Archives of Razi Institute, Vol. 76, No. 5 (2021) 1255-1267

DOI: 10.22092/ari.2021.356135.1785

Original Article

Measurement of SARS-CoV-2-Specific Humoral and Cellular Immunity in Coronavirus Disease 2019 Convalescent Health Care Workers in Iraq

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Received 7 September 2021; Accepted 4 October 2021
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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the *Coronaviridae* family which led to a global pandemic. However, available knowledge on adaptive immunity in convalescent individuals is limited. The present study was conducted on 191 convalescent coronavirus disease 2019 (COVID-19) health care workers (HCW); moreover, it evaluated the cell-mediated immunity of 122 recovered HCW and the level of anti-receptor binding domain (RBD) IgG antibodies of 181 recovered HCW. Cellular and humoral immune responses were identified over time from one to eight months post recovery with varying disease severity using MTT proliferation assay and enzyme-linked immunosorbent assay. Analysis of lymphocyte proliferation with S1 protein in mild-moderate and severe HCW revealed an insignificant difference with an increase in the maximum and third quartile (Q3) from one to eight months after COVID-19 recovery. Antibody levels in mild-moderate and severe recovered HCW were insignificantly different from post-COVID 19 recovery (P>0.05); in addition, the median, maximum, and Q3 values of anti-RBD IgG were close to each other over the time intervals from one to eight months post recovery. These data suggest that many convalescent HCW enrolled in this study were re-exposed to the virus without the development of symptoms indicating the role of cell-mediated and humoral immunity in preventing symptomatic reinfection. This study reveals that a robust immunity developed after mild, moderate, and severe COVID-19 that could last for several months post recovery.

Keywords: Cell-Mediated Immunity, Coronavirus Disease 2019 (COVID-19), Humoral Immunity, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

1. Introduction

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, along with a series of similar symptoms of pneumonia collectively known as Coronavirus Disease 2019 (COVID-19). As the virus globally spread, the World Health Organization (WHO) declared it a worldwide pandemic (1, 2). SARS-CoV-2 belongs to the *Coronaviridae* family and contains two major structural proteins, namely nucleoprotein which is found inside the virus, and spike (S) protein that protrudes from the viral surface. The S glycoprotein is a large trimeric glycoprotein composed of a polypeptide chain (from 1,100 to 1,600 residues in length) and responsible for cell attachment and viral fusion (3, 4). The S protein is used as a target for characterizing the immune response to SARS-CoV-2 (5). It is divided into two regions S1 and S2 subunits. The S1 subunit is a V-shaped polypeptide with four distinct domains of A, B, C, and D, and domain B functions as the receptor-binding domain (RBD) (6). Several studies have shown that the virus is attached to

the cells by the interaction of RBD with cellular receptor angiotensin-converting enzyme 2 (ACE2) (6,7), followed by viral fusion into the cell. Subsequently, the active viral replication and release of the virus from lung cells lead to the development of symptoms (8).

COVID-19 is characterized by fever, headache, dry cough, dyspnea, and pneumonia. Although most SARS-CoV-2 infections are not severe, some patients are required to be hospitalized (9). The host immune system produces SARS-CoV-2 specific antibodies and T cells that can bind to viral proteins through their antigen receptors and then begin to secrete molecules that help control the infection. Single-cell RNA sequence analysis of bronchoalveolar lavage fluid of COVID-19 patients revealed an increase in CD8 T cell infiltrate with clonal expansion (10). The recovery from disease indicates the development of adequate adaptive immunity that is successful in the fight against infection (11), and dysregulation in host immune response to viral infection results in immunopathology (12-13). It is found that disease severity is associated with lymphocytopenia and an increase in the level of pro-inflammatory cytokines, such as interleukin 6 (IL-6), interleukin-5, and interleukin13 (14-16).

Acute respiratory distress syndrome (ARDS) may develop from excessive inflammation and lymphocytopenia. Cell destruction causes the patients to require the mechanical ventilator for several weeks or it may even lead to death (17). Protective immunity mainly arise from T cell detected in the blood of convalescent COVID-19 patients with antiviral activity (18,19), and in recovery patients with asymptomatic to mild disease, SARS-CoV-2 specific antibody starts to decrease after 2-3 months from recovery (20). These antibodies can neutralize the virus and prevent infection (21). Health care workers (HCW) are more susceptible to infection and reinfection than other fractions of the population due to close contact with the virus (22). Therefore, they are needed for longitudinal studies with longer time frames to discover and analyze the key features of SARS-CoV-2 adaptive immunity.

In this study, blood was collected from convalescent HCW to investigate how long SARS-CoV-2 humoral and cellular immunity could last in the circulation after recovery from COVID-19.

2. Materials and Methods

2.1. Human Subjects

Convalescent COVID-19 HCW were selected from different stages of disease (mild, moderate, and severe) according to the WHO (23) classification. The WHO defined the stages of the disease as follows: 1) severity of the disease associated with oxygen saturation ≤ 89% at rest and signs of respiratory distress, such as severe dyspnea and chest pain, and 2) mild-moderate COVID-19 patient requires non-hospitalization and is associated with oxygen saturation higher than 90% at rest and symptoms, such as fever, headache, cough, shortness of breath, pneumonia, loss of smell and taste, nausea, vomiting, and diarrhea.

Convalescent HCW (n=191) were assigned to one of the four groups in the study. The samples were collected from patients one (n=45; up to 25 and 20 samples were in mild-moderate and severe stages, respectively), three (n=45; up to 25 and 20 samples were in mild-moderate and severe stages, respectively), five (n=53; up to 34 and 19 samples were in mild-moderate and severe stages, respectively), and eight months (n=48; up to 26 and 22 samples were in mild-moderate and severe stages, respectively) post COVID-19 recovery.

Up to 5 ml of the whole blood was collected from each donor. Up to 2 ml in serum separator tube was used in enzyme-linked immunosorbent assay (ELISA), and up to 3 ml blood was utilized in citrated tube for peripheral blood mononuclear cell (PBMC) isolation.

2.2. Peripheral Blood Mononuclear Cell Isolation

PBMC was isolated from citrated blood by density gradient sedimentation as previously described (24) to prepare a pure population ready for use in MTT assay. For PBMC isolation, the whole blood was diluted with RPMI 1640 (US biological, USA) media and gently layered above the equal volume of lymphosep (biowest,

France) in Falcon tube and centrifuged for 20 min at 400G. In the end, erythrocytes and granulocytes that have a higher density than mononuclear cells settled as sediments to the bottom of tubes. Mononuclear cells forming a creamy thin layer were observed at the interface between plasma and lymphosep. Mononuclear cells were gently removed by automatic pipette and washed twice using washing buffer (phosphate buffer saline [PBS] and 2% fetal bovine serum) or RPMI 1640 media. Complete RPMI 1640 media was added to pelleted cells to make the final lymphocyte suspension. The cells were counted, and the viability percentage was estimated using a hemocytometer and Trypan blue staining.

2.3. Recombinant SARS-CoV-2 S1 Preparation

Recombinant SARS-CoV-2 S1 (Elabscience, USA) at the concentration of 50 ug/ml was diluted with PBS (Bioworld, USA) to reach the concentration of 10 ug/ml and stored at -20˚C in aliquots to minimize freezing/thawing cycles.

2.4. Proliferation Assay (MTT Assay)

As described in a previous study (25), the final PBMC concentration adjusted to be 1×10^6 cell/ml and approximately 13×10^4 cells in 135 ul complete RPMI 1640 media was plated for each COVID-19 convalescent individual. Following that, it was incubated with recombinant SARS-CoV-2 S1 protein at the concentration of 1 ug/ml for three days at 37˚C. Negative control wells lacked the recombinant protein. Afterward, the centrifugation, followed by the aspirate of the media was conducted, and 50 ul of MTT (Elabscience, USA) was added to each well according to the instruction kit and incubated for 3 h at 37˚C. At the end of time, the supernatant was removed by automatic pipette, and 100 ul of dimethyl sulfoxide was added, shook for 10 min, and mixed by pipetting to completely dissolve formazan crystals. It was then read using an ELIZA reader at 490 wavelengths.

The proliferation of cells=(OD of stimulated cell with protein/ OD of negative control)×100%

2.5. Serology

Anti SARS-CoV-2 RBD IgG antibody was detected using ELISA **(**Sunlong biotech**,** Cat num. SL321Hu-1, China). The procedure was done according to the manufacturer's protocol. Firstly, 100 ul of diluted samples and standard were added into appropriate wells and incubated for 1 h at 37˚C. The liquid was removed, and 100 ul of biotin-conjugated antibody (1×) was added into each well for 30 min at 37˚C. The solution was removed and washed three times with 300ul of wash buffer (1×). The remaining liquid was removed by snapping the plate onto absorbent paper, and 100 ul of streptavidin-conjugated HRP (1×) was added to each well and incubated for 30 min at 37˚C. Subsequently, the washing process was repeated 5 times, and 90 ul of TMB substrate was added to each well and incubated for 15 min at 37˚C. Following that, 50 ul of stop solution was added to each well and mixed by tapping the side of the plate. The plate was read using a microplate reader at 450 nm.

3. Results

3.1. SARS-CoV-2 Cell-Mediated Immunity Started from One Month and Continued to Eight Months Post Recovery

To assess the virus-specific cell-mediated immunity (CMI), peripheral blood mononuclear cells were isolated from 122 convalescent HCW, and it was then treated with SARS-CoV-2 S1 protein, followed by MTT assay to measure the proliferation of SARS-CoV-2 memory cells. SARS-CoV-2 CMI was found to be developed in convalescent HCW one-month post recovery and was established eight months post recovery. For 28 convalescent subjects at one-month post recovery (17 non-severe and 11 severe cases), the mean values of the proliferation index (PI) with S1 protein were obtained at 55.53 and 17.25 for severe and mild-moderate cases, respectively, which were significantly higher in severe cases (P>0.05). This suggests the development of specific SARS-CoV-2 immune cells as shown in figure 1.

For 39 convalescent subjects at eight months post recovery (20 severe and 19 non-severe cases), the result showed that the mean values of PI with S1 protein for severe and mild-moderate cases were 114.34 and 140.13, respectively, as shown in figure 2. This suggests an established SARS-CoV-2 specific cellular immunity after eight months from COVID-19 recovery.

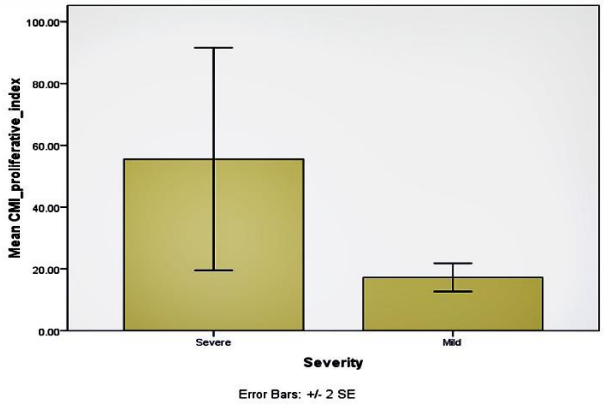

Figure 1. Proliferation index with S1 protein of the health care workers after one-month recovery

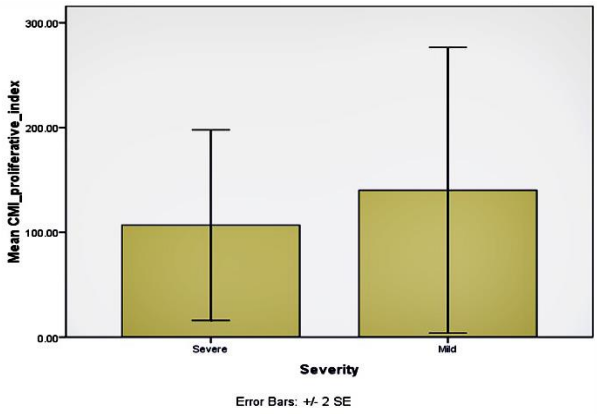

Figure 2. Peripheral blood mononuclear cell proliferation with S1 protein of the health care workers after eight-month recovery

3.2. Characteristics of SARS-CoV-2 cell-mediated immunity in mild-moderate convalescent subjects

The CMI was analyzed in a total of 72 mild-moderate convalescent subjects as shown in figure 3. The mean values of lymphocytes PI with S1 protein were 17.2553, 40.6836, 40.4574, and 140.13 for one, three, five, and eight months, respectively. Intriguingly, the PI mean value for eight months was higher than the PI values of one, three, and five months. Furthermore, the PI mean values for three and five months were higher than that of the one month.

The lymphocyte PI was then compared using boxplot which is a standardized way of displaying the distribution of data based on a five-number summary (minimum, first quartile [Q1], median, third quartile [Q3], and maximum); moreover, it can indicate outliers and what their values are. Boxplot showed that the maximum and Q3 were increasing steadily from one month to eight months post recovery; however, the median, unlike the mean values, were not increasing steadily as shown in figure 4. However, there was an insignificant difference between lymphocytes PI in one and three months (P=0.26), one and five months (P=0.18), as well as one and eight months (P=0.08).

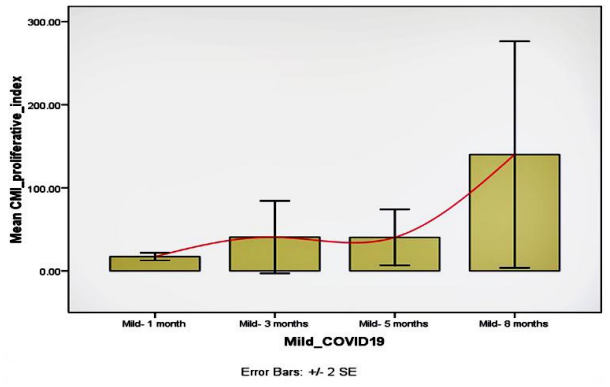

Figure 3. Cell-mediated immunity of mild-moderate COVID-19 recovered health care workers

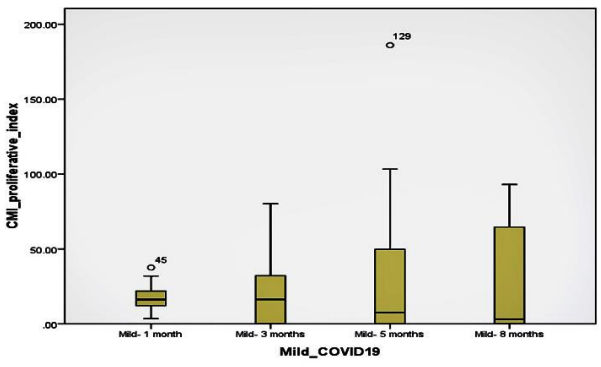

Figure 4. Lymphocytes proliferation index of mild-moderate COVID-19 recovered health care workers using a boxplot

3.3. Characteristics of SARS-CoV-2 Cell-Mediated Immunity in Severe COVID-19 Convalescent Subjects

The CMI was analyzed in a total of 50 convalescent severe subjects. The mean values of PI with S1 protein were 55.5325, 222.3081, 97.4961, and 114.3402 for one, three, five, and eight months, respectively. Lymphocytes PI for three months was higher than those of one, five, and eight months as shown in figure 5. There was an insignificant difference among one and three months (P=0.4), one and five months (P=0.41), as well as one and eight months (P=0.3).

The CMI of severe cases was compared using a boxplot. The result showed that the maximum and Q3 of lymphocytes PI were highest in the group five months post recovery, followed by eight months post recovery as shown in figure 6.

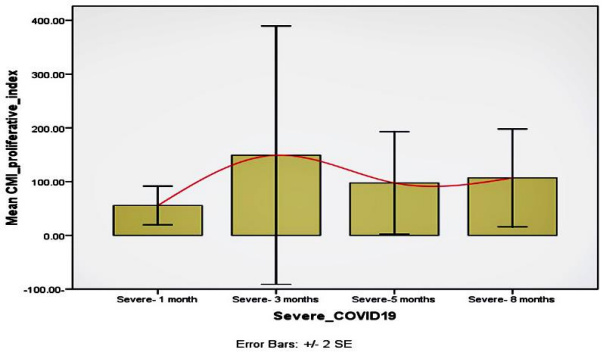

Figure 5. Proliferation index with S1 protein of severe COVID-19 recovered health care workers

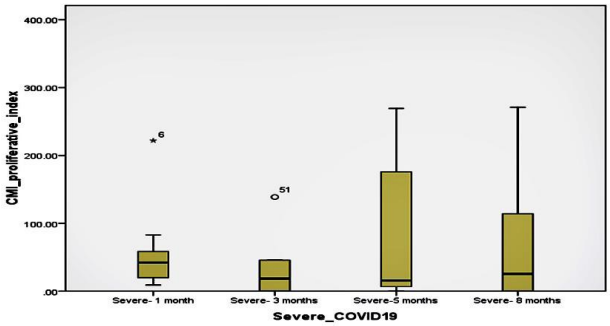

Figure 6. Proliferation index with S1 of severe COVID-19 recovered health care workers using a boxplot

3.4. Convalescent COVID-19 Health Care Workers Exhibited Persistent Anti-RