Original Article

Seroprevalence of Anti-SARS-CoV-2 IgG and IgM Antibodies among Government Employees in Iran

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ABSTRACT

The COVID-19 disease emerged in Wuhan, China, in December 2019 and quickly became a global health threat. Around 6,947,192 people have been killed around the world so far, including 146,292 in Iran. In addition to the definitive diagnosis of the disease by RT-PCR, immunological and serological tests can check the anti-SARS-CoV-2 N protein antibody titer in people at different stages of infection with acceptable sensitivity and specificity. The serological examination is an effective and efficient method for determining the prevalence of the disease, especially when asymptomatic cases are present or the diagnosis of symptomatic cases is incomplete. The study examined the seroprevalence of COVID-19 at the Razi Vaccine and Serum Research Institute (RVSRI) and the Agricultural Research, Education, and Extension Organization (AREEO). A total of 493 blood samples were collected from volunteers in June 2020 in AREEO, and 380 samples were collected in June and July 2020 in RVSRI. The total number of volunteers from both organizations was 873. Standard ELISA kits were used to measure IgG and IgM antibodies against SARS-CoV-2. A statistical analysis of the obtained data was conducted using SPSS (version 22.0). Among the total 873 volunteers examined in RVSRI and AREEO, 10.5% had elevated serum titers either for IgM or IgG, 3.55% of whom were women and 6.95% were men. Generally, 8.8% of people tested positive for IgM, which showed a recent infection with COVID-19 in people at that time and partially indicated the start of a new wave of COVID-19. In RVSRI, 3.42% of people with positive IgM titers (positive or negative IgG titers) were women and 5.53% were men. In AREEO, 3.02% were women and 5.72% were men. The seroprevalence rate of COVID-19 in RVSRI was 11.6%, 4.2% of which were women and 7.35% were men, with no significant difference between women and men. The COVID-19 seroprevalence in AREEO was 9.7%, 3.22% of which were women and 6.5% were men, with no significant difference between women and men.

Keywords: ELISA, IgM, IgG, SARS-CoV-2, Seroprevalence

1. Introduction

Coronavirus disease (COVID-19), which is caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), first appeared in China in 2019 and quickly spread throughout the world in 2020. COVID-19 was declared an emergency by the World Health Organization on February 10, 2020 (1), and a pandemic on March 21, 2020 (2). Around 6,947,192 people have been killed worldwide, including 146,292 Iranians. On February 30, 2020, a definitive positive test for Corona infection was reported in the province of Qom for the first time in Iran. It is as critical to understand the epidemiology of a disease as it is to treat the disease to prevent an epidemic from spreading. The rate at which a disease spreads during epidemics is affected by the number of infected individuals, the ability of the virus to transmit, and the number of susceptible individuals (3). The COVID-19 disease can manifest as an asymptomatic, mild disease or as a life-threatening condition that necessitates hospitalization and leads to death (4).

Some individuals may acquire COVID and not display any symptoms, which is called asymptomatic COVID-19. COVID-19 poses several critical challenges, including the role played by asymptomatic individuals in disease transmission (5). In fact, all infected individuals play a crucial role in disease transmission since asymptomatic or mildly symptomatic cases often remain unscreened or do not report their illness to public health authorities (6).

Due to its high sensitivity and specificity, the real-time polymerase chain reaction (RT-PCR) method is considered the gold standard for the detection of the causative COVID-19 virus (7). This method was not widely available across the country until several months after this study was conducted. Additionally, SARS-CoV-2 infection is characterized by a wide range of clinical manifestations, ranging from no symptoms to respiratory distress (8). An individual who is asymptomatic or mildly infected may not be tested, and thus their infection status will remain unknown. Therefore, there is a potential risk of underestimating the magnitude of the SARS-CoV-2 infection in the population.

A valuable tool for predicting the spread of the disease in an epidemic is the evaluation of changes in the disease prevalence over time, which is imperative when deciding on healthcare needs. Serological tests can detect COVID-19 antibodies in individuals, considering that antibodies produced by the immune system may remain in the body for a long time after infection. It provides the possibility of identifying those who have been exposed to the disease, regardless of whether they exhibit symptoms or not, which is extremely valuable for determining the number of infected individuals. There have been several studies performed before and after July 2020 in Iran that have looked at geographical separation (9,10) or certain groups of people (11).

