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Evaluation of the Level and Duration of SARS-Cov-2 Neutralizing Antibodies in COVID-19 Convalescent Healthcare Workers

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Abstract

Background The neutralizing antibodies (nAbs) are the main factor for the protective immunity against viruses.

Objective To evaluate the level and duration of anti-receptor-binding domain (RBD) nAbs for 8 months in convalescent Coronavirus disease-2019 (COVID-19) patients with a history of mild-moderate and severe disease.

Methods Up to 160 sera from COVID-19 convalescent hospital healthcare workers (HCWs) with a history of mild-moderate and severe disease at 1-, 3-, 5-, and 8-month post-recovery using in-house developed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-RBD neutralizing competitive enzyme-linked immunosorbent assay (ELISA).

Results SARS-CoV-2 nAbs persisted till 8 months post-recovery in most convalescent patients, the level of nAbs in severe disease group was higher than in mild-moderate disease group; moreover, anti-RBD nAbs mean concentrations in COVID-19 convalescent HCWs tend to increase over time after infection. Similarly, frequency of samples with positive anti-RBD nAbs also tends to increase over time post-recovery.

Conclusion The protective immunity to SARS-CoV-2 in HCWs was found to last for a long time exceeding 8 months after recovery. The increase in the mean level of nAbs over time in COVID-19 convalescent HCWs might be attributed to the frequent asymptomatic re-exposures in HCWs.

Keywords COVID-19, neutralizing antibodies, anti-RBD nAbs, long-term immunity, SARS-COV-2-RBD neutralizing ELISA assay

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List of abbreviations: ACE2 = Angiotensin-converting enzyme 2, BMI = Body mass index, COVID-19 = Coronavirus disease-2019, cVNT = Conventional virus neutralization test, ELISA = Enzyme-linked immunosorbent assay, HCWs = Healthcare workers, HRP = Horse-reddish peroxidase, nAbs = Neutralizing antibodies, pVNT = Pseudovirus-based virus neutralization test, RBD = Receptor-binding domain, PCR = Polymerase chain reaction, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2, S = Spike, TMPRSS2 = Transmembrane protease serine 2, TMB = Tetramethylbenzene

Introduction

The coronavirus disease-2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the SARS-related coronavirus species [1-4]. COVID-19 includes a variety of clinical syndromes, from asymptomatic cases to mild flu-like illness to severe illness that demands hospitalization, primarily due to pulmonary complications [3,5,6]. Despite the fact that SARS-CoV-2 primarily affects the respiratory system, new evidence suggests that COVID-19 also affects the vascular system, causing thrombotic microangiopathy and thrombosis in a variety of organs, including the lungs [7-9]. As a result, it is

no surprise that patients with pre-existing cardiovascular diseases, hypertension, and other comorbidities are at a higher risk [10]. For entry into target cells, SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a receptor, and for activation of the viral spike (S) protein, it uses transmembrane protease serine 2 (TMPRSS2), a cellular serine protease [11,12]. Antibodies that target the receptor-binding domain (RBD) of the S protein are particularly interesting because they can prevent virus infection and spread by blocking virus entry into cells. Additionally, these neutralizing antibodies (nAbs) may be used in passive antibody therapy [13-15]. As a result, it is not surprising that antibody responses to SARS-CoV-2 have gotten a lot of attention as a way to accurately assess infection prevalence [13,16]. Regarded anti-SARS-CoV-2 antibody responses are not known to be long-lasting, especially when considering evidence that antibody responses to other coronaviruses are changeable and transient [17-20]. It is also unclear whether all COVID-19 patients, particularly those with mild disease, will produce enough SARS-CoV-2 nAbs to prevent re-infection in comparison with those with severe COVID-19. Therefore, in the present study, it was applied in-house developed SARS-COV-2 RBD neutralizing ELISA assay to qualitatively and quantitatively assess the level and duration of nAbs against SARS-CoV-2 infection primarily in sera collected at different time points post-recovery from convalescent individuals' cohorts with mild-moderate and severe infection with COVID-19.

Methods

Study population

This is a cross-sectional study conducted at the Microbiology Department, College of Medicine, Al-Nahrain University during the period between December 2020 and September 2021. The study included a recovered COVID-19 frontline Healthcare workers (HCWs) at many hospitals in Baghdad. A total of 160 serum samples were collected randomly from convalescent COVID-19 HCWs that volunteered to donate. All donors had previously polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection. Serum samples were collected at different time points (1-, 3-, 5-, and 8-months post-recovery), each time point covered 20 individuals with a history of mild-moderate disease and 20 individuals with a history of severe disease.

Inclusion criteria

(1) Ex COVID-19 patients who had previously positive SARS-CoV-2 PCR
(2) Informed consent
(3) Age >18 years.

Exclusion criteria

(1) Refusal to give informed consent
(2) Contraindication to venipuncture
(3) Vaccinated individuals.

