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## Screening for SARS-CoV-2 IgM and IgG antibodies among healthcare workers: A single-center study in Egypt

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### Abstract

Coronavirus disease 2019 (COVID-19) pandemic has become a global public health disaster, spreading throughout the world. In order to accurately determine the extent of the pandemic, it is important to accurately identify the prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection among healthcare workers (HCWs). This study intended to determine the prevalence of SARS-CoV-2 infection among HCWs and examine its correlation with the demographic characteristics of the study participants prior to the implementation of the vaccination campaign. In this cross-sectional study included 431 HCWs from Suez Canal University Hospital in Ismailia, Egypt. Their sera were screened for SARS-CoV-2 antibodies using a one-step novel coronavirus (COVID-19) IgM/IgG antibody test from Artron, Canada. Positive cases were then confirmed using nasal swab real-time reverse transcriptase PCR from Viasure, Spain. Of the 431 study participants, 254 (58.9%) were males and 177 (41.1%) females. The majority of participants, 262 (60.8%), were younger than 30 years old, 150 (34.8%) between 30 and 40 years old, and only 19 (4.4%) older than 40 years old. Out of the total samples, 26 (6%) tested positive for SARS-CoV-2 IgM, while 19 (4.4%) tested positive for both IgM and IgG. The majority of the samples, 386 (89.6%), tested negative for both IgG and IgM. There was no association between the prevalence of SARS-CoV-2 and either sex or age of study participants. In conclusion, during the study period, the prevalence of SARS-CoV-2 infection among healthcare workers at Suez Canal University Hospital in Egypt was relatively low. Additionally, there was no significant correlation observed between the prevalence of positive cases and either age or sex.

**Keywords:** Novel coronavirus, SARS-CoV-2 IgM, SARS-CoV-2 IgG, immune response, antibodies screening, rapid test

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## Introduction

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and is a newly identified viral disease that was first identified in Wuhan, China, in December 2019.<sup>1</sup> Due to the high levels of contagion, the severity of the illness, and the number of countries affected, the World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020.<sup>2</sup>

The range of disease manifestations caused by COVID-19 has surprised the world, with symptoms ranging from mild to severe pneumonia, acute respiratory distress syndrome, and even death.<sup>3</sup> However, there are several reports indicating that many individuals may be carriers of the virus without displaying any symptoms for several weeks.<sup>4,5</sup>

In order to safely end shutdowns and control the spread of COVID-19, many public health authorities in various countries have emphasized the importance of implementing a systematic program for mass diagnostic testing. Seroprevalence surveys can be a valuable tool in this effort, as they utilize serology tests to detect antibodies against the virus. Antibodies are produced in response to infections. By conducting serologic assays, it is possible to estimate the infection rates of the population, including individuals who may have had mild or asymptomatic infections or who were never tested despite showing symptoms. Such surveys are currently being conducted in numerous locations worldwide.<sup>6</sup> Serologic assays estimate population-based infections, including mild/asymptomatic cases or untested symptomatic individuals. Ongoing investigations are underway globally.

Healthcare workers (HCWs) are the frontline workforce for clinical care of suspected and confirmed COVID-19 cases. Consequently, they are presumably exposed to a higher risk of acquiring the disease than the general population.<sup>7</sup> Understanding the risk factors of SARS-CoV-2 infection during clinical setting is urgently needed, which, not only provides the HCWs with essential guidance of self-protection, but also helps policy makers to

formulate appropriate measures to control infection in the hospital setting. Therefore, the main objective of this study was to assess the prevalence of SARS-CoV-2 antibodies among HCWs who have been in contact with COVID-19 patients.

## Subjects and Methods

### *Study population*

The Research Ethics Committee of the Faculty of Medicine, Suez Canal University reviewed and approved the study protocol (reference no, 4437, May 2021). A total of 431 HCWs were recruited from Suez Canal University Hospital, Ismailia, Egypt starting from June 20 till August 16, 2022.

