Original article

Antibody profile in symptomatic/asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected Saudi persons

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected persons could be symptomatic or asymptomatic. Asymptomatic and symptomatic patients can transmit SARS-CoV-2. This study aimed to study the humoral immune response in Saudis who are Covid-19 symptomatic and asymptomatic patients. We created three types of enzyme-linked immunosorbant assays (ELISAs) to reveal IgG and IgM antibodies (Abs) against SARS-CoV-2. The developed ELISAs were designed to detect Abs against SARS-CoV-2 N, S and N + S proteins. A number of Covid-19 symptomatic (153) and asymptomatic (84) RT–PCR-confirmed patient sera were used to evaluate the ELISAs and to determine the IgG and IgM antibody profile in those patients. The sensitivity and specificity of these ELISAs were evaluated using pre-Covid-19 pandemic serum samples. The results revealed the existence of anti-SARS-CoV-2 IgG and IgM Abs in Covid-19 symptomatic and asymptomatic Saudi persons. The use of SARS-CoV-2 N and S proteins in the same ELISA greatly increased the detectability of infection. In conclusion, the Covid-19 symptomatic and asymptomatic Saudi persons demonstrated both IgG and IgM antibody profile with higher titer in symptomatic patients. The use of N + S proteins as antibody capture antigens greatly increased the ELISA sensitivity.

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1. Introduction

The newly emerged Covid-19 pandemic is brought about by a virus named the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is one of the Coronaviridae family, (Ge et al., 2020). Covid-19-infection is characterized by fever, vomiting, fatigue, dry cough, dyspnea, diarrhea, and smell/taste loss (Mohamud et al., 2020). There are some works done on the symptom severity manifested by Covid-19-infected patients and few data is obtainable about the antibody profile of SARS-CoV-2-

positive asymptomatic. Asymptomatic type of SARS-CoV-2-infection is defined as a SARS-CoV-2-infected person who is confirmed positive for viral nucleic acid by RT-PCR testing with no clinical symptoms (indicated above). Several researches stated that asymptomatic SARS-CoV-2 infections play a significant part in the disease transmission (Zhang et al., 2020), so, one of the best controls of the Covid-19 pandemic is to early identifying and quarantine asymptomatic persons.

Several studies demonstrated the detection of immunoglobulin (Ig) M (IgM) and IgG and few studied IgA against SARS-CoV-2 (Grossberg et al., 2021; Xiao et al., 2020). Investigating the anti-SARS-CoV-2 antibody profile in Covid-19 patients is a need to characterize the advancement of the disease, distinguish patients with mild symptoms or asymptomatic, detect delayed symptom beginning, and anticipate expected long haul immunity (Asselah et al., 2021).

In symptomatic Covid-19-infected persons, the kinetic of the IgG and IgM is antibodies (Abs) is studied by many instigators (Cervia et al., 2021; Zhao et al., 2020). At the very early stage of

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infection, there are undetectable antibody levels (Wang et al., 2020). It was stated that IgM Abs are detectable four days post Covid-19 symptom onset and then decline to imperceptible levels after one month. IgG Abs are detectable one week post infection, peak around 25 days and keep raised (Lou et al., 2020). Until the moment, RT-PCR is the only method to detect the asymptomatic. Numerous elements influence the results of the RT-PCR coming about in about 30% bogus negative rates (Cai et al., 2020).

It is crucial to create serological assays, and improve the early detection of asymptomatic Covid-19 persons to create more reasonable procedures for the control of the pandemic. This better settled through studding the elements of IgG and IgM Abs reactions in the asymptomatic Covid-19 infections. In this study, we investigated RT-PCR confirmed asymptomatic and symptomatic Covid-19 infected Saudi persons and determined their IgG and IgM antibody profile.

2. Material and methods

2.1. Samples

Research experiments conducted in this article were reviewed and approved by the Research Ethics Committee at King Khalid University (HAPO-06-B-001). All examinations were done following all guidelines, regulations, legal, and ethical standards as required for human. Patient informed consent was not a need as we got coded serum samples without patient information.

Study samples included symptomatic (n = 153) and asymptomatic (n = 84) Covid-19-infected patient sera that are affirmed positive by RT-PCR. Stored serum samples several years before the emergence of the Covid-19 pandemic were used as negative control for Covid-19-infection. Some of these samples are healthy blood donors (n = 100) and some are other viruses infected (hepatitis B virus (HBV, n = 50), hepatitis C virus (HCV, n = 50), and cytomegalovirus (CMV, n = 50).

