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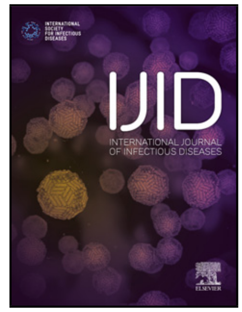

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Dramatic Rise of Seroprevalence Rates of SARS-CoV-2 Antibodies among Healthy Blood Donors: The evolution of a Pandemic

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Highlights

- Seroprevalence rates of SARS-CoV-2 provide important epidemiological data
- Dramatic rise form 0% to 27.4% of seroprevalence rates in blood donors
- The dramatic rise reflects widespread intracommunity transmission

- Seropositive blood donors are mostly young, 18-40 year old

Abstract

Background: Seroprevalence studies of SARS-CoV-2 antibodies are useful in assessing the epidemiological status in the community and the degree of spread.

Objective: To study the seroprevalence rates of SARS-CoV-2 antibodies among healthy blood donors in Jordan, at various points of time as the pandemic evolves in the community.

Methods: A total of 1374 blood donor were tested for the SARS-CoV-2 total immunoglobulin antibodies in 3 groups.

The first and second group: 746 and 348 individuals were tested in June and September-2020 respectively. The 3rd group of 292 were tested in February-2021. We utilized a qualitative assay (specificity: 99.8%, sensitivity: 100%).

Results: The first 2 groups (January-September, 2020) when the number of confirmed Covid-19 cases were several hundred-3000 showed a seroprevalence rate of 0% (95% CI 0.00%-0.51%). The 3rd group (early February 2021), when the number reported confirmed case has reached 100 folds that of September 2020, revealed a seroprevalence of 27.4% (95% CI 22.5%-32.9%).

Conclusions: a dramatic rise in seroprevalence of SARS-CoV-2 antibodies was seen among healthy blood donors in Jordan in parallel with wide-spread intracommunity transmission of the disease. This information is useful to assess the degree of herd immunity and provides for better understanding of the pandemic.

Key words: Covid-19, seroprevalence, blood donors, SARS-CoV-2 antibodies

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has resulted in more than 106 million cases of confirmed infection and more than 2.3 million deaths worldwide as of February 11th 2021 (European Centre for Disease Prevention and Control, 2021).

Population based Seroprevalence studies are extremely important to understand the evolution of the pandemic and to estimate infection rates and prevalence. They are important for calculating absolute risks of the infection as well as death rates and to predict the spread of the virus in communities based on the level of herd immunity after infections and or vaccination. They are also important for planning and monitoring the impact of implementation and relaxation of epidemic mitigation policies (Busch and Stone, 2021)

The true prevalence of the infection is believed to be several times more than the number of PCR confirmed cases because of the large number of asymptomatic infections and or mild infections that went untested especially early in the pandemic

(Huang et al., 2020, McLaughlin et al., 2020, Busch and Stone, 2021). The ratio of estimated to reported infections can range up 12.5 (Bajema et al., 2020).

Numerous population seroprevalence studies were conducted (Bajema et al., 2020, Lai et al., 2020, Chughtai et al., 2020, McLaughlin et al., 2020, Naranbhai et al., 2020, Sam et al., 2021, Sutton et al., 2020, Menachemi et al., 2020, Silveira et al., 2020, Kar et al., 2021, Vena et al., 2020 ,Pollán et al., 2020, Bogogiannidou et al., 2020, Poustchi et al., 2020., Shields et al., 2020, Ng et al., 2020, Figueiredo-Campos et al., 2020, Stringhini et al., 2020, Havers et al., 2020, Ho et al., 2020, Xu et al., 2020, Qutob et al., 2020, Capai et al., 2020 ,Sood et al., 2020, Godbout et al., 2020, Rostami et al., 2020) in efforts to estimate the true prevalence of the COVID-19 infection.

The largest seroprevalence study was the one by Bajema et al. from the USA which showed by September 2020 that the estimated population seroprevalence to be less than 10% in the majority of tested communities, although it ranged from 0 up to 23.3% in the highest hit areas. Obviously the wide variation reflects the level of transmission in the tested communities and number of PCR confirmed reported cases. (Bajema et al., 2020).

