

# Seroprevalence of SARS-Cov-2 IgG antibodies in patients at a single center in Saudi Arabia

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**BACKGROUND:** The coronavirus disease 2019 (COVID-19) pandemic has had a massive impact on public health as well as the economy. Understanding the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection among undiagnosed individuals is important for developing an informed pandemic response.

**OBJECTIVE:** Investigate the prevalence of undiagnosed COVID-19 disease.

**DESIGN:** Cross-sectional.

**SETTING:** Tertiary care center in Madinah, Saudi Arabia.

**SUBJECTS AND METHODS:** All participants were on follow-up visits to various clinics and had not been previously diagnosed with COVID-19. Enzyme-linked immunosorbent assay was used to specifically assess the anti-spike IgG antibody seropositivity in serum samples. We associated the seropositivity rates of the participants with age, body mass index (BMI), nationality, blood groups, and sex with uni- and multivariate analyses.

**MAIN OUTCOME MEASURES:** Seropositivity for IgG anti-spike antibodies against SARS-CoV-2.

**SAMPLE SIZE AND CHARACTERISTICS:** 527 subjects, with a median (interquartile percentiles) age of the 527 subjects was 34 (24-41).

**RESULTS:** Of the 527 samples, about one-fourth (n=124, 23.5%) were positive for anti-spike IgG antibody against SARS CoV-2. Age was associated with anti-spike IgG antibody positivity ( $P<.002$ ). Participants >30 years were more likely to be seropositive (28-29%) than younger participants (15.4%). Additionally, seropositivity was associated with female gender ( $P<.001$ ) and a higher BMI ( $P<.006$ ). In the multivariate logistic regression, age >30, female gender and BMI >40 were associated with seropositivity.

**CONCLUSION:** The percentage of seropositive individuals reflects the high level of undiagnosed COVID-19 patients among the population. Our results will help in a better evaluation of the public health measures applied during the COVID-19 pandemic and any future public health crises.

**LIMITATIONS:** Sample size was small, single-center study and no rural areas were included.

**CONFLICT OF INTEREST:** None.

By March 2022, more than 2 years after the initial identification of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the worldwide pandemic has touched almost 224 countries, and affected more than 400 million confirmed COVID-19 cases and almost 6 million deaths. (<https://www.worldometers.info/coronavirus/>, accessed on 2 March 2022). The novel SARS-CoV-2 concerns health care authorities as the virus can be disseminated around the globe through asymptomatic individuals.<sup>1</sup> This concern becomes more important in resource-limited areas with an absence of serological assays<sup>2</sup> and mass testing for SARS-CoV-2.<sup>3</sup> Surveillance data on viral RNA have been unable to determine the number of asymptomatic infections.<sup>4</sup> Based on a meta-analysis, the fraction of asymptomatic individuals was estimated between 20% to 31%.<sup>4</sup> Moreover, reports of asymptomatic infection that relied on serotype detection of anti-N and anti-S viral proteins estimated the prevalence of undiagnosed COVID-19 to be around 21%.<sup>5</sup> Evidence from clinical and population studies suggests that asymptomatic individuals may be as likely as individuals with symptoms to spread SARS-CoV-2 infection.<sup>6,7</sup> Thus, accurate estimation of the undiagnosed COVID-19 disease in populations is essential to build a model that reflects the transmission of the virus globally. In fact, many countries are conducting seroepidemiological studies on COVID-19 to approximate an accurate prevalence of the disease, particularly of asymptomatic cases.<sup>8-10</sup> Performing seroprevalence investigations have several advantages. They help in recognizing the disease burden and measuring the necessity for vaccine coverage and effectiveness. They are furthermore important to estimate the likelihood and effects of re-infections.<sup>11</sup> Additionally, it would be an important tool to measure the sustainability of naturally acquired immunity for those with a confirmed history of infection or asymptomatic individuals.<sup>12</sup>

Several nationwide studies have been conducted in Saudi Arabia concerning asymptomatic individuals. For example, a study conducted in Maternity and Children's Hospital, Madinah, showed that over 58% of children and 50.2% of women were seropositive to SARS-CoV-2 anti-spike IgG.<sup>13</sup> Another national report on the seroprevalence of COVID-19 among blood donors in Saudi Arabia showed a prevalence of 11%, with an apparent disparity between regions.<sup>14</sup> In the current study, we aimed to investigate the prevalence of undiagnosed COVID-19 disease among the outpatients making follow-up visits to the general clinics at the Taibah University Medical Center, Madinah, Saudi Arabia. In addition, the study aimed to investigate the

relationships between the specific IgG antibodies of SARS-CoV-2 with age, sex, nationality, blood groups and body mass index (BMI) among undiagnosed COVID-19 disease subjects.

## SUBJECTS AND METHODS

This study was designed to investigate the seroprevalence of SARS-CoV-2 IgG antibodies in adults who had not been previously diagnosed with COVID-19. Subjects were on follow-up visits to general walk-in clinics at the Medical Center of Taibah University, Madinah, Saudi Arabia. Sampling was by convenience, with subjects responding to posters with information on study objectives and target groups. Contact information was included in the posters for any queries and details concerning the arrangement for the visit to collect the data and blood samples. The study was also advertised through healthcare workers at the medical center. Data were collected between mid-December 2020 to 31 April 2021. The minimum numbers of participants needed for this study were 162 women and 162 men, based on the equation suggested by Charan and Biswas,<sup>15</sup> using  $\alpha=0.05$ , an expected proportion of 30% positive SARS-CoV-2 anti-spike (anti-S) IgG cases, and a precision of 5%.

Subjects had no history of COVID-19 disease (no illness or had not experienced COVID-19 symptoms as self-reported by the subjects). Individuals were excluded if diagnosed previously with COVID-19 disease by RT-PCR, vaccinated (single or multiple doses, self-reported by the subjects) against COVID-19, or had experienced unusual symptoms within the previous two weeks. Additionally, those who were at close contact with confirmed SARS-CoV-2 infected patients and experienced symptoms of the COVID-19 disease were also excluded.

Blood samples (3-5 mL) were collected in plain vacutainer tubes. After coagulation, samples were centrifuged for 10 minutes at 1500g. Sera were then immediately transferred into 1.5 mL tubes and kept at -70°C until assayed. The study was approved by the College of Applied Medical Sciences Ethical Committee with IRB no. MLT /2020/56/217. Informed consent was acquired from all participants prior to inclusion in the study and after a full explanation of the study objectives and procedures.

Enzyme-linked immunosorbent assay was performed according to the published procedure.<sup>16</sup> Briefly, the ELISA 96-well plate was coated with the full-length spike protein antigen of SARS-CoV-2 at 4°C for overnight with 2 µg per well (Sinobiological, China; <https://www.sinobiological.com/>). Plates were then washed 5

times using a washing solution. Diluted serum samples (1:100) were then added to wells at 100  $\mu$ L/well and incubated for 30 minutes at room temperature. Following plate washing, a 100  $\mu$ L/well of alkaline phosphatase conjugate IgG antibody (Sigma Aldrich, United States) was added for 30 minutes. Plates were then washed 5 times and 100  $\mu$ L/well of p-NPP substrate was added and kept in dark for 30 minutes. The reaction was terminated by adding 100  $\mu$ L/well of stopping solution (1.2 N sodium hydroxide, Reagecon, United Kingdom). Plates were loaded in a plate reader (ELX800, BioTek) for optical density measurement at 405 nm.

Data were analyzed using the IBM SPSS, version 23 (IBM Corp, Armonk, NY, USA). Multivariate regression analyses were used to analyze associations among seropositivity and demographic variables. A *P* value  $\leq .05$  indicates statistically significant differences.

## RESULTS

The median (interquartile percentiles) age of the 527 subjects was 34 (24-41) (**Table 1**). The percentage of

males was 50.3%. The majority were Saudi (85.8%) and young adults (18-40 years: 68.9%). About one-third were either obese (28.8%) or morbidly obese (6.1%). The overall seropositivity of the specific anti-spike IgG SARS CoV-2 antibody was 23.5% (n=124).

In a univariate analysis, age group was associated with anti-spike IgG antibody positivity ( $P < .002$ ) (**Table 2**). Participants of 30 years of age or older were more likely to be seropositive (28-29%) than younger participants (15.4%) (**Figure 1**). However, anti-spike antibody levels as reflected by measured optical density were similar across different age groups.