### *Methods*

Sera of all study subjects were tested for SARS-CoV-2 antibodies (IgG and IgM) using rapid screening, one step novel coronavirus (COVID-19) IgM/IgG antibody tests (catalog number A03-51-322, Artron, Canada), according to manufacturer's instructions.<sup>8,9</sup>

### *Principle of the assay*

The assay is a qualitative in vitro test that detects IgM/IgG antibodies to SARS-CoV-2. A positive result indicates past exposure to the virus, but not necessarily an ongoing infection. Positive IgM or IgM/IgG results suggest a recent infection, which should be confirmed by nasal swab PCR. The test uses immobilized antibodies on a nitrocellulose strip and colloidal gold conjugated to COVID-19 antigens. When specimen and assay buffer are added, antibodies, if present, bind to COVID-19 conjugates, forming an antigen-antibody complex that migrates through the strip. If the complex meets the corresponding immobilized antibody, a reactive result is confirmed by a burgundy-colored band. Absence of a colored band indicates a non-reactive result. Specimen types can include whole blood, serum, or plasma.

HCWs who tested antibody positive were examined by nasal swab SARS-CoV-2 polymerase chain reaction (PCR), using real-

time (RT)-PCR detection kits (VIASURE, CerTest Biotec, Zaragoza, Spain), according to manufacturer's instructions. The assay is intended for the specific detection of SARS-CoV-2 in nasal swabs of patients with clinical presentation suggestive of COVID-19. RNA was extracted from respiratory samples, amplified using the commercial RT-PCR kits. The isolated RNA was transcribed generating complementary DNA by reverse transcriptase. This was followed by amplification of a conserved region of ORF1ab and N genes for SARS-CoV-2 using specific primers and a fluorescent-labeled probe. ORF1ab refer to two open reading frames, ORF1a and ORF1b, found in genomes of coronaviruses, encode polyproteins essential for virus replication, transcription, and other processes and, N genes, nucleocapsid, a type of structural protein of coronavirus, is a structural protein that plays a critical role in the assembly of new viral particles. The real-time PCR detection system (CFX96 Real-Time System C 1000 Thermal Cycler, Biorad, Switzerland) was used to detect fluorescent reporter dye probes specific for SARS-CoV-2. The thermal cycling conditions were 15 min at 45 °C for reverse transcription, 2 min at 95 °C for PCR initial denaturation, 10 sec at 95 °C for denaturation and 50 sec at 60 °C for annealing/extension (data collection) and 1 cycle at 95 °C and 45 cycles at 95 °C and 60 °C, according to the instructions of the RT-PCR kits manufacturer. Positive and negative controls were included in each run to generate valid results.

#### Statistical Analysis

Data were tabulated using a statistical spreadsheet program and analysis of these data was done by using Statistical Package for the Social Sciences (SPSS) version 20 software (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov was used to verify the normality of distribution of variables. Comparisons between groups were evaluated using Chi-square test (Monte Carlo). ANOVA was used for comparing between the different categories. Significance of the obtained results was judged at the 5% level.

## Results

All 431 study subjects were stratified according to their ages into 3 age groups: under 30 years old, between 30 and 40 years old and older than 40 years. A total of 262 individuals (60.8%) were under 30 years old, 150 (34.8%) between 30 and 40 years old while only 19 (4.4%) were greater than 40 years old. The age ranged between 20 and 70 years old, with mean  $\pm$  SD of  $29.53 \pm 5.99$  and median of 28 years. Of the 431 study subjects, 254 (58.9%) were males and 177 (41.1%) females.

A total of 386 (89.6%) individuals tested negative for SARS-CoV-2 antibodies (IgG and IgM). The cases who tested positive for IgM only were 26 (6%) while 19 (4.4%) tested positive for both IgG and IgM. Regarding the distribution of studied subjects according to their occupation, 290 (67.3%) belonged to nursing jobs either supervisor or nurse, 27 (6.3%) hospital service workers and 94 (21.8%) physicians and 20 (4.6%) secretary workers (Table 1).

**Table 1.** Distribution analysis of the 431 studied cases.

	No. (%)
Sex	
Male	254 (58.9%)
Female	177 (41.1%)
Age (years)	
<30	262 (60.8%)
30 – 40	150 (34.8%)
>40	19 (4.4%)
Min. – Max.	20.0 – 70.0
Mean $\pm$ SD.	29.53 $\pm$ 5.99
Median (IQR)	28.0 (26.0 – 32.0)
Results	
Negative	386 (89.6%)
Positive IgM	26 (6%)
positive IgG & IgM	19 (4.4%)
Occupation	
Nursing	290 (67.3%)
Service workers	27 (6.3%)
Physicians	94 (21.8%)
Secretary	20 (4.6%)

Table 2 shows the relational analysis between the previous results and the demographic data of study subjects. There was no relation between the prevalence of SARS-CoV-2 infection with either age or sex. Of the 386 individuals with negative antibodies to SARS-CoV-2, 222 (57.5%) were males and 164 (42.5%) females. Of the 26 IgM positive cases, 18 (69.2%) were males and 8 (30.8%) females. And, of the 19 HCWs IgM and IgG positive cases, 14 (73.7%) were males and 5 (26.3%) females ( $p > 0.05$ ).