Those population- based seroprevalence studies varied in the targeted populations tested and the recruitment strategies used which may explain some of the variation. Among the populations tested are healthy blood donors (Sughayer et al., 2020, Slot et al., 2020, Nesbitt et al., 2021, Gallian et al., 2020, Olariu et al., 2021, Younas et al., 2020, Banjar et al., 2021, Uyoga et al., 2021, Busch and Stone, 2021, Martinez-Acuña et al., 2020, Saeed et al., 2021, Slot et al., 2020, Fiore et al., 2021). Again the seroprevalence among healthy blood donors and other population groups studied varied according to the community tested and the time of testing in terms of the pandemic evolution (Lai et al., 2020, Rostami et al., 2020)

In this longitudinal study we aimed to estimate the seroprevalence of Covid-19 among healthy blood donors in Jordan at different points in time to assess the degree of community spread and herd immunity and to further understand the evolution of the pandemic. This, to our knowledge is the first longitudinal seroprevalence study in blood donors and the first from Jordan.

Methods:

Study population:

The study population consisted of 3 groups of healthy blood donors. The distribution of the donors in terms of the period of donation are detailed in Table 1. The first group were randomly chosen from donors attending the blood bank between January and June 2020. There were no specific criteria based on which they were chosen. We aimed to include donors from January and February 2020 even though the first case of Covid-19 in Jordan was recorded in March of the same year. The number for each month was arbitrarily chosen. The smallest number in the first group was in June because the first group was tested in the first week of June. The second and third group were randomly chosen in the first half of September and late January-early February respectively. The second group were chosen at that period to reassess the situation at the beginning of full society opening that started in early September. The 3rd group were chosen at that period to assess the impact of COVID-19 first wave that peaked in January of 2021.

Samples:

Left over sera and or plasma collected routinely during the process of blood or apheresis platelet donations were used for the study. The donors were healthy asymptomatic subjects between the ages of 18 and 63 who underwent routine screening to determine their acceptability for donation as per standard practice. The sera were tested in 3 batches at 3 different points in time. The first batch consisting of 746 samples representing the period of January to June 2020 was tested in June of that year. The second batch of 348 samples were collected and tested in September of 2020 while the third batch of 292 samples were collected during the period of January 28-Februay 5, 2021 and tested in the same period. Thus the total number of donors tested in the study was 1374.

Testing Methodology:

The samples were tested according to the manufactures recommendations using a commercially available FDA approved kit for total immunoglobulins against SARS-Cov-2. The test was performed on Cobas 6000 or Cobas Pro, Roche analyzers, using the Elecsys-Anti-SARS-CoV-2 kit (Roche Diagnostics GmbH, Mannheim, Germany). This test is a qualitative assay that uses an electrochemiluminescence method (ECLIA) which is an immunoassay for the in vitro qualitative detection of total antibodies (IgA, IgM and IgG) to SARS-CoV-2 in human serum and plasma. The assay uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV-2. The analyzer automatically calculates the cutoff based on the measurement of 2 calibrators. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff). If the COI is < 1.0 then the assay is non-reactive (negative for anti SARS CoV 2 antibodies) and if COI is ≥ 1.0 then the assay is considered reactive (positive for anti SARS CoV 2 antibodies).

The test was validated by the manufacturer using 5272 samples including blood donors, diagnostic routine, other corona viruses and common cold panels. The specificity was determined to be 99.8% (95% CI, 99.7%-99.9%) while the sensitivity was 100% (95% CI, 88.3%-100.0%).

In house validation using serum samples obtained from previously RT-PCR- confirmed Covid-19 infected patients who have recovered at least one month prior to sampling was performed at 2 different times: in June using 4 samples and in September using 6 more samples.

Statistical methods:

Descriptive statistics were used to analyze the results. Chi square statistics were used to compare the seropositive donors' characteristics versus the seronegative ones. Student's T-test was used to compare the mean ages of the seropositive and seronegative donors. We adjusted the estimated crude prevalence rate to the test's sensitivity and specificity using an online tool: http://www2.univet.hu/users/jreiczig/CI4prevSeSp/calc02/index.php as. per methods described by Lang and Reiczjkl (Lang and Reiczigel, 2014).

Results:

The in-house validation of the 10 samples from known confirmed previously infected patients reveled positive results for the presence of total anti SARS-CoV-2 nucleocapsid antibodies.

The demographics of the donors and the period of donation are summarized in table 1. The donors were mostly males (86%) and from the capitol city of Amman, Jordan (78%).

The first group was previously reported in a preprint (Sughayer et al., 2020) and revealed along with the second group a seroprevalence rate of 0% in each group (95% CI 0.00%, 0.51%).

The third group on the other hand which represents the most recent period of January 28- February 5, 2021, showed a positive serological test for the SARS CoV-2 in 80 of 292 donors; a crude seroprevalence rate of 27.4%. The adjusted estimated seroprevalence rate is 27.3% (95% confidence intervals 22.5% and 32.9%). The age of the entire group ranged between 18 and 65 years. The mean age of the seronegative donors and seropositive donors were (Mean ±SD) 31.4 ±9.75 and 29.7 ±8.56 respectively. There was no statistically significant difference in the mean age of the 2 groups (p: 0.13). The demographics and characteristics of the seropositive donors in comparison to the seronegative ones are shown in Table 2. Most of those who tested positive (85%) were in the age group of 18-40 years. However there was no statistically significant difference between the seropositive and negative donors in terms of gender, age, blood group or residence. Males and females were almost equally affected (27.6% vs 26.3%).

One fifth of the seropositive donors were retrospectively found to have been confirmed positive for the Covid-19 infection by the PCR test. Forty seven (58.8%) were not known to have the disease and so did not undergo or underwent a PCR test with negative result. There was no information with regards to previous infection for 17 of the seropositive donors.

Discussion:

The importance of serological testing for the SARS CoV-2 antibodies have been previously highlighted (Busch and Stone, 2021, Raoult, 2021). Among the advantages of such testing is the understanding of the evolution of the pandemic in terms of having a rough estimation of the prevalence of the infection. This will serve health planners and decision makers to properly enforce or relax mitigation measures. And most importantly at these times as vaccines are being rolled out it serves in estimating the risk rates for the infection, the degree of herd immunity and helps prioritizing vaccine recipients. .

In this study we measured the seroprevalence rates in healthy blood donors at three points in time. The results are striking in that they show a dramatic change from 0% early and in the middle of the pandemic, up to 27.4% in February of 2021. These findings would be reasonable if we consider the cumulative number of confirmed cases around these times in Jordan. Figure 1 shows the cumulative daily cases of Covid-19 in Jordan. In the first period up till June 2020 there were only several hundred confirmed cases which increased gradually to around 3000 cases in early September (WHO, 2021). However towards the end of September and afterwards the cumulative number of cases started a steep rise so that on Februay 5, 2021 the cumulative number of confirmed cases was more than 100 folds that of September 2020. It is worth mentioning that a strict lockdown was in effect till early June that was gradually relaxed over three months with full opening of all sectors including schools and international travel in September 2020. It is clear that the first wave of the Covid-19 pandemic in Jordan has actually started in late September when the community was fully open and the intracommunity spread became evident. Before that the several hundred cases were actually limited to transmission within known specific hot foci. This explains the extremely low seroprevalence initially found in June and September of last year, as the infection transmission was under strict control with quarantine routinely imposed on all contacts of index cases.

The current (early February 2021) crude seroprevalence rate of 27.4%, if can be generalized to the population at large would mean that the number of cases is roughly 2.7 million in a population of 10 million in Jordan. This, if true, means that there are 8 cases for every confirmed case. This high ratio of estimated to reported cases is similar to some of the highest ratios reported by Bajema et al. in the USA (Bajema et al., 2020). Looking at it from a different angle we can also see that in our rather small sample one fifth of the donors were previously confirmed positive by the PCR test so we may assume a ratio of estimated to reported cases to be 5 at the lowest estimate.

Most of our seropositive donors (85%) were young; below 40 years of age. Other studies have shown inconsistent findings with either similar age group distribution to our cohort or the opposite with higher rates in older individuals (Bajema et al., 2020 ,Martinez-Acuña et al., 2020, Vena et al., 2020, Olariu et al., 2021). Never the less this finding regarding the age helps in setting the vaccination priority for those older than 40 years.