Participants with a BMI  $>30$  were more likely to be seropositive than other groups ( $P < .006$ ) (**Figure 2**). Conversely, anti-spike antibody levels as presented by measured optical density were similar across different BMI groups. The anti-spike IgG antibody seropositivity was associated with female gender ( $P < .001$ ). In addition, the mean (SD) level of spike IgG antibody was significantly ( $P < .01$ ) higher in females compared to males (1.86 [0.11] vs 1.35 [0.12], respectively). The distribution

**Table 1.** Characteristics of participants (n=527).

Age	
12 to < 18	6 (1.1)
18 to <30	195 (37.0)
30 to $\leq$ 40	168 (31.9)
>40	158 (30.0)
Gender	
Female	261 (49.5)
Male	266 (50.5)
Nationality	
Saudi	452 (85.8)
Non-Saudi	75 (14.2)
Body mass index	
<18.5	26 (4.9)
18.5 to <25	151 (28.7)
25 to <30	173 (32.8)
30 to <40	145 (27.5)
$\geq$ 40	32 (6.1)
Antibody test result	
Positive	124 (23.5)
Negative	403 (76.5)

Data are n (%).

**Table 2.** Demographic and clinical factors associated with seropositivity.

	Positive	Negative	P value
Age groups (years)			
18 to <30	31 (15.4)	170 (84.6)	.002
30 to <40	47 (28.0)	121 (72.0)	
$\geq$ 40	46 (29.1)	112 (70.9)	
Gender			
Male (n=266)	43 (16.2)	223 (83.8)	.001
Female (n=261)	81 (31.0)	180 (69.0)	
Nationality			
Saudi (n=452)	103 (22.8)	349 (77.2)	.199
Non-Saudi (n=75)	21 (28.0)	54 (72.0)	
Body mass index			
<25	32 (18.4)	142 (81.6)	.006
25 to <30	35 (20.7)	134 (79.3)	
30 to <40	43 (28.3)	109 (71.7)	
$\geq$ 40	14 (43.8)	18 (56.3)	
Blood group			
A	39 (24.1)	123 (75.9)	.888
B	21 (25.6)	61 (74.4)	
AB	4 (18.2)	18 (81.8)	
O	60 (23.0)	201 (77.0)	

Data are n (%).

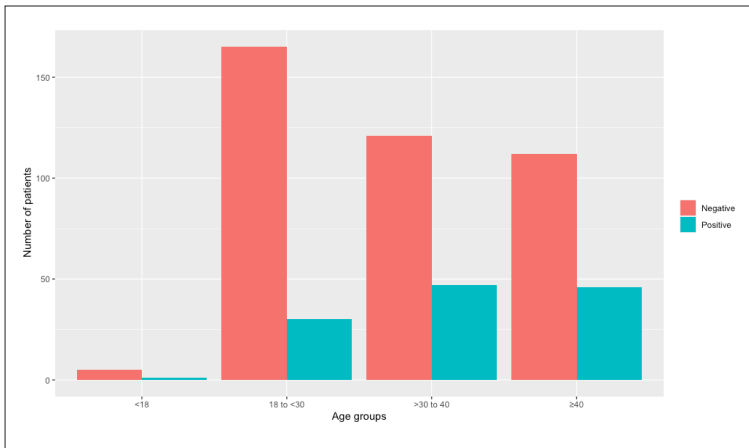


Figure 1. Number of seropositive subjects by age groups ( $P<.002$ ).

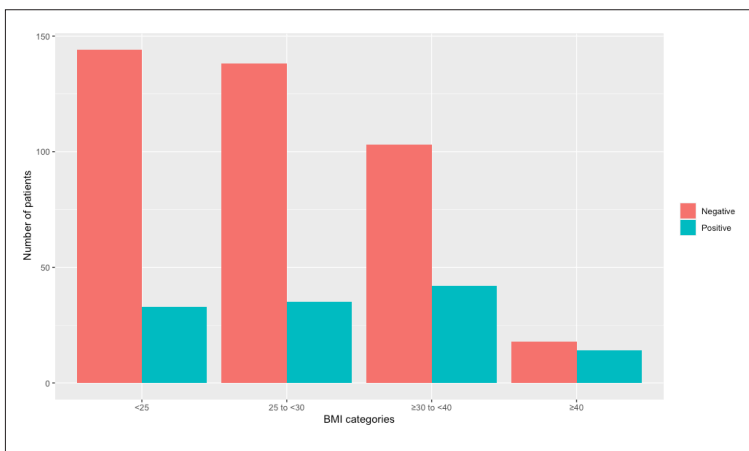


Figure 2. Seropositivity by BMI category.