Regarding age groups, of the 386 individuals with negative antibodies to SARS-CoV-2 231 (59.8%) were under 30 years, 139 (36%) in the 30-40 age group while 16 (4.1%) were more

than 40 years old. For the IgM positive cases, 20 (76.9%) were under 30 years, 5 (19.2%) in the 30-40 age group while only one (3.8%) was older than 40 years old.

Finally, the double positive IgG and IgM group had 11 (57.9%) HCWs under 30, 6 (31.6%) between 30 and 40 while only 2 (10.5%) greater than 40 ( $p \geq 0.05$ ). The mean age  $\pm$  SD of the negative SARS-CoV-2 antibodies group was  $29.53 \pm 5.72$ , for the IgM positive group,  $28.23 \pm 6.26$  while for the double positive IgM and IgG group  $31.16 \pm 9.88$  ( $p \geq 0.05$ ). To conclude, there was no difference between the 4 occupation groups regarding SARS-CoV-2 exposure (Table 2).

**Table 2.** Relation between results and demographic data of the 431-study sample.

Demographic data	Results			p value
	Negative (n = 386)	Positive IgM (n = 26)	Positive IgG & IgM (n = 19)	
Sex				
Male	222 (57.5%)	18 (69.2%)	14 (73.7%)	NS
Female	164 (42.5%)	8 (30.8%)	5 (26.3%)	
Age (years)				
<30	231 (59.8%)	20 (76.9%)	11 (57.9%)	<sup>MC</sup> NS
30 – 40	139 (36%)	5 (19.2%)	6 (31.6%)	
>40	16 (4.1%)	1 (3.8%)	2 (10.5%)	
Min. – Max.	20.0 – 70.0	22.0 – 55.0	22.0 – 65.0	NS
Mean $\pm$ SD	$29.53 \pm 5.72$	$28.23 \pm 6.26$	$31.16 \pm 9.88$	
Median (IQR)	28.0 (26 – 32)	26.50 (25 – 28)	28.0 (25 – 32)	
Occupation				
Nursing	257 (66.6%)	20 (76.9%)	13 (68.4%)	<sup>MC</sup> NS
Workers	25 (6.5%)	1 (3.8%)	1 (5.3%)	
Doctors	86 (22.3%)	4 (15.4%)	4 (21.1%)	
Secretary	18 (4.7%)	1 (3.8%)	1 (5.3%)	

$\chi^2$ : Chi square test, MC: Monte Carlo, F: F ANOVA test.  $P > 0.05$  is not significant (NS).

## Discussion

This work aimed to evaluate the seroprevalence of SARSCoV-2 antibodies among HCWs in Suez Canal University Hospital in Ismailia, Egypt. This sample of individuals represented high risk group as they were serving confirmed positive COVID-19 patients. The IgM seroprevalence was 6% among the 431 HCWs, and 4.4 % were both IgG and IgM positive. This result suggests that the community prevalence of the disease is much lower than HCWs percentage of the

infection which is still so far from the 67% herd immunity percentage which is expected to achieve the protection against the pandemic spread.<sup>10</sup> There was no correlation with either age, sex, or specific health worker occupation. Our results showed that the HCWs seroprevalence was higher but still close to those predicted by the WHO which expected the seroprevalence of SARS-CoV-2 infection to be about 2-3% of the whole population.<sup>11</sup> This could be due to different factors including different sample population nature, high

exposure rate to infection because of contacting COVID-19 patients regularly. In addition, other factors could also contribute to this difference including less compliance to safety precautions measures especially under the insufficiency conditions of personal protective equipment, including face shield, eye, and body protection, which occurred at the beginning of the pandemic as well as different cultural practices, higher social interaction and finally, the variable diagnostic sensitivity of the used assay. Probably, these infections included cases with no or only mild symptoms which did not require medical consultation. This highlights the importance of public screening plans to detect infective cases with no apparent symptoms and hence limit the spread of the disease. False negative or false positive rapid screening test results could still be obtained, which could probably lead to underestimation or overestimation of the true seroprevalence value of SARS-CoV-2 infection among those individuals, respectively. Our study data are, to our knowledge, the first estimates to be assessed among HCWs in Ismailia region, Egypt.