2.2. Detection of IgG/IgM anti-SARS-CoV-2 antibodies using commercial ELISA

All samples used in this work were investigated for harboring anti-SARS-CoV-2 Abs. The detection of IgM and IgG anti-SARS-CoV-2 nucleoprotein (N) Abs was done using Human COVID-19 Nucleocapsid (N) IgM ELISA Kit (Cat: MBS3809907, MyBioSource.com) and Human COVID-19 Nucleocapsid (N) IgG ELISA Kit (Cat: MBS3809906, MyBioSource.com) respectively. The detection of IgM and IgG anti-SARS-CoV-2 spike protein (S) Abs was done using COVID-19 Human IgM IgG Assay Kit (Cat: KA5826, Abnova). The detection of IgG and IgM anti-SARS-CoV-2 N + S proteins were done using Human Anti-2019 nCoV(N + S) IgG ELISA Kit (Cat# MBS7608199; MyBioSource.com) and Human Anti-2019 nCoV(N + S) IgM ELISA Kit (Cat# MBS7608200; MyBioSource.com) respectively. All ELISAs were run following each corresponding manufacturer's instructions.

2.3. Detection IgG/IgM anti-SARS-CoV-2 antibodies using in-house ELISA

2.3.1. Preparation of the ELISA plates

For the preparation of in-house ELISA assays, each well of the ELISA plates (Flat bottom, UltraCruz® ELISA Plate, Santa Cruz Biotechnology) was coated with 50 μL carbonate/bicarbonate coating buffer (12.5 mM Sodium bicarbonate, 87.5 mM Sodium carbonate, pH 9.6, Techno Pharmchem) containing 4 μg/mL protein. The first plate group was coated with SARS-CoV-2 N recombinant protein (Cusabio Technology LLC., China), the second plate group was

coated with SARS-CoV-2 S-RBD recombinant proteins (Cusabio Technology LLC., China) and the third group was coated with both N and S proteins at 4 μg/mL protein each. Plates were kept at 4 °C for 17 h, washed one time utilizing wash buffer (0.05% (v/v) Tween-20 in phosphate-buffered saline (PBS, 0.0027 M KCl, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.0018 M and KH_2PO_4, pH 7.4 (PBST), all from Sigma-Aldrich) and 200 μL blocking buffer (PBST + 2% bovine serum albumin (BSA, Sigma- Aldrich) were added to each well. Plates were kept at ambient temperature for 3 h followed by two washes and then kept at −20 °C till use.

2.3.2. Standardization of serum and secondary antibody dilutions

To determine the optimal serum dilution to be used in detecting IgG/IgM Abs against SARS-CoV-2, ten anti-SARS-CoV-2 Abs positive and ten anti-SARS-CoV-2 Abs negative sera were serially diluted (1:2, 1:10, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600 and 1:3200) in blocking buffer. The conjugates, goat anti-Fc fragment of human IgG horse radish peroxidase (HRP) enzyme-conjugated secondary Abs and goat F(ab')2 anti-human IgM Abs (Novus Biologicals) were tested at the dilutions 1:10,000, 1:20,000, 1:30,000 and 1:50000 to detect human IgG and IgM respectively.

Two sets of the plate groups were used to discover human IgG and IgM Abs against SARS-CoV-2 separately. Each serum sample dilution (in duplicates, 100 μL/well) was added to corresponding wells of each plate group (N, S or N + S). The plates were kept at 37 °C for 55 min and then washed thrice with PBST. To corresponding wells of the first set (N, S or N + S coated plates), different dilutions (100 μL/well) of goat anti-human IgG Fc fragment HRP-conjugated secondary Abs were added, and goat F(ab')2 anti-human IgM secondary Abs (100 μL/well of each dilution) were added to separate wells of the second set. All plate sets were kept at 37 °C for 45 min followed by 5 extensive washes. To all wells, substrate (3,3′,5,5′-Tetramethylbenzidine, 100 μL/well, Sigma-Aldrich) was added and plates were kept in the dark for 11 min. When the color in wells developed, the reaction was stopped utilizing 1 N HCl (100 μL/well) and color intensity of each well was measured at 450 nm via the 800™ TS, BioTek spectrophotometer. The best serum dilution was used in later work.