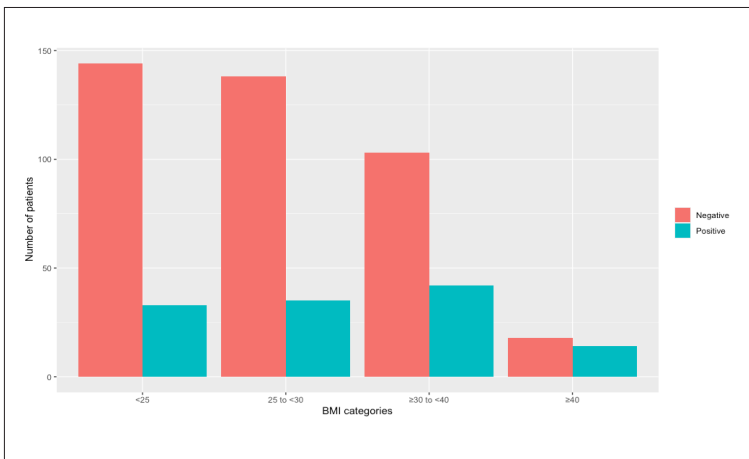


Figure 3. Age distribution by gender for seropositive subjects.

of seropositivity by gender is shown in **Figure 3**. Blood group and nationality had no effect on seropositivity and anti-S IgG levels were detected (data not shown). In the multivariate logistic regression analysis, female gender, age older than 30 years, and a BMI of more than 40 were associated with seropositivity (**Table 3**).

## DISCUSSION

Timely identification of undiagnosed COVID-19 patients is vital for disease control in fighting against the novel virus. Presently, the pandemic of SARS-CoV-2 is ongoing with high morbidity and significant mortality. Inadequate pre-existing immunity to the novel virus is assumed to lead to more cases.<sup>17</sup> There is a necessity for high sensitivity as well as high specificity in the serological assays to recognize the amount of infection in populations.<sup>18</sup> We conducted the current study as input to Saudi Arabia's national serosurvey, considering the frequency of SARS-CoV-2 antibodies in Madinah. We inspected the seroprevalence among participants who were presenting for follow-up visits at the Medical Center of Taibah University and who had no previously confirmed diagnoses or symptoms of COVID-19 disease. Our data showed that 23.5% of the participants were seropositive for anti-spike IgG antibodies even though they had not been diagnosed with COVID-19. Nevertheless, this might not be an exact approximation of the fraction of undiagnosed COVID-19 disease in the overall population. For that reason, the proportion of undiagnosed COVID-19 disease needs to be determined through population screening.<sup>19</sup> Furthermore, the percentage of undiagnosed COVID-19 disease would be greater as many infected people possibly will be neglected by polymerase chain reaction (PCR) testing. Thus, it would be preferable to perform a timely PCR in addition to serological testing to more precisely evaluate the undiagnosed COVID-19 disease fraction.<sup>20</sup>

Numerous studies have reported asymptomatic patients in small cohorts.<sup>21-23</sup> For example, at the stage of viral screening, nearly 57% SARS-CoV-2 cases in Washington state of the United States were asymptomatic.<sup>24</sup> About 45% of SARS-CoV-2 cases remained undiagnosed.<sup>24</sup> As a control measure for public health, serological and clinical features of asymptomatic carriers are not well-studied.<sup>25</sup> Undiagnosed persons with COVID-19 disease are unintentionally accountable for SARS-CoV-2 transmission within communities. We showed in our study that a quarter of the subjects were seropositive against the viral spike protein. Our finding is similar to our previous serosurvey study on blood donors where about 19% of blood donors who were