A study conducted in Spain to assess the seroprevalence of SARS-CoV2 infection among 578 HCWs starting from March 28 till April 9, 2020, found that 9.3 % of the assessed individuals were seropositive for IgM and/or IgG and/or IgA. Their results are even higher than ours and this could be related to the different burden of the pandemic in Spain than in Egypt in addition to possible different diagnostic sensitivity of the utilized diagnostic techniques.<sup>12</sup>

Surprisingly, a cross-sectional study was done among 635 HCWs of an Indian teaching university hospital in Kerala region, they demonstrated 0% seroprevalence. As the authors explained, this could be due to the strict infection control and risk management measures they were conducting, and which was acknowledged by the WHO.<sup>13</sup>

Another recent study, performed in 364-bed hospital in New York, USA, during March and April 2020 showed the seroprevalence of SARS-CoV-2 infection among HCWs to be around 10% which is again higher than our results. This could be explained by the higher sample size of

the tested population in addition to the higher burden of disease in the USA. Moreover, like our results, they found no differences regarding sex, age, or specific occupation in the hospital.<sup>14</sup>

Similarly, a study performed on 2992 HCWs in a hospital in California, during May and June 2020 reported a seroprevalence of 1.13%. However, they found statistically significant differences regarding age but not for specific occupations. This low estimate could be explained by the originally low community prevalence (approximately 4.4%) during the investigation period in addition to other factors such as good risk management measures.<sup>15</sup>

Other studies which were done in other areas in USA, used different diagnostic techniques as well as different study subjects' inclusion criteria, other than HCWs. For example, a study assessed the seroprevalence of SARS-CoV-2 infection in San Francisco, California found a seroprevalence rate of 2.5% to 4.2% which is also close to our results.<sup>16</sup>

Another retrospective, cross-sectional study in New York city started from February till April 2020 and collected more than 5000 samples, demonstrated seroprevalence results of 19.3% which is much higher than ours. One possible reason for this result could be the higher sample size which could represent more ideally the true percentage of the infection.<sup>17</sup> One more study, also in New York state, has investigated samples collected during April 2020 and found a high incidence, reaching 22.7% of the tested population.<sup>18</sup> The higher incidence rate estimated from this study could be due to different sample population sources (grocery stores) in addition to the larger sample size.

Whether the presence of SARS-CoV-2 antibodies makes the infected persons immune or not is still controversial and to which extent and until when is also another mystery.<sup>19</sup> This needs higher sample size studies to be conducted on different populations and to measure the SARS-CoV-2 IgG titer through quantitative ELISA technique to allow monitoring of the titer, and to determine whether it will fade or keeps its same level throughout several months after the first estimation date. This will help to identify the persistence period of these antibodies and



hence its protective effects on the immune system.

Moreover, the exact mechanism of humoral antibody response against SARS-CoV-2 is still unclear and the exact time of emergence of its specific antibodies is also controversial. Some researchers reported that the median duration of SARS-CoV-2 IgG specific antibodies development to be about 14 days after the initial infection.<sup>20, 21</sup>

Finally, while this study could provide additional data for the HCWs seroprevalence of SARS-CoV-2 infection in Egypt. Further multicenter studies with larger sample size and longer duration are required to better clarify the true burden of the disease in our area in addition to the kinetics of humoral immune system against novel viruses.

In conclusion, the seroprevalence of SARS-CoV-2 antibodies was relatively low and agreeing with other studies worldwide. Only a few differences were detected which could be due to different sample size, geographically different burden of the disease, different infection control protocols, different compliance to these measures and/or finally to different cultural practices. Non-significant correlation with age, sex or specific occupation was found which could suggest recommending implementation of extensive screening plans regardless for those factors.

### Author Contributions

All authors contributed equally in this work including the collection of samples, laboratory work, manuscript writing, statistical analysis and paper supervision.

### Declaration of Conflicting Interests

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### Ethical approval

The Research Ethics Committee of the Faculty of Medicine, Suez Canal University reviewed and approved the study protocol (reference no, 4437, May2021).

### Informed consent

A verbal informed consent was obtained from all participants before being included in the study.

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