Ethical approval

This study was approved by the Institutional Review Board, College of Medicine, Al-Nahrain University (No. 202011112 on 8/12/2020). After obtaining the consent, participants filled out a baseline questionnaire, and blood samples were drawn. The study was conducted by COVID-19 contact restrictions using appropriate measures for infection prevention.

Data collection

Participants were asked to provide information about their age, sex, weight, height, general health status (e.g. cardiovascular or respiratory diseases, diabetes mellitus, pregnancy, any autoimmune diseases, etc.); moreover, patients were asked for the history of the previous COVID-19 infection including the severity of COVID-19 signs and symptoms, minimum level of oxygen saturation (SpO_2), hospital admission, date of the positive COVID-19 PCR test result, date of enrolment and the date of recovery or negative COVID-19 PCR test result.

SARS-CoV-2 RBD neutralizing enzyme-linked immunosorbent assay (ELISA) assay

Using an in-house developed in vitro SARS-CoV-2 RBD neutralizing ELISA assay prepared in a recent study [21], the qualitative and quantitative detection of circulating neutralizing/blocking antibodies in serum samples was done in an isotype-independent manner. For quantitative assay, standards preparation was performed by a serial dilution of the stock purified recombinant concentrated anti-SARS-CoV-2 Spike nAbs to get four points for the standard curve to calculate the unknown nAbs concentration in the samples. Thus, the anti-SARS-CoV-2 spike nAbs standards concentration was 10000 ng/ml, 1000 ng/ml, 100 ng/ml, and 0 ng/ml. Sample diluent served as the zero standard (0 ng/ml). For qualitative assay, the prepared anti-Human SARS-CoV-2 highest standard of 10000 ng/ml as positive control and the sample diluent as negative control, and the optimal established cut-off value of the assay were used.

To perform this competitive assay, a calculated volume of receptor-binding domain labeled with horse-reddish peroxidase (RBD-HRP) working solution to fit 10 ng per well was mixed with a volume ratio of 1:1 with serum samples at a final dilution of 1:10 and each the freshly prepared standards. The mixture was then incubated at 37°C for 1 hour to allow the neutralization reaction to occur. For binding reaction, 100 µL of each standard mixture and each sample mixture were transferred to the wells of the human ACE2 coated microplate and incubated in dark at 37°C for 1 hr. The unbound HRP-RBD, as well as any HRP-RBD bound to non-nAb, will be captured on the plate by binding to hACE2, whereas the circulating nAb- RBD-HRP complexes remain in the supernatant and are washed away 4 times with wash buffer. That is, if nAbs are present in the serum, the interaction of ACE2-RBD can be neutralized (inhibited/blocked) by specific nAbs in patient serum, just like in Conventional virus neutralization test (cVNT) or Pseudovirus-based virus neutralization test (pVNT). For substrate reaction, one-component tetramethylbenzene (TMB) substrate was added to each well and incubated in the dark at 37°C for 20 minutes. Then, stop solution was added to each well to quench the reaction. The absorbance was read in the microtiter plate reader (BioTek) at 450 nm immediately after adding the stop solution.

Depending on the amount of nAbs present in convalescent sera, the binding of SARS-CoV-2 S RBD to ACE2 would be blocked to various degrees that should correlate with the optical density of this enzyme-linked immune sorbent-based assay. The serum samples with more nAbs show a lower signal intensity.

Statistical analysis

The data were processed using statistical package for social sciences (SPSS) version 16.0.0, Microsoft Excel 2010, and Graphpad Prism version 7.04. The data of the current study were scrutinized carefully in terms of being parametric or non-parametric using normality tests. Accordingly, the proper statistical tests were used. Student t-test and analysis of variance (ANOVA) test were used for parametric data to measure the significance of difference in means taking into account whether variables of analysis sharing different or equal variance. For qualitative nominal data, Pearson's chi-square test, with or without Yate's correction, Fisher Exact test, and McNemar test were used to measure significance of hypothesis for association. Correlation coefficient tests, odd ratio, correlation coefficient, among variables were used to assess the nature of correlation in terms of positive, negative or indifference.

Results

Descriptive data of study population

To estimate the level and persistence of the response of anti-SARS-CoV-2-RBD nAbs in a sample of Iraqi convalescent COVID-19 HCWs, the validated in-house neutralizing ELISA assay was used; a total of 160 sera of hospital's COVID-19 convalescent HCWs who had previously PCR-confirmed SARS-CoV-2 infection were collected at various intervals of post-recovery time (1, 3, 5, and 8 months post-recovery); each time interval included 20 HCWs

with a history of mild-moderate disease and 20 HCW with a history of severe disease. Up to 62.5% (100/160) of participants were females. Up to 19.4% (31/160), 48.1% (77/160), and 32.5% (52/160) of participants were normal weight, overweight and obese, respectively. Of note, 66.2% (106/160) of participants were without medical co-morbidities while 33.8% (54/160) were with different types of co-morbidity. Among the co-morbidities reported by the participants, diabetes mellitus (DM) of 11.2% (18/160), cardiovascular disease of 11.9% (19/160), asthma of 6.2% (10/160), and others of 4.4% (7/160). Following the serological testing by in-house SARS-CoV-2 RBD neutralizing ELISA kit, it was demonstrated that 71.9% (115/160) of the COVID-19 convalescent HCWs in all groups were positive to anti-RBD nAbs presence, while 28.1% (45/160) of HCWs were negative.