We conducted a study to examine the prevalence of antibody titers in employees of two governmental organizations: a vaccine and serum production facility located in Karaj, Iran, under the name Razi Vaccine and Serum Research Institute (RVSRI) and the Agricultural Research, Education, and Promotion Organization (AREEO) located in Tehran, Iran. Participants in this study included 380 individuals from RVSRI and 483 individuals from AREEO.

2. Materials and Methods

2.1. Sample Collection

This study was conducted at RVSRI, Karaj, Iran, from June to July 2020 to investigate COVID-19 seroprevalence. With their written consent, 380 volunteers from RVSRI were included in the study, 135 of whom were women and 245 were men. In addition, 493 volunteers were also included from AREEO organizations, 155 of whom were women and 338 were men. Blood samples were collected from patients with appropriate personal protective equipment (Table 1).

2.2. ELISA Assay

A sample of clotted blood (2 ml) was collected from each participant. Upon separation, the serum was

Table 1. The prevalence of serum IgG and/or IgM (either or both positive) in each organization and in all volunteers

	IgM or/and IgG positive		
Oraganization	positive	Negative	Total
RAZI	44 (11.6%)	336 (88.4%)	380
AREEO	48 (9.7%)	445 (90.3%)	493
Total	92 (10.5%)	781 (89.5%)	873

stored at -20°C until used. ELISA kits (Pishtaz Teb Co., IR. Iran) were used to detect anti-SARS-CoV-2 IgG and IgM antibodies in serum samples from 873 participants. The sensitivity and specificity of the IgG kit were 94.1% and 98.3%, respectively, according to the manufacturer. After the infection of the IgM kit, the sensitivity was 30.7% for 0-7 days, 85.4% for 7-14 days, and 78.4% for 15 days and more, and the specificity was 99.4%. The 96-well microplate (coated with N protein) was poured with 100 µl of diluted serum (1:50) and held for 30 min at 37°C to detect specific IgM or IgG antibodies. A 100 µl solution of enzyme conjugate was added to each well after washing and then incubated at 37°C for another 30 min. Once the second wash cycle had been completed, 100 µl of the substrate was poured into each well and incubated for 15 min at 25°C. Finally, a stop solution was added to the wells to terminate the reaction. A microplate reader at 450 nm was used to determine the optical density of each well within 30 min.

2.3. Statistical Analysis

Some statistical tests were used to analyze the obtained data using SPSS (version 22.0), including the Chi-Squared and Fisher's Exact tests. The results were considered significant if the *P*-value was less than 0.05.

3. Results

A total of 380 participants from RVSRI, Karaj, Iran, and 493 from AREEO, Tehran, Iran, were enrolled in this study in June and July 2020. SARS-CoV-2 was detected in 10.5% of the participants using IgM and IgG antibody tests (Table 1), and 8.8% of participants from these two governmental organizations had IgM titers. Data and future events confirmed that this COVID-19 outbreak occurred in Iran and that another outbreak had begun (Figure 1). The seroprevalence of COVID-19 among AREEO participants was 9.7%, 3.23% of which were females and 6.5% were males (Figure 2 and Table 1), with no significant difference between women and men ($P>0.05$). COVID-19 seroprevalence at RVSRI was 11.6%, 4.23% of which were females and 7.35% were males, with no significant differences between males and females ($P>0.05$) (Figure 3 and Table 1).

The two organizations had an emerging seroprevalence of COVID-19. In total, 8.8% of people had an IgM titer, and 1.7% had an IgG titer but no IgM titer, indicating a significant increase in recent infections and possibly a new wave in the near future (Figure 1). In terms of the ongoing spread of COVID-19, 8.95% of people at RVSRI had a high IgM titer (and a negative or positive IgG titer), 3.42% of whom were women and 5.53% were men, and at the AREEO, 8.74% of participants were IgM positive (IgG negative or positive), 3% of whom were women and 5.72% were men (Figures 2 and 3). Our data showed no significant difference in COVID-19 outbreak rates between men and women in either organization based on the number of participants (Table 1). A total of 34 people at RVSRI and 43 people at AREEO had elevated IgM titers (positive and negative IgG titers), equal to 8.95% and 8.7% of volunteers, respectively, with no significant differences between the two organizations (Figures 2 and 3).

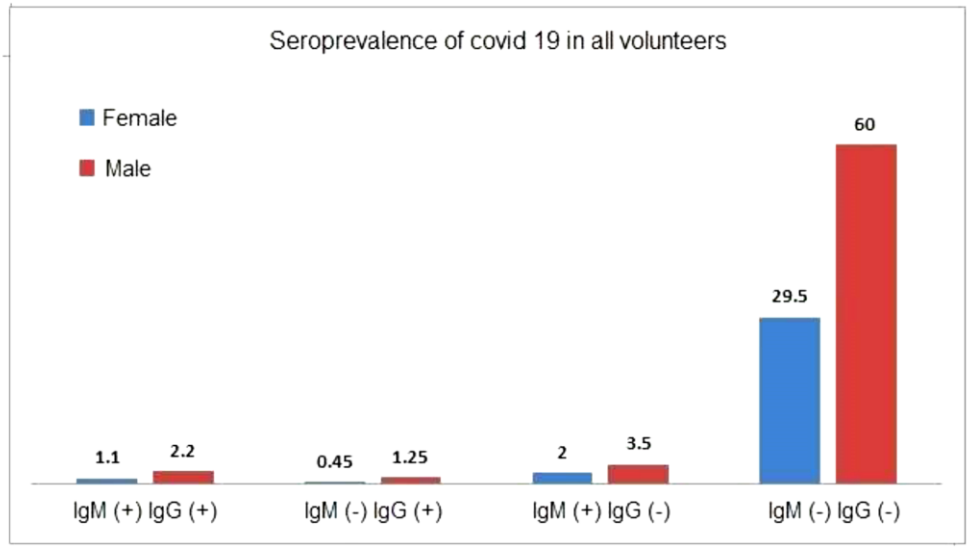

Figure.1. Seroprevalence of Covid 19 in all volunteers by gender

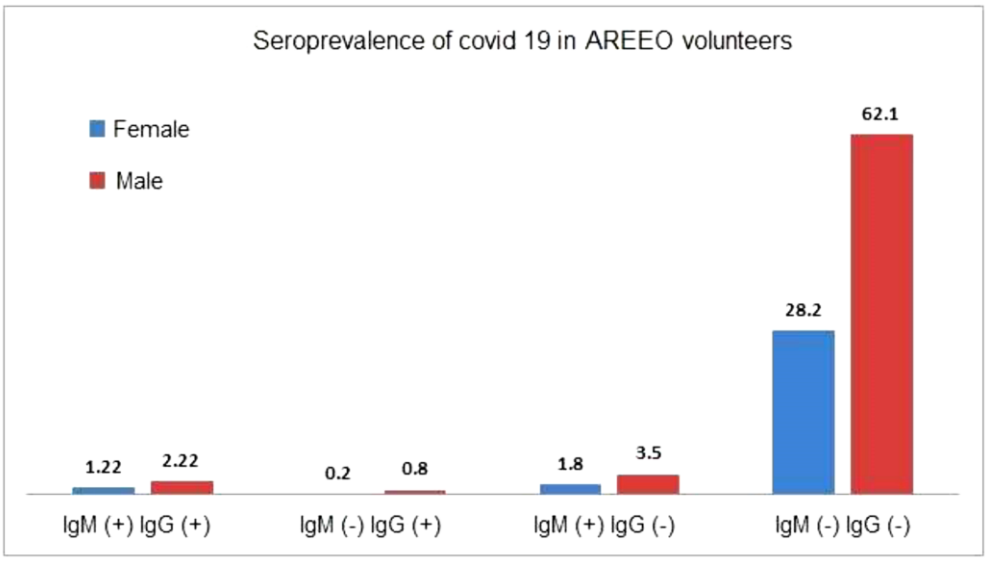

Figure.2. Seroprevalence of covid 19 in AREEO volunteers by gender

Additional tests were performed on these volunteers after an examination by a doctor. Retests were conducted if necessary. IgM antibody titers increased in some cases, requiring the doctor to place those suspected of active infection under home quarantine. About 1.7% of participants had positive IgG and negative IgM titers (Figure 1). Approximately 2.6% of participants from RVSRI (10 people) and 1% of AREEO (5 people) were involved (Figures 2 and 3). After examination by the institute doctor, no signs of an active infection were observed. There was a history of infection in most of these people. They were tested a few months before the study. Based on their medical histories and the process of antibody production in their bodies, it was determined that these people had recovered.

