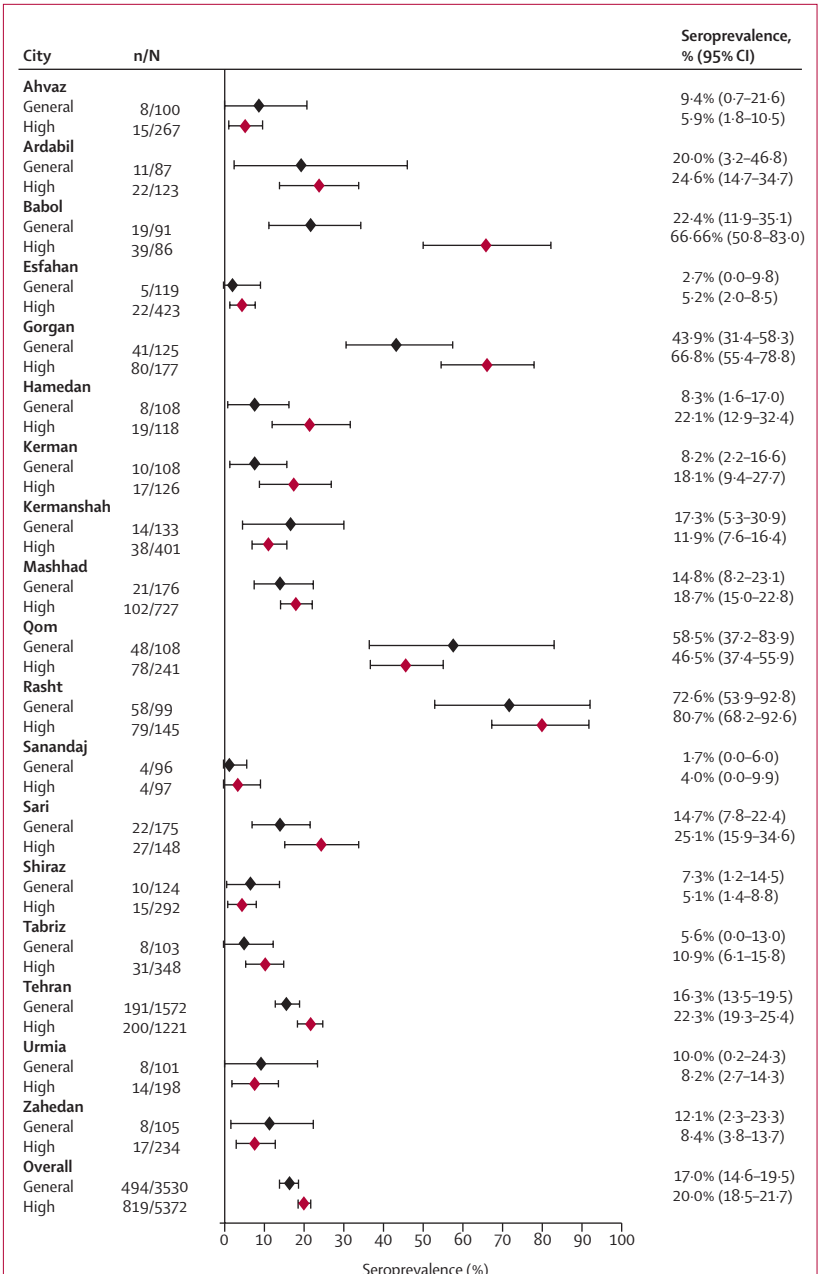

Figure 3: **Seroprevalence of severe acute respiratory syndrome coronavirus 2 in general populations and high-risk populations by city**
All seroprevalence estimates are adjusted for scenario 1 test performance.

As stated by WHO and shown in a few other studies, because of frequent or close social interactions and the possibility of asymptomatic transmission, the risk of SARS-CoV-2 transmission in certain occupations might be increased.[9,20–22] However, consistent with another seroprevalence study from Guilan province in Iran, the seroprevalence estimates in our study did not vary

	Sample size, n	Seropositive participants, n	Seroprevalence, %			
			Crude	Weighted*	Adjusted for test scenario 1	Adjusted for test scenario 2
Front-line doctors and nurses	1245	209	16·8% (14·8–19·0)	15·9% (13·9–18·0)	21·6% (18·6–24·9)	20·1% (17·3–23·1)
Non-front-line health-care workers	1156	162	14·0% (12·1–16·1)	13·5% (11·3–15·9)	18·0% (14·6–21·6)	16·8% (13·6–20·1)
Pharmacy employees	620	101	16·3% (13·5–19·4)	15·5% (12·5–18·6)	21·0% (16·5–25·8)	19·5% (15·3–24·0)
Taxi drivers	718	101	14·1% (11·6–16·8)	14·1% (11·4–16·9)	18·8% (14·7–23·2)	17·5% (13·7–21·6)
Cashiers of supermarket chains	753	110	14·6% (12·2–17·3)	16·1% (12·9–19·2)	22·0% (17·1–26·8)	20·5% (15·9–24·9)
Bank employees	880	136	15·5% (13·1–18·0)	14·2% (12·1–16·5)	19·1% (15·8–22·6)	17·7% (14·7–21·0)
Overall high-risk population	5372	819	15·3% (14·3–16·2)	14·9% (13·8–16·0)	20·0% (18·5–21·7)	18·6% (17·2–20·2)

Seroprevalence data are % (95% CI). *Weighted by city population size.

Table 3: **Seroprevalence of severe acute respiratory syndrome coronavirus 2-specific IgG and IgM antibodies in high-risk populations**

by occupation.[7] The similar seroprevalence estimates between general population and high-risk groups observed in our study might be explained by a low compliance of the general population with applied health regulations (eg, physical distancing), and by inadequate personal protective equipment, including medical devices, in high-risk occupational environments such as hospitals.[20–22] Therefore, the type of occupation could meaningfully contribute to the elevated risk of SARS-CoV-2 exposure if infection control measures were effectively applied to the general population. In the case of insufficient prevention strategies, most people would have a high risk of exposure outside of their working environment, essentially nullifying any safety precautions applied there.

To our knowledge, this is the first multicentre seroprevalence study in Iran to report the seroprevalence of anti-SARS-CoV-2 antibodies in the general population and in individuals employed in occupations with a high risk of exposure across 18 cities. In this study, the overall prevalence estimates were adjusted not only for sex, age, and city population, but also for test performance using different scenarios. Despite these strengths, our study has some limitations that should be noted. First, individuals with a history of COVID-19 symptoms might be more willing to participate in the study, resulting in an inflated proportion of individuals with positive tests and overestimated prevalence. However, because participants were randomly selected from the population and the response rate to the first study invite was high, the potential effect of this limitation on our findings is expected to be low. Second, the Pishtaz Teb SARS-CoV-2 ELISA kits were not fully assessed before this study and required further validation. Additionally, the sensitivities of the tests were lower than those of tests used in other countries.[14] To address these limitations, we did additional testing to assess the test performance under monitored conditions and adjusted all the seroprevalence estimates for the test characteristics. Third, in a few cities, such as Tehran, because of a strict lockdown during the data collection period, we could not recruit participants from some high-risk occupations (eg, pharmacy workers). Therefore, the number of samples was not commensurate with the population of those cities. To resolve this issue and achieve the required overall sample size, more individuals from the high-risk populations of cities with less strict lockdown policies were recruited. However, because of the smaller sample sizes in the cities with strict lockdowns, the estimates for individual cities should be interpreted with caution.

In conclusion, the findings of this study imply that prevalence of seropositivity is likely to be much higher than the reported prevalence rates based on confirmed COVID-19 cases in Iran. Despite the high seroprevalence estimates in a few cities, the low overall prevalence estimates highlight the fact that a large proportion of the population in Iran is still uninfected. The similar seroprevalence estimates across high-risk occupations in this study could indicate that the currently applied infection control measures might be inadequate or not appropriately adhered to or enforced. As such, there is an urgent need for public health policies and adequate personal protective equipment among front-line workers to prevent the potential increase in patient load in hospitals across the country, especially during the second wave of infection.[23]

Contributors

HP, MD, ZM, ASh, SK, and RM contributed to the study design, analysis plan, implementation of the research, and manuscript writing. ASh and SK contributed to sample preparation and laboratory testing. ZM and MSh contributed to data cleaning and analysis. HP, ZM, MSh, and RM had access to all data and verified the data. All others have contributed in implementation, data and sample gathering, and manuscript editing.

Declaration of interests

We declare no competing interests.

Data sharing

The study protocol and individual participant data that underlie the results reported in this Article, after de-identification (text, tables, figures, and

appendices) can be shared with investigators whose proposed use of the data has been approved by the independent review committee of Tehran University of Medical Sciences and Digestive Diseases Research Institute. Data can be provided for individual participant data meta-analysis or other projects comparing the seroprevalence estimates in different regions. The proposals should be directed to the corresponding author at dr.reza.malekzadeh@gmail.com. To gain access, data requesters will need to sign a data access agreement, confirmed by RM as the senior author of the manuscript and the project leader.

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References

1 WHO. Coronavirus disease 2019 (COVID-19) situation report—75. April 4, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200404-sitrep-75-covid-19.pdf?sfvrsn=99251b2b_4 (accessed June 29, 2020).

2 Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis* 2020; **34:** 101623.

3 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323:** 1239–42.

4 Bendavid E, Mulaney B, Sood N, et al. COVID-19 antibody seroprevalence in Santa Clara County, California. *medRxiv* 2020; published online April 30. https://doi.org/10.1101/2020.04.14.20062463 (preprint).

5 Vogel G. Antibody surveys suggesting vast undercount of coronavirus infections may be unreliable. April 21, 2020. https://www.sciencemag.org/news/2020/04/antibody-surveys-suggesting-vast-undercount-coronavirus-infections-may-be-unreliable (accessed June 29, 2020).

6 WHO. Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection. March 17, 2020. https://apps.who.int/iris/bitstream/handle/10665/331656/WHO-2019-nCoV-Seroepidemiology-2020.1-eng.pdf?sequence=1&isAllowed=y (accessed June 29, 2020).

7 Shakiba M, Hashemi Nazari SS, Mehrabian F, Rezvani SM, Ghasempour Z, Heidarzadeh A. Seroprevalence of COVID-19 virus infection in Guilan province, Iran. *medRxiv* 2020; published online May 1. https://doi.org/10.1101/2020.04.26.20079244 (preprint).

8 Popovich N, Sanger-Katz Margot. The world is still far from herd immunity for coronavirus. May 28, 2020. https://www.nytimes.com/interactive/2020/05/28/upshot/coronavirus-herd-immunity.html (accessed June 29, 2020).

9 WHO. Considerations for public health and social measures in the workplace in the context of COVID-19. May 10, 2020. https://www.who.int/publications/i/item/considerations-for-public-health-and-social-measures-in-the-workplace-in-the-context-of-covid-19 (accessed June 29, 2020).

10 Rosenberg ES, Tesoriero JM, Rosenthal EM, et al. Cumulative incidence and diagnosis of SARS-CoV-2 infection in New York. *Ann Epidemiol* 2020; **48:** 23–29.

11 Johns Hopkins University of Medicine Coronavirus Resource Center. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU) https://coronavirus.jhu.edu/map.html (accessed Nov 18, 2020).

12 Efron B. Bootstrap methods: another look at the jackknife. *Ann Stat* 1979; **7:** 1–26.

13 Sood N, Simon P, Ebner P, et al. Seroprevalence of SARS-CoV-2-specific antibodies among adults in Los Angeles County, California, on April 10–11, 2020. *JAMA* 2020; **323:** 2425–27.

14 Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020; **396:** 535–44.

15 Zhuang Z, Zhao S, Lin Q, et al. Preliminary estimation of the novel coronavirus disease (COVID-19) cases in Iran: a modelling analysis based on overseas cases and air travel data. *Int J Infect Dis* 2020; **94:** 29–31.

16 Salomon JA. Defining high-value information for COVID-19 decision-making. *medRxiv* 2020; published online April 8. https://doi.org/10.1101/2020.04.06.20052506 (preprint).

17 Verity R, Okell LC, Dorigatti I, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. *Lancet Infect Dis* 2020; **20:** 669–77.

18 Ghaffarzadegan N, Rahmandad H. Simulation-based estimation of the spread of COVID-19 in Iran. *Syst Dyn Rev* 2020; **36:** 101–29.

19 Byambasuren O, Cardona M, Bell K, Clark J, McLaws ML, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: systematic review and meta-analysis. *medRxiv* 2020; published online Sept 13. https://doi.org/10.1101/2020.05.10.20097543 (preprint).

20 Mutambudzi M, Niedzwiedz CL, Macdonald EB, et al. Occupation and risk of COVID-19: prospective cohort study of 120,075 UK Biobank participants. *medRxiv* 2020; published online May 23. https://doi.org/10.1101/2020.05.22.20109892 (preprint).

21 Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. *JAMA* 2020; **323:** 1406–07.

22 Chu DK, Akl EA, Duda S, et al. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. *Lancet* 2020; **395:** 1973–87.

23 Devi S. COVID-19 resurgence in Iran. *Lancet* 2020; **395:** 1896.