Descriptive qualitative data of the study

The descriptive statistics of the age, body mass index (BMI), and anti-SARS-CoV-2-RBD nAbs concentration is demonstrated in table 1. The mean, median, and range of age was 37.1 and 35, (21-72) years, respectively, and the mean, median, and range of BMI was 28.44, 28.05, (15.59-42.02) kg/m^2, respectively. Also, the mean concentration of the anti-SARS-CoV-2-RBD nAbs in HCWs (n=160) was 5370.3 ng/ml while, the median concentration was 1546.3 ng/ml.

Table 1. The descriptive statistics of the age, body mass index and anti-SARS-CoV-2-RBD neutralizing antibodies concentration

Variable		Statistic	Std. Error
Age (yr)	Mean	37.11	0.87
	Median	35.00	
	Std. Deviation	11.00	
	Minimum	21.00	
	Maximum	72.00	
BMI (kg/m^2)	Mean	28.45	0.34
	Median	28.05	
	Std. Deviation	4.33	
	Minimum	15.59	
	Maximum	42.02	
Anti-RBD neutralizing IgG antibodies (ng/ml)	Mean	5370.3	2337.82
	Median	1546.3	
	Std. Deviation	29571.3	
	Minimum	0.03	
	Maximum	293000	

Age and BMI in the different groups of study

The mean age of COVID-19 convalescent HCWs has no statistical significance when compared with different COVID-19 severity groups at different time points (p-value >0.05). Nevertheless, HCWs with a history of severe COVID-19 disease were a bit older than those with a history of mild-moderate COVID-19

disease. The mean BMI with different disease severity was found to be significant (p<0.023). It was found that the mean BMI in groups of convalescent HCWs with a history of severe COVID-19 disease was higher than in convalescent HCWs groups with a history of mild-moderate COVID-19 disease as shown in table 2.

Table 2. Mean body mass index values of HCWs in all different disease severity groups at the different times points

		BMI (kg/m^2)		
Study groups	**N**	**Mean**	**Std. Deviation**	**P value**
Mild/moderate-1-month	20	27.87	5.16	0.023
Severe-1-month	20	28.37	4.07	
Mild/moderate-3-month	20	28.13	4.49	
Severe-3-month	20	29.23	3.08	
Mild/moderate-5-month	20	25.95	3.13	
Severe-5-month	20	30.19	2.67	
Mild/moderate-8-month	20	27.49	4.38	
Severe-8-month	20	30.36	5.70	
Total	160	28.45	4.33	

The relationship of level of anti-SARS-COV-2-RBD nAbs and the disease severity in different study groups

The statistically significant relationship between the mean concentration of anti-SARS-CoV-2-RBD nAbs and the different groups of convalescent HCWs with different disease severity was proven (p=0.016; p<0.05). It was observed that the mean level of anti-SARS-CoV-2-RBD nAbs in convalescent HCWs groups with a history of severe disease was higher than in convalescent HCWs groups with a history of mild-moderate disease when comparing without looking at the difference between the post-recovery time points of each group (Table 3).

The changing over time of the level of anti-SARS-CoV-2-RBD nAbs among the HCW groups recovered from the mild-moderate disease

The mean concentration of anti-RBD nAbs in COVID-19 convalescent HCWs groups with a history of mild-moderate disease significantly changed with different time points post-recovery (p<0.05). The mean level of nAbs in mild-moderate groups of COVID-19 convalescent HCWs were in a trend of increasing over time (1-, 3-, 5-, and 8-months post-recovery) as shown in figures 1. That mean level of nAbs did not wane over time in COVID-19 convalescent HCW groups, even for those recovering from the mild-moderate disease.