Among all participants, the titers of five people were in the suspicious range. Blood sampling and re-testing were done, and based on the second test results, they were placed in the relevant group.

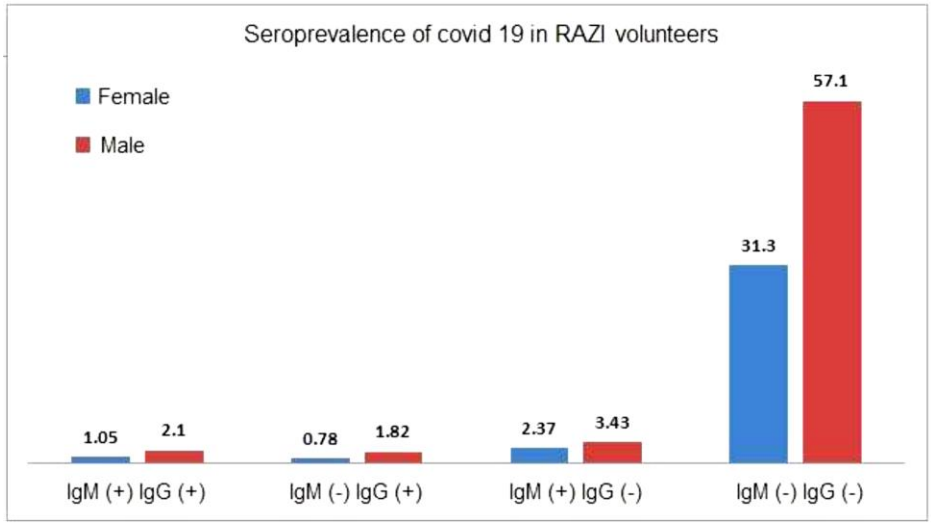

Figure.3. Seroprevalence of covid 19 in RAZI volunteers by gender

4. Discussion

As soon as COVID-19 emerged in Wuhan, China, in December 2019, it became a global threat and a pandemic. Globally, it has killed about **6,947,192** people, including 146,292 Iranians. Moreover, this disease has caused a lot of economic losses in Iran and around the world. Since the outbreak of the disease in Iran, there has been concern about providing the possibility for people to be examined in organizations and offices. This way, if a person has a hidden form of the disease, they enter a quarantine to prevent the spread of the disease. Different studies report varying proportions of asymptomatic infections, ranging from 4% to 41% (12). As a result, rapid screening methods are critical to identifying suspicious people and preventing the spread of the pandemic. RT-PCR is the definitive diagnostic method for the disease, but in August 2020, it was far too expensive and time-consuming for extensive screening of people. While RT-PCR can definitively diagnose Coronavirus infection, immunological and serological tests can detect Coronavirus antibodies in people at various stages of infection. Serological testing is a fast, inexpensive, and powerful way to determine the prevalence, especially in asymptomatic cases (13).

When antibodies are detected in individuals' serum samples, it may indicate that they are infected with the SARS-CoV-2 virus and that their immune system has responded to the virus. The immune system produces antibodies in response to infection; therefore, it is possible to detect these antibodies in the blood of individuals who have already been exposed to infection, whether they are asymptomatic or suffering from symptoms. It may take between one and three weeks for the body to produce antibodies after the first symptoms appear. After infection with SARS-CoV-2, antibodies can develop in about two to three weeks. Serological tests may not detect current SARS-CoV-2 infections or diagnose current COVID-19 infections with certainty. Positive serological test results indicate a past or recent infection, but the non-existence of antibodies in negative serological test results can have many implications. Consequently, it is very critical to interpret personal performance in light of these results. In most cases, negative serological results indicate that a person is not infected with the disease. Even so, the infection cannot be definitively ruled out since the immune system may not have developed a strong antibody response, or there may not have been enough time to produce antibodies while the person is in the window period (antibody production process). The window period is a period following an infection that lasts from one to three weeks.