2.3.3. Detection of IgG/IgM anti-SARS-CoV-2 antibodies in all samples

All serum samples (symptomatic/asymptomatic Covid-19-positive, healthy blood donors, HBV-positive, HCV-positive, and CMV-positive) were diluted 1: 400 in blocking buffer. Anti-SARS-CoV-2 N, S, and N + S IgG/IgM Abs were detected in all diluted sera using the same method described above.

2.4. Checking of the assay specificity and sensitivity

To check the specificity of the in-house prepared ELISA plate groups, all Covid-19-infected sera and all other viruses (HCV, HBV and CMV)-infected sera were compared for their reactivity toward N, S, and N + S proteins found in different coated plates. The investigation included both antibody types (IgG and IgM) against SARS-CoV-2. The sensitivity of the in-house prepared ELISA plate groups was checked by serially diluting (1:2–1:32000) all serum samples under test (negative and positive) in blocking buffer. All serum dilutions were examined for the harboring of IgG and IgM Abs directed to SARS-Cov-2.

3. Statistical analysis

All experimental data outcomes were expressed as the medians (interquartile ranges, IQRs), and group characteristics were expressed as number (%). Significant differences between each two groups were specified utilizing the Mann–Whitney *U* test

using the SPSS V20 software (SPSS Inc., Chicago, Ill., USA). A p value < 0.01 is considered as significant.

Cut-off value was calculated according to Wiederschain (Wiederschain, 2009) as the mean OD value at a wavelength of 450 nm of negative controls plus 3 value of their standard deviations (SD). The sensitivity of all in house made ELISA was determined according to Ráez-Bravo et al. (Ráez-Bravo et al., 2016) as follows:

100 (The number (no.) of true positive samples/the total no. of true positive samples + false negatives).

The specificity of all in house made ELISAs was determined as follows:

100 (The no. of true negative samples/the total no. of true negative samples + false positives).

4. Results

4.1. Anti-SARS-CoV-2 IgG/IgM antibodies using commercial ELISA

Anti-SARS-CoV-2 IgG/IgM antibody positivity was checked using ready-to-use commercial ELISA kits. Results are summarized in table 1.

4.2. Optimal dilution of serum and secondary antibodies

Covid-19-positive and negative sera were diluted up to 1:3200. Negative sera showed no faint background starting from the dilution 1:4. The positive sera showed good positive reaction up to 1:1600, then turned faint positive at dilution 1:3200. We used the dilution 1:400 in subsequent work to avoid missing of weekly positive samples. The goat F(ab')2 anti-human IgM and goat anti-Fc fragment of human IgG horse radish peroxidase (HRP) enzyme-conjugated secondary Abs best dilutions were at 1:20,000 and 1:30,000 respectively.

4.3. Anti-SARS-CoV-2 proteins IgG and IgM antibodies in symptomatic/asymptomatic persons.

In-house ELISAs were used to detect the IgG and IgM Abs responses against N, S and N + S proteins in all serum samples. The IgG antibody-positivity in asymptomatic persons was 96.43% (81/84) against N and S proteins (Table 2). The same picture was obtained in symptomatic persons, where 100% (153/153) were IgG antibody-positive against N and S proteins (Table 2).

There were a non-significant increase (p = 0.0266) in anti-S protein IgG Abs level in symptomatic persons over that in asymptomatic ones (Fig. 1A). The interquartile range (IQR, Fig. 1B) of Abs levels (as expressed by optical density) was 0.24 for symptomatic persons and 0.2334 for the asymptomatic persons.

There were a non-significant increase (p = 0.0278) in anti-N protein IgG Abs level in symptomatic persons over that in asymptomatic ones (Fig. 2A). The interquartile range (IQR, Fig. 2B) of Abs levels (as expressed by optical density) was 0.163 for symptomatic persons and 0.2175 for the asymptomatic persons.

Testing of anti-N and S proteins IgM Abs (Table 3) revealed 82.35% (126/153) anti-S protein IgM antibody positivity in symptomatic persons and 89.29% (75/84) in asymptomatic Covid-19 infected persons. The IgM antibody response was different against N protein, where it showed 100% (153/153) in symptomatic persons and 85.71% (72/84) in asymptomatic Covid-19 infected persons (Table 3).

There were a non-significant increase (p = 0.165) in anti-S protein IgM Abs level in symptomatic persons over that in asymptomatic ones (Fig. 3A). The interquartile range (IQR, Fig. 3B) of Abs levels (as expressed by optical density) was 1.1196 for symptomatic persons and 0.5808 for the asymptomatic persons.