Table 3. Mean level of anti-SARS-COV-2-RBD neutralizing antibodies in convalescent HCW groups with different disease severity and different time points post-recovery

Study groups	N	Anti-RBD neutralizing IgG antibodies (ng/ml) Mean	Std. Deviation	P value
Mild/moderate-1-month	20	301.46	857.81	
Severe-1-month	20	1897.60	2782.02	
Mild/moderate-3-month	20	779.56	1224.13	
Severe-3-month	20	2571.60	1819.75	0.016
Mild/moderate-5-month	20	1634.00	1951.96	
Severe-5-month	20	2484.70	2115.46	
Mild/moderate-8-month	20	2694.30	1723.07	
Severe-8-month	20	30599.00	80759.85	
Total	160	5370.30	29571.31	

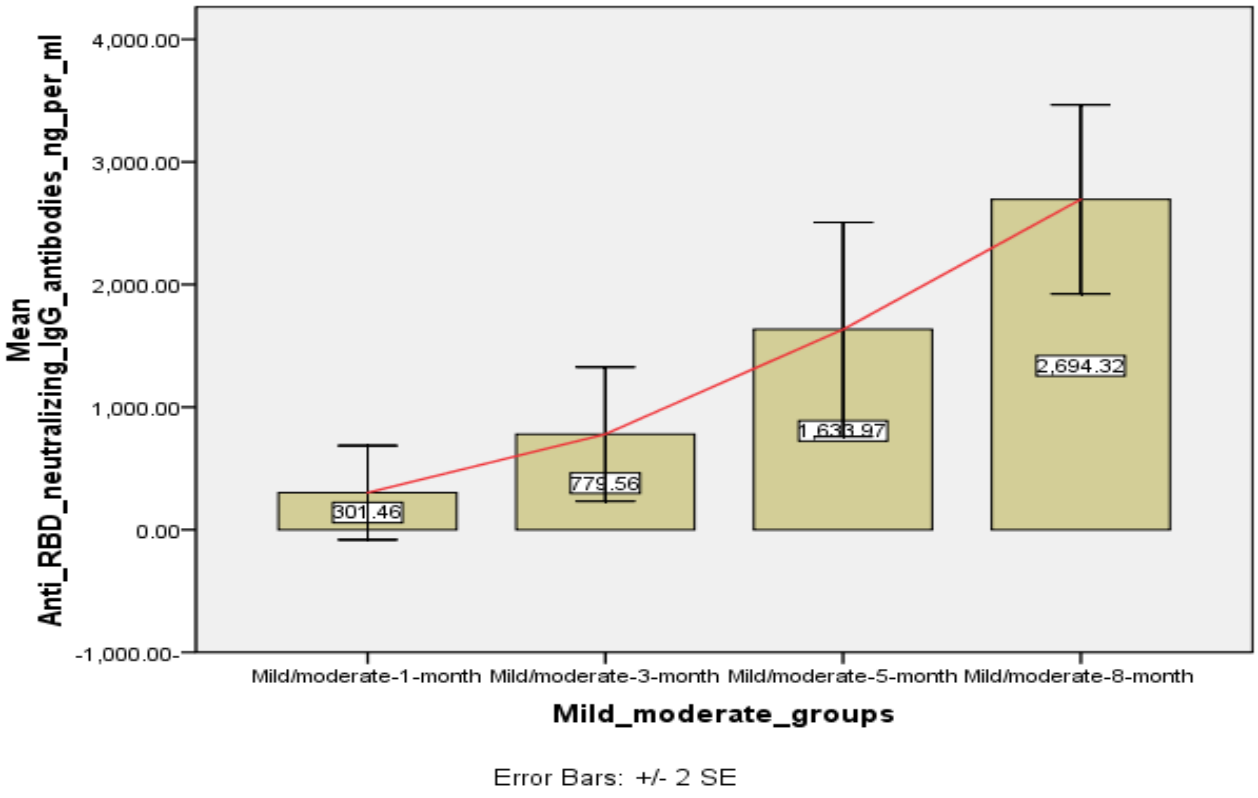

Figure 1. The changes of the mean level of nAbs in mild-moderate groups of COVID-19 convalescent HCWs over time

The changing over time of the level of anti-SARS-CoV-2-RBD nAbs among the HCW groups recovered from the severe disease

Despite the mean concentration of anti-RBD nAbs in COVID-19 convalescent HCWs groups with a history of severe disease had no statistically significant difference among different time points post-recovery (p=0.07; p>0.05), the mean level of nAbs in severe groups of COVID-19 convalescent HCWs tends to increase over time (1, 3, 5, and 8 months post-recovery) (Figure 2).

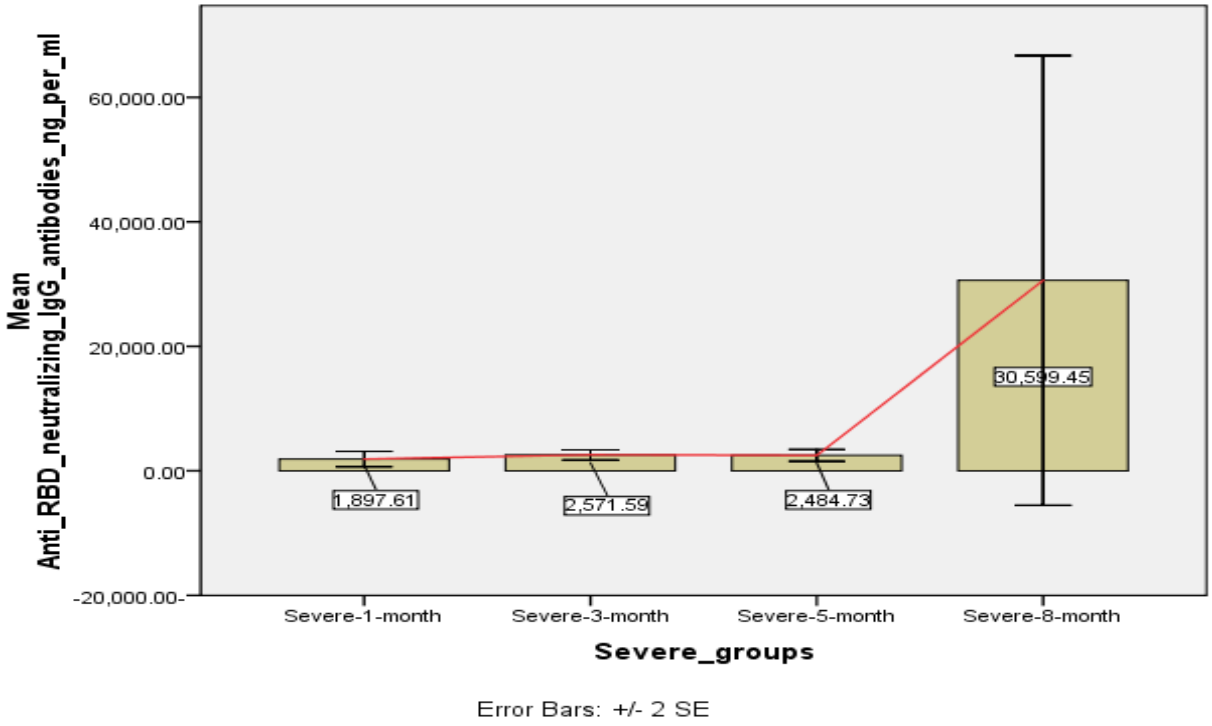

Figure 2. The changes of the mean level of nAbs in severe groups of COVID-19 convalescent HCWs over time

The association of the positive anti-SARS-CoV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCWs groups with a history of mild -moderate disease

The evaluation of the association between the presence of detectable anti-SARS-CoV-2-RBD nAbs and time intervals post-recovery was performed using the chi-square test. For COVID-19 convalescent HCWs groups with a history of the mild-moderate disease, a total of 80 sera grouped according to the time interval post-recovery were assessed for the presence of detectable anti-RBD nAbs using the in-house SARS-CoV-2-RBD neutralizing ELISA assay used in this study. The frequencies of samples with positive anti-RBD nAbs were found to increase over time as shown in figure 3, reflecting the increasing level of anti-SARS-CoV-2-RBD nAbs in mild-moderate groups of COVID-19 convalescent HCWs over time and indicating the persistence of the nAbs until the eight-month post-recovery. The frequencies of convalescent HCWs with positive anti-RBD nAbs were 4/20 (20%), 11/20 (55%), 12/20 (60%), and 19/20 (95%) at 1, 3, 5, and 8 months after recovery, respectively (P = 0.0001).

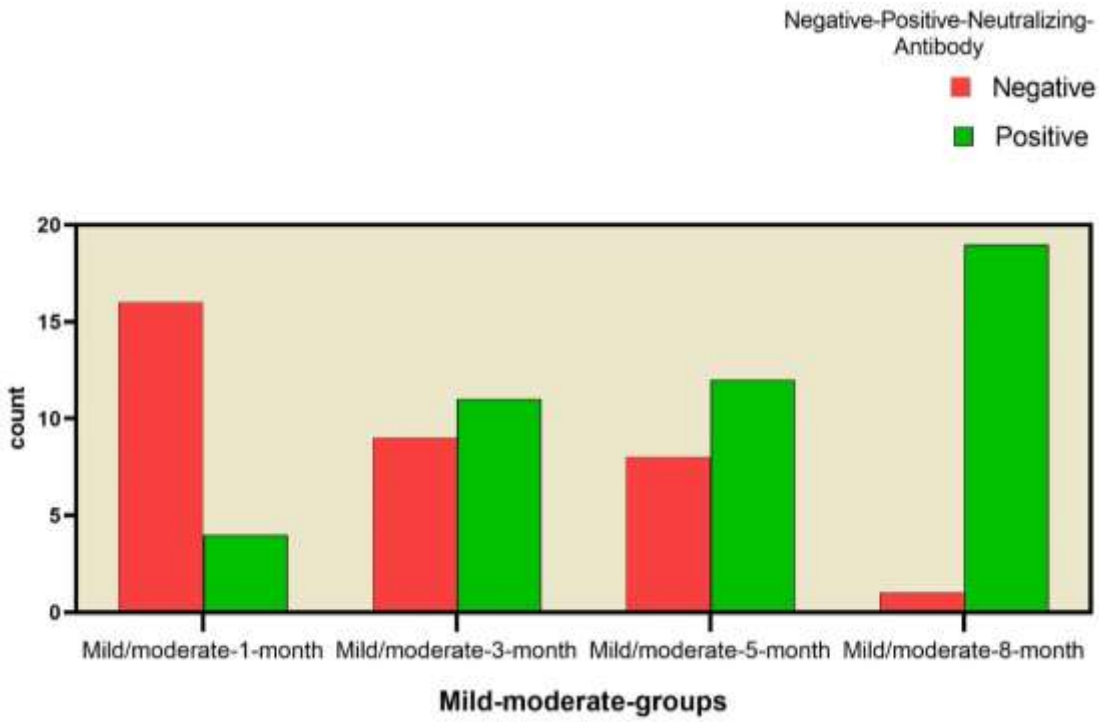

Figure 3. Illustration for the association of the positive anti-SARS-COV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCW groups with a history of mild-moderate disease by displaying the increasing frequencies of samples with positive anti-RBD nAbs versus the negative

The association of the positive anti-SARS-CoV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCWs groups with a history of severe disease

Of a total of 80 sera collected at different time intervals post-recovery from COVID-19 convalescent HCWs with a history of the severe disease, the frequencies of samples reading a positive anti-RBD nAbs were determined for each time group using the in-house neutralizing ELISA kit. The frequencies of samples reading positive anti-RBD nAbs in the different severe groups of COVID-19 convalescent HCWs were relatively close to each other and slightly tend to increase over time as shown in figure 4, reflecting a fluctuating level of anti-SARS-CoV-2-RBD nAbs but generally mildly increasing over time. The frequencies of convalescent HCWs with positive anti-RBD nAbs were 12/20 (60%), 20/20 (100%), 18/20 (90%), and 19/20 (95%) at 1, 3, 5, and 8 months after recovery, respectively (P = 0.001).

The association of the sex of the study population with the positive anti-SARS-CoV-2-RBD nAbs post-recovery and with the presence of co-morbidities

It was shown that sex grouping has no association with the presence of detectable anti-SARS-CoV-2-RBD nAbs post-recovery (p=0.683) or the presence of co-morbidities (p=0.546). The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by sex was 73/100 (73%) and 42/60 (70%) for females and males, respectively. The frequency of COVID-19 convalescent HCWs with co-morbidities by sex was 32/100 (32%) and 22/60 (36.6%) for females and males, respectively.

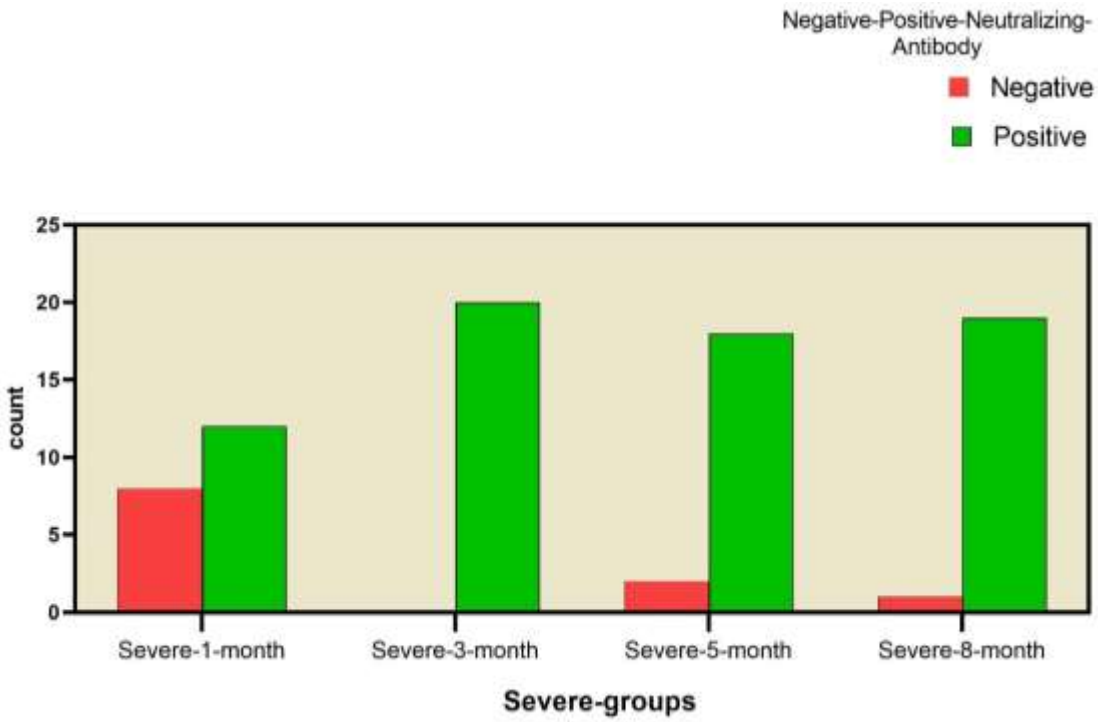

Figure 4. Illustration for the association of the positive anti-SARS-COV-2-RBD neutralizing antibodies with the post-recovery time in COVID-19 convalescent HCW groups with a history of severe disease by displaying the increasing frequencies of samples with positive anti-RBD nAbs versus the negative

The association of obesity, co-morbidities, and type of co-morbidity of the study population with the positive detection of anti-SARS-CoV-2-RBD nAbs post-recovery

The current study revealed that no significant difference in the frequencies of COVID-19 convalescent HCWs with positive detectable nAbs by obesity (p=0.798), co-morbidities (p=0.5), or type of co-morbidity (p=0.909). The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by obesity was 21/31 (67.7%), 57/77 (74%), and 37/52 (71%) for normal and overweight, and obese, respectively. The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by co-morbidities was 78/106 (73.5%) for those who had no co-morbidities and 37/54 (68.5%) for those who had co-morbidities. The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by type of co-morbidity was 13/18 (72.2%), 13/19 (68.4%), 7/10 (70%), and 4/7 (57.1%) for DM and cardiovascular disease, asthma, and other, respectively.

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and the co-morbidities of COVID-19 convalescent HCWs

The mean concentrations of anti-SARS-CoV-2-RBD nAbs were borderline significantly different between ones with comorbidities versus those without comorbidities (p=0.055). It was observed that the mean concentration level of anti-SARS-CoV-2-RBD nAbs of COVID-19 convalescent HCWs having co-morbidities was higher than those having no co-morbidities as illustrated in figure 5.

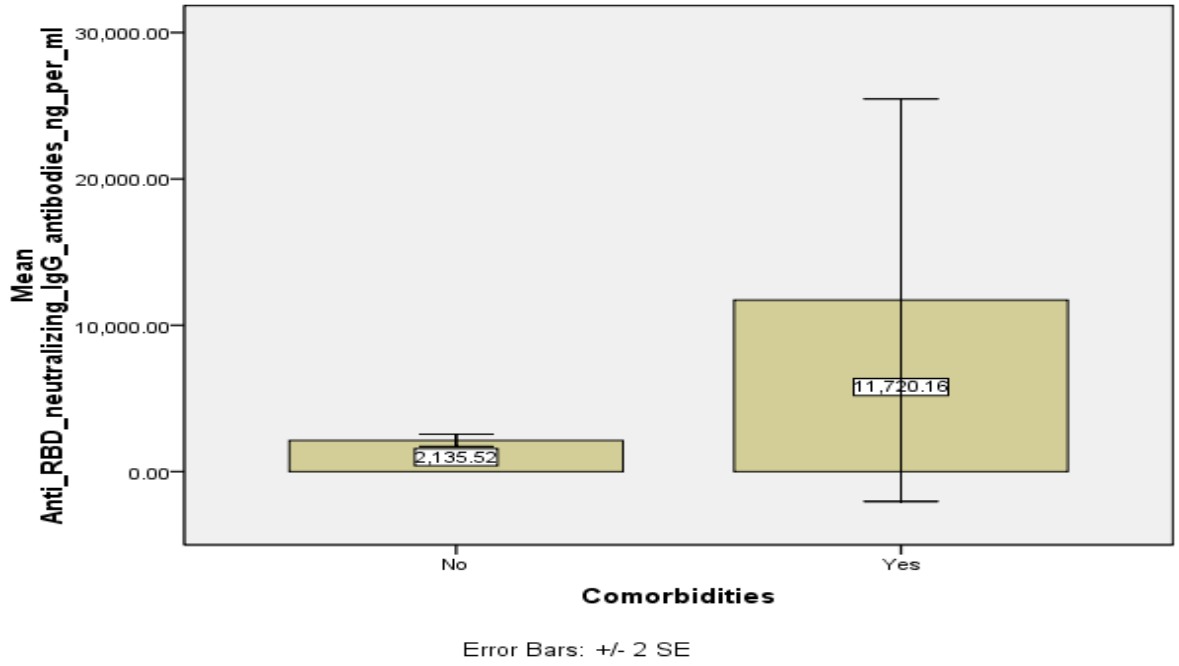

Figure 5. Relationship between the level of anti-SARS-CoV-2-RBD nAbs and co-morbidities of the COVID-19 convalescent HCW in different study groups

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and type of co-morbidity of the COVID-19 convalescent HCWs

The mean concentrations of anti-SARS-CoV-2-RBD nAbs were not statistically significantly different when compared among different groups of COVID-19 convalescent HCWs who grouped according to the type of co-morbidity ($p>0.05$).

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and obesity of the COVID-19 convalescent HCWs

There was no statistically significant difference in the mean concentrations of anti-SARS-CoV-2-RBD nAbs among the different obesity groups of COVID-19 convalescent HCWs ($p>0.05$).

Discussion

Better diagnostic tests and treatment, as well as the development of effective vaccines are necessary to help control COVID-19 pandemic; moreover, clarifying the neutralizing memory arsenal against SARS-CoV-2 will enable a clear explanation of immune responses and the needed strategies to combat this viral infection. In this study, the anti-RBD nAbs levels and their persistence in 160 COVID-19 convalescent HCWs with a history of mild-moderate and severe disease were monitored at various intervals (1, 3, 5, and 8 months) of post-recovery time. Similar to a previous study [22], convalescent HCWs with high BMI were associated with more severe disease and were more likely to have an increased level of anti-RBD nAbs.

In the terms of the relationship between the level of nAbs and severity, this study found that the mean level of anti-SARS-CoV-2-RBD nAbs in convalescent HCWs groups with a history of severe disease was higher than in convalescent HCWs groups with a history of mild-moderate disease, as reported by others [23-26]. It is possible that disease severity as measured by a symptom density score, influences the initial magnitude of antibodies, resulting in weaker humoral responses in mildly ill patients but instead strong stimulation of short-lived plasmablasts by inflammatory prolonged disease in those with severe disease. Following infection or immunization, an initial peak, as well as an early decline of antibodies, is usual, since most short-lived antibody secreting

plasmablasts concerned for the early antibody peak has dropped dead by month three [27]. However, findings in this study showed that anti-RBD nAbs persisted until the eight months post recovery in most convalescent individuals and this indicates that protective immunity against SARS-CoV-2 may relatively be long-lasting enough for reasonable annual vaccination programs especially in front line HCWs, as described in previous studies [24,28], but contrasted with another study that suggested unlikely long term protective immunity on-line with the other coronaviruses [29]. As was stated before, long-lived plasma cells are responsible for the longer-term retention of anti-RBD nAbs during months 8 and thereafter [29].

In addition, this study revealed that the mean level of nAbs tends to increase over time among all mild-moderate groups as well as severe groups of the COVID-19 convalescent HCWs. A similar study demonstrated the increase of anti-RBD titer over time [28] but other studies revealed contrasting findings with a decrease in anti-RBD nAbs over post-recovery time [30,31]. The observed increase in anti-RBD nAbs possibly belongs to the reboots from asymptomatic re-exposure that occurred in individuals at high risk such as HCWs despite the development of an efficient humoral immune response after the symptomatic initial infection. The mean concentration of anti-RBD nAbs in HCWs with mild-moderate and severe disease at eight months post-recovery might be exposed more than once to SARS-CoV-2 resulting in multiple folds higher the mean concentration of nAbs in 8-month than in 1-month post-recovery as a baseline. Recent studies have indicated that asymptomatic SARS-CoV-2 reinfections [24,32,33], as well as, symptomatic COVID-19 re-infections in HCWs are frequent when compared to the general population even though with the presence of high protective immunity [29,32].

Other investigations showed that antibodies to SARS-CoV-2 have been linked to a lower incidence of SARS-CoV-2 reinfection in healthcare workers for up to seven months following infection [34,35]. Notably, a study with similar results explained the increase in the level of nAbs over time linked to the preferential detection of increasing higher-affinity antibodies and considered the secondary re-exposure unlikely due to low circulation of the virus [35]. But another research suggested that despite herd protection from vaccination or infection, SARS-CoV-2 may continue to circulate throughout community populations [36].

In addition, the current study showed that the frequency of samples with positive anti-RBD nAbs from HCWs with mild-moderate and severe diseases tends to increase over time, confirming the boosting effect caused by re-infections, which also occurred in HCWs with waning or seroconverted nAbs levels, explaining the increased proportion of positive samples. Therefore, even for HCWs who already have recovered from a mild COVID-19 disease and have antibodies detected in routine serologic tests, continue the use of personal protective equipment to support infection prevention and control, at least until strong indicators of immunological protection are established.

In conclusions, anti-RBD nAbs persisted until the eight-months post-recovery in most of convalescent patients. The mean level of nAbs in mild-moderate and severe COVID-19 convalescent HCWs groups tends to increase over time, this possibly belongs to the reboots from asymptomatic re-exposure that occurred in individuals at high risk such as HCWs despite the development of an efficient humoral immune response after the symptomatic initial infection. Frequency of samples with positive anti-RBD nAbs in COVID-19 convalescent HCWs tends to increase over time, confirming the boosting effect caused by re-infection, which also occurred in those with waning nAbs levels, explaining the increased proportion of positive samples. Severe cases tend to develop higher level of nAbs to SARS-CoV-2.

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Author contribution

Mousa: Did the laboratory work and wrote the article. Dr. Abdulamir: Supervision of the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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