undiagnosed COVID-19 disease were seropositive to the anti-spike protein antigen.<sup>23</sup> However, other local studies showed different seroprevalence percentages among those undiagnosed with COVID-19 disease. A recently released study showed an overall seroprevalence estimation of about 11% in Saudi Arabia (5.1% in Riyadh, 1.5% in Jazan, 18.4% in Qassim, 20.8% in Hail, 14.7% in the eastern region [Alahsa governorate], and 18.8% in Makkah.<sup>14</sup> Makkah and Madinah are the two Holy cities in Saudi Arabia and share similar characteristics in terms of pilgrimages and visitors. Therefore, that could be a reason for the similar seroprevalence of anti-spike antibody percentage. Another national study showed that the seroprevalence of anti-spike IgG antibodies for the country was 1.4% (12/837).<sup>26</sup> That large difference between our study and the latter could be due to the sample size of the earlier study, as it consisted of only 73 samples from Medina (4.1% seropositive) and was conducted over 6 days in May 2020 during the early days of the pandemic. These variations between the studies could be due to the sensitivity of the immunoassays used in the studies. Additionally, it could be due to the date of the studies as that will have an impact on the reported outcomes.

Interestingly, we found that seropositivity in females was significantly higher than in males. Our result is in agreement with several studies that showed high seropositivity for COVID-19 antibody among undiagnosed females compared to undiagnosed males.<sup>27,28</sup> In addition, female patients are more likely to develop long COVID-19.<sup>29,30</sup> Immune response variations have also been described among male and female patients with COVID-19, which might account for these differences in seroprevalence and long-term disease.<sup>29</sup>

We found that participants with a BMI of 40 or more had a higher seropositivity rate than subjects with a BMI under 40. Consistent with our finding, a retrospective study in adult COVID-19 symptomatic patients showed that patients with BMI >30 were more likely to be hospitalized for acute and critical care when compared with those of BMI <30.<sup>31</sup> Therefore, further investigations to correlate the BMI with antibody levels in undiagnosed COVID-19 disease individuals are required.

Remarkably, SARS-CoV-2 can be powerfully transmitted albeit no symptoms are seen in asymptomatic carriers.<sup>32</sup> Collectively, scientific reports have confirmed a high percentage of undiagnosed COVID-19 disease carriers, thus supporting extensive viral screening specifically in at-risk peoples. Early recognition of undiagnosed COVID-19 disease individuals may be accomplished by methodical screening in an intensified surveillance system.<sup>27</sup> The study participants were recruited from the

**Table 3.** Factors associated with seropositivity among undiagnosed COVID-19 subjects (n=521).

	B	P value	Adjusted odds ratio (95% CI)
Age			
18-<30	Reference		
30-<40	.801	<b>.005</b>	2.228 (1.278-3.884)
≥40	.828	<b>.004</b>	2.289 (1.309-4.004)
Gender			
Male	Reference		
Female	.908	<b>&lt;.001</b>	2.479 (1.607-3.823)
Nationality			
Saudi	Reference		
Non-Saudi	.161	.592	1.174 (.653-2.113)
Body mass index			
<25	Reference		
25-<30	-.067	.816	.935 (.531-1.646)
≥30-<40	.214	.459	1.238 (.703-2.180)
≥40	.869	<b>.043</b>	2.385 (1.030-5.523)
Blood groups			
A	Reference		
B	.095	.772	1.100 (.578-2.092)
AB	-.232	.699	.793 (.244-2.571)
O	.002	.993	1.002 (.620-1.620)

Multivariate logistic regression. Model summary measures: deviance 536.715, omnibus test of coefficients (chi-square 38.342,  $P < .001$ ), Cox Snell R square 0.070, Nagelkerke R square 0.106

Health Center at Taibah University. The center provides medical services to faculty members, employees, students, and their families despite of their nationality, sex, and educational level. Therefore, the study population represents to a good extent the adult population in the Madinah region, Saudi Arabia. However, including more health centers from the city in future investigations will provide better representations of the Madinah population. In addition, the study included adult subjects. Expanding the investigation to include children is recommended in future investigations.

In conclusion, the seropositivity rate reveals the degree of undiagnosed COVID-19 patients among the studied population. According to our findings, undiagnosed COVID-19 disease represents a high fraction of the Madinah population. Further investigations to include more individuals and different regions in the country are required. Moreover, the present results and



those of others add more information to the scientific community for a better evaluation of the public health measures applied during the COVID-19 pandemic and during any future public health crises.

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