There were a significant increase (p = 0.0001) in anti-N protein IgM Abs level in symptomatic persons over that in asymptomatic ones (Fig. 4A). The interquartile range (IQR, Fig. 4B) of Abs levels (as expressed by optical density) was 0.552 for symptomatic persons and 0.1815for the asymptomatic persons.

Testing of anti-N + S mixed proteins IgG and IgM Abs (Table 3) revealed 100% (153/153) anti-N + S mixed proteins IgG and IgM antibody positivity in symptomatic Covid-19 infected persons. The IgM and IgG antibody response showed 98.81% (83/84) in asymptomatic persons (Table 4).

4.4. Specificity and sensitivity of the In-house assays

Regarding the sensitivity, the standardized preparations could detect the anti-SARS-CoV-2 positive sera up to high dilution reaching 1:16000 and faintly at 1:32000. Regarding the specificity, there were weak reaction to HIV and HCV positive sera at a serum dilution up to 1:8. These reactions were not detectable at dilutions starting from 1:16.

5. Discussion

Since the Covid-19 pandemic started, enormous number of ELISA and lateral flow assays has been developed to detect the disease infection. This study created in-house ELISAs and compared them with comparable commercial ones to screen the antibody profile of symptomatic and asymptomatic Covid-19-infected Saudi persons. In this study, we used a panel of samples that were RT–PCR affirmed as symptomatically or asymptomatically Covid-19-infected. Negative controls, represented in pre-pandemic samples, were utilized to evaluate the performance of the assays. In this investigation, we detected IgG and IgM Abs directed to SARS-CoV-2. This is because some investigators reported that that IgM Abs could be detected three days post-Covid-19-infection as a first line of humoral immunity and followed by IgG antibodies after seven days (Lei et al., 2020; Zhao et al., 2020).

Before we start checking of available serum samples, we examined these samples for the acquisition of anti-SARSA-CoV-2 IgG and IgM Abs specially those antibodies against N, S or combined N + S proteins. Results showed the presence of some antibody-negative samples, either IgG or IgM, in the RT-PCR affirmed positive ones. Analogous findings were got by other investigators (Wang et al., 2020) where they found some cases are RT-PCR con-

Table 1

Number of symptomatic and asymptomatic IgG and IgM antibody-reactive samples against SARS-CoV-2 N, S and N + S proteins as detected by commercial ELISA kits.

	Anti-SARS-CoV-2 IgG Abs (number)				Anti-SARS-CoV-2 IgM Abs (number)			
	Symptomatic		asymptomatic		Symptomatic		asymptomatic	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
N	147	6	74	10	152	1	80	4
S	145	8	73	11	151	2	79	5
N + S	147	6	75	9	153	0	81	3

Table 2

Number and percent of IgG antibody positive symptomatic and asymptomatic persons against N and S proteins.

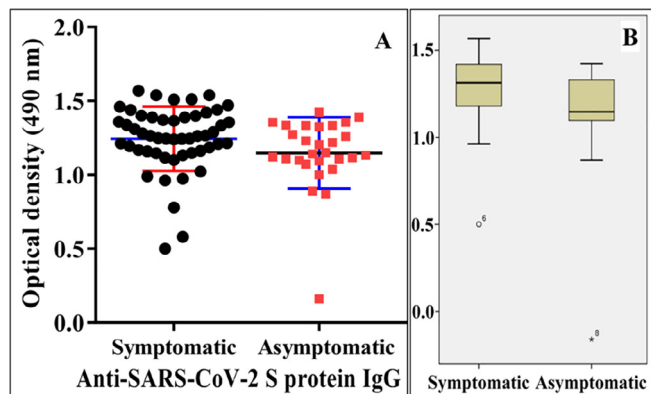|	Surface protein (IgG)				Nucleoprotein protein (IgG)			
	Symptomatic		asymptomatic		Symptomatic		asymptomatic	
	Number	%	Number	%	Number	%	Number	%
Positive	153	100	81	96.43	153	100	81	96.43
Negative	0	0	3	3.57	0	0	3	3.57
Total	153	100	84	100	153	100	84	100