Those with a positive IgG antibody but no IgM antibody are argued to have produced antibodies against COVID-19. The possibility of a previous infection is also high. Past infections may not cause symptoms in some patients. It is

argued that people with negative IgG and IgM panels may not be infected with COVID-19. One's immune system may actively produce antibodies associated with a recent infection in people with no IgG but a positive IgM panel. Those who have both IgG- and IgM-positive panels are believed to be actively developing antibodies against an infection that began more than 14 days earlier.

The relationship between specific antibody levels and SARS-CoV-2 infection was not well defined at the time of this study. Even though animal challenge studies showed protective antibody responses in the short term, long-term protection for humans requires further studies (14, 15). It is also possible that some tests cross-react with other viruses, such as those responsible for the common cold. As a result, false positive test results may occur. Coronavirus infection does not always lead to detectable antibodies. In some cases, antibody levels may decline over time to undetectable levels. During the early stages of infection, IgM and IgG antibodies are absent. Therefore, serological tests do not definitely indicate SARS-CoV-2 infection (16).

The Ministry of Health in Iran published the seroprevalence of COVID-19 from different provinces during the pandemic every month, based on which the seroprevalence of COVID-19 was 21.2% in June and July 2020 in Tehran province. According to the findings of this study, the seroprevalence rate of COVID-19 was 11.6% in RVSRI, 9.7% in AREEO, and 10.5% in all volunteers, which was lower than the reported seroprevalence of COVID-19 at that time in Tehran (21.2%). As Tehran is close to Karaj, its results can also be applied to Karaj (17). For two key reasons, seroprevalence is lower among employees than in the general population. First, people with a history of illness or infection may not have participated in the study. This is because the disease was unknown at the time and people were unwilling to enter quarantine. Furthermore, government employees may have been instructed to comply with health protocols in offices and are more aware of using masks and complying with health conditions than the average person, making them more likely to protect themselves against virus infections (17). In contrast to AREEO, RVSRI has production departments where people must perform certain production processes in small spaces for long periods, which may explain the lower seroprevalence in AREEO than RVSRI. Based on the statistical results and comparing them to the serological prevalence in the city, it can be argued that increasing public knowledge, observing health protocols, and using methods such as quarantine in cases of disease or suspected cases can significantly reduce the spread of the disease. As shown in figure 1, the rate of newly developed seroprevalence of COVID-19 (positive IgM titer) in the two organizations was 8.8%, which indicates a significant increase in newly developed infections and suggests the possibility of a new wave of COVID-19 in the near future.

In this study, 1.7% of participants had an IgG titer but no IgM titer, indicating that few people were infected during the previous wave. This study was conducted close to the beginning of the COVID-19 outbreak in the country (early February 2020), and thus its ratio to new cases is partly related to the drop in antibody titer over time. Therefore, it can be concluded that the significant difference between the new and old cases of COVID-19 infection indicates that the next wave of infection is likely to be larger. As time passed, this issue became apparent. According to RVSRI data, 3.42% of people with positive IgM titers (positive or negative IgG titers) were women and 5.53% were men, while in the AREEO organization, 3.02% were women and 5.72% were men. In either group, there was no significant difference in the rate of new COVID-19 outbreaks between women and men based on the number of participants.

According to this study, SARS-CoV-2 seroprevalence was 10.5% among RVSRI and AREEO employees in June and July 2020. Serological assays, such as ELISA, have a high degree of ease and capability in screening and examining the general situation of society, which is necessary to make important decisions. The method also demonstrated that it could be used to control infections in small communities and offices.

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Authors' Contribution

Study concept and design: M. D.

Acquisition of data: M. D. and MH. ZJ.

Analysis and interpretation of data: M. D. and MH. ZJ.

Drafting of the manuscript: M. D.

Critical revision of the manuscript: MH. ZJ.

Statistical analysis: M. D.

Ethics

It is declared that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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