Our findings are also in line with previous studies which showed that early in the pandemic the seroprevalence of Covid-19 in blood donors or other nontargeted populations was very low ranging from 0% to 2.0% (Erikstrup et al., 2021, Godbout et al., 2020, Qutob et al., 2020, Xu et al., 2020, Ho et al., 2020, Banjar et al., 2021, Nesbitt et al., 2021Slot et al., 2020, Fiore et al., 2021, Saeed et al., 2021). However other studies especially those from hardly hit communities early in the pandemic showed relatively high rates up to 23% (Percivalle et al., 2020).

Our current high seroprevalence rate (as of early February 2021) is similar to that of communities that were hardly affected by the pandemic such as that of New York City and Chelsea, Massachusetts (Bajema et al., 2020, Naranbhai et al., 2020).

With regards to blood group association with decreased risk for Covid-19 infection in blood group O (Gallian et al., 2020) our limited data did not reveal such an association.

Our study to our knowledge is the first longitudinal study of covid-19 seroprevalence in healthy blood donors. It shows the evolution of the pandemic and the dynamics and hidden magnitude of the problem.

There are some limitations in our study including the small number of the blood donors in the 3rd group. In addition the blood donors are mostly males and of younger age groups. However the age groups represented in our study constitute around 60% of the Jordanian population, the rest being mostly children under the age of 18. Thus it may be difficult to draw generalization as there may be selection bias and nonrepresentation of the entire population. Further studies are recommended to include children and the elderly.

In conclusion a dramatic rise in seroprevalence of SARS-CoV-2 from almost 0 to 27.4% in healthy blood donors was seen as the Jordanian community entered into the peak of the first wave of COVID-19. An estimate of the true prevalence is achieved through this longitudinal serological study leading to much better insight and understanding of the evolution of the pandemic in Jordan.

Several gaps are present, however. These include the limitations previously mentioned especially the small number of the donors and the bias of age and gender and the lack of full representation of the entire community.

Nevertheless, we think this study represents an important addition to our means of controlling and fighting COVID-19.

The authors declare no conflict of interest.

No external funding was obtained.

Ethical approval:

The institutional review board (IRB) at King Hussein Cancer Center approved the study.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure 1

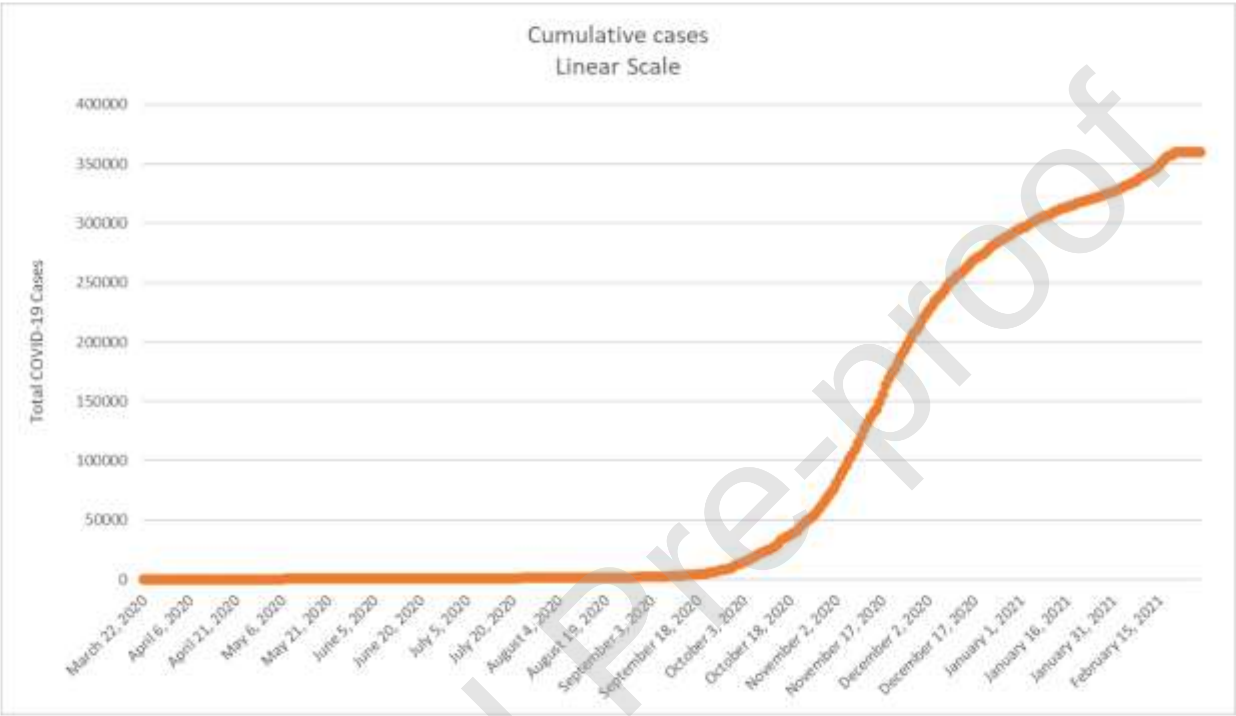
Cumulative cases
Linear Scale
Total COVID-19 Cases
400000
350000
300000
250000
200000
150000
100000
50000
0
March 22, 2020
April 6, 2020
April 21, 2020
May 6, 2020
May 21, 2020
June 5, 2020
June 20, 2020
July 5, 2020
July 20, 2020
August 4, 2020
August 19, 2020
September 3, 2020
September 18, 2020
October 3, 2020
October 18, 2020
November 2, 2020
November 17, 2020
December 2, 2020
December 17, 2020
January 1, 2021
January 16, 2021
January 31, 2021
February 15, 2021

Table 1

Demographics of the 1374 Healthy Donors- No. (%)	
Male	1177(85.7%)
Female	197 (14.3%)
Residential area: Amman West Amman	1077 (78.4%) 706 (65.6%)
East Amman	371 (34.4%)
District outside Amman North	297 (21.6%) 128 (9.3%)
Center	138 (10.1%)
South	31 (2.3%)
Period	
January-2020	104 (7.6%)
February-2020	89 (6.5%)
March-2020	90 (6.6%)
April-2020	147 (10.7%)
May-2020	229 (16.7%)
June-2020	75 (5.4%)
Septemper-2020	348 (25.3%)
28 Jan- Feb 5th, 2021	292 (21.3%)

Table 2

Comparison of seronegative and seropositive donors

Category		Seropositive donors 80 of 292	Seronegative donors 212 of 292	p-value	Crud Prevalence Rate for Seropositive Donors
number of donors		80 (%)	212 (%)		27.4%
Male		70 (87.5%)	184 (86.8%)	0.9	27.6%
Female		10 (12.5%)	28 (11.8%)		26.3%
Age (yr)	18-30	47 (58.8%)	116 (54.7%)	0.4	28.8%
	31-40	21 (26.3%)	52 (24.5%)		28.8%
	41-50	11 (13.7%)	24 (11.3%)		31.4%
	51-65	1 (1.2%)	11 (5.2%)		8.3%
	Unknown	0	9 (4.2%)		
Blood group:	O	33 (41.3%)	88 (41.5%)	0.5	27.3%
	A	23 (28.8%)	73 (34.4%)		24.0%
	B	15 (18.7%)	37 (17.5%)		28.8%
	AB	9 (11.2%)	14 (6.6%)		39.1%
Rhesus blood type:	+	70 (87.5%)	194 (91.5%)	0.3	70 (26.5%)
	-	10 (12.5%)	18 (8.5%)		10 (35.7%)
Residential Location	North	7 (8.8%)	33 (15.6%)	0.3	17.5%
	Center including Amman	68 (85.0%)	168 (79.2%)		28.8%
	South	5 (6.2%)	11 (5.2%)		31.3%

History of past-Covid-19: PCR-Confirmed past infection	16 (20%)	0	NA	NA
Negative/not done PCR	47 (58.8%)	180 (84.9%)		
No information available	17 (21.2%)	32 (15.1%)		