Fig. 1. Anti-S protein IgG antibody level in symptomatic and asymptomatic Covid-19 infection (A) and the interquartile ranges of symptomatic and asymptomatic Anti-S protein IgG antibody level (B).
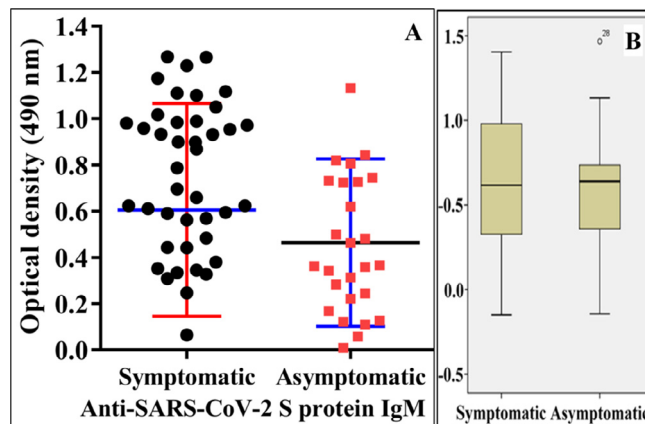

Fig. 2. Anti-N protein IgG antibody level in symptomatic and asymptomatic Covid-19 infection (A) and the interquartile ranges of symptomatic and asymptomatic Anti-N protein IgG antibody level (B).

Fig. 3. Anti-S protein IgM antibody level in symptomatic and asymptomatic Covid-19 infection (A) and the interquartile ranges of symptomatic and asymptomatic Anti-S protein IgM antibody level (B).
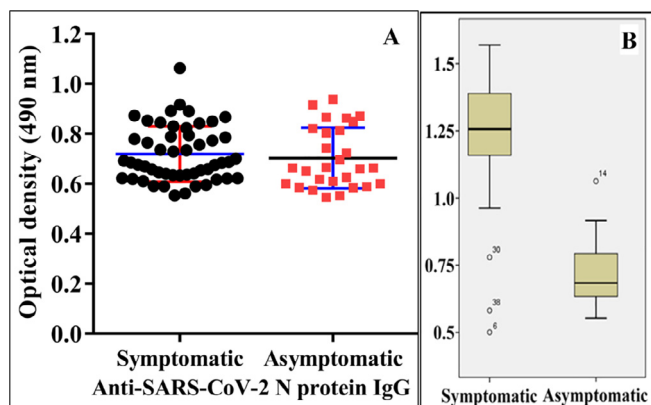

Fig. 4. Anti-N protein IgM antibody level in symptomatic and asymptomatic Covid-19 infection (A) and the interquartile ranges of symptomatic and asymptomatic Anti-N protein IgM antibody level (B).
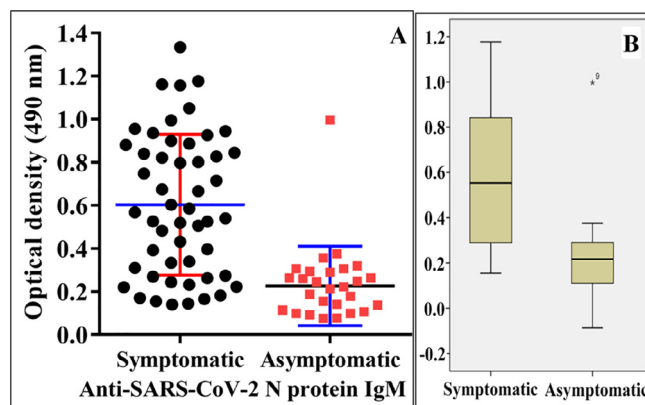
firmed but with no IgG and IgM antibody responses. In contrary to these findings, some investigators tested RT-PCR negative samples and found that they were Covid-19-infected asymptomatic cases and they have antibodies either IgG type alone or both IgG and IgM types (Lei et al., 2020).

Before we start running the ELISA assays, we titrated both the serum and the two conjugates to determine the optimal serum and conjugate dilutions. Results showed that the sera were reactive up to dilution of 1:3200. We determined to use dilution of 1:400 to avoid any escape of low antibody titer samples. Also, at

Table 3

Number and percent of IgM antibody positive symptomatic and asymptomatic persons against N and S proteins.

	Surface protein (IgM)				Nucleoprotein protein (IgM)			
	Symptomatic		asymptomatic		Symptomatic		asymptomatic	
	Number	%	Number	%	Number	%	Number	%
Positive	126	82.35	75	89.29	153	100	72	85.71
Negative	27	17.65	9	10.71	0	0	12	14.29
Total	153	100	84	100	153	100	84	100

Table 4

Number and percent of anti-N + S mixed proteins IgM and IgG Abs in symptomatic and asymptomatic persons.

	N + S proteins (IgG)				N + S proteins (IgM)			
	Symptomatic		asymptomatic		Symptomatic		asymptomatic	
	Number	%	Number	%	Number	%	Number	%
Positive	153	100	83	98.81	153	100	83	98.81
Negative	0	0	1	1.19	0	0	1	1.19
Total	153	100	84	100	153	100	84	100

this dilution, no cross-reactivity of negative controls was noticed. Some investigators used nearly similar dilution (1:300) to detect anti-SARS-CoV-2 Abs (Dufloo et al., 2020). Also, conjugate dilution was tested to get the best reading with minimal background.

Utilizing the in-house ELSA to detect anti-N and S proteins IgG Abs, all RT-PCR confirmed samples (symptomatic and asymptomatic) were positive against N proteins but some samples (3/84) were negative against S protein. The titer of the anti-SARS-CoV-2 IgG Abs was higher in the symptomatic serum samples than that of the asymptomatic ones. This differences in antibody titer was also found by other researchers (Dufloo et al., 2020).

When testing in-house ELSA to detect anti-N and S proteins IgM Abs, some samples were shown to be negative. In symptomatic cases, the negativity was directed only against S protein but not against N protein. In asymptomatic cases, there were negative IgM Abs cases against both S and N proteins, but, the number of the negatives against N protein in asymptomatic is more than in symptomatic cases. Some studies pointed out that early antibody immune responses (here represented by IgM Abs) were N protein-targeted (Guo et al., 2020; Liu et al., 2020), while another study revealed that immunoassays targeting the spike protein are more specific than the N-based assays in detecting Covid-19 infection (Burbelo et al., 2020). Our study is in agreement with the statement that early antibody immune responses were N protein-targeted (Guo et al., 2020; Liu et al., 2020).

When we combined both antigens (N and S) in the same ELISA, antibody negativity dropped to the minimum. This indicates that screening of asymptomatic cases, either RT-PCR positive or negative, is better done using combined S and N proteins as target antigens to capture antibodies in human sera. Detection of asymptomatic Covid-19 infection is very important as this group of virus carries is very dangerous to individuals in their contact. Here, we studied the antibody profile of asymptomatic and symptomatic Covid-19 infected Saudi persons. The anti-N, S and N + S proteins IgG and IgM Abs were screened in both groups. Different controls, including normal or other viruses-infected sera, were used in this study. In the current study, it was shown that Saudi asymptomatic individuals have both IgG and IgM Abs directed to SARS-CoV-2 with unknown time of virus acquisition. Our finding that the antibody titer in Saudi asymptomatic persons is lower than that of Saudi symptomatic persons, till if not numerically significant, is in agreement with other's work (Long et al., 2020; Sekine et al., 2020).

Further, the detection of the asymptomatic Covid-19 infection is a need and can be fulfilled through random screening of the population (Gudbjartsson et al., 2020) as the asymptomatic infections may be higher among the population and some asymptomatic cases may not be detected by RT–PCR testing. Serological testing should be side by side with RT-PCR testing as some workers detected negative RT-PCR case with positive antibodies (Dufloo et al., 2020; Long et al., 2020). Also, other investigation stated that viral shedding is continued in respiratory tract at least 10 days after onset of the Covid-19 (Wang et al., 2020; Young et al., 2020).

6. Conclusions

In conclusion, this study reflected a very good representation of the in-house ELISAs with excellent diagnostic value in discovering anti-SARS-CoV-2 IgG and IgM Abs. This investigation demonstrated that SARS-CoV-2-positive asymptomatic Saudi individuals have IgG and IgM Abs as humoral immune responses against SARS-CoV-2 and the titer of these antibodies is slightly lower than those detected in SARS-CoV-2-positive symptomatic Saudi individuals. Combining of N and S proteins in the same ELISA as antibody-capture antigens greatly increased the Covid-19 infection detection power. Also, screening of asymptomatic cases which are either RT-PCR positive or negative is highly recommended using combined S and N proteins ELISA as target antigens to capture antibodies in human sera.

Competing Interests

All authors state that they haven't any financial/commercial conflict of interest regarding this work.

Author contributions

EHI conceived and designed the ELISAs and experiments. MA, MK and EHI performed the experiments, analyzed the data and wrote the paper. HAG supervised the team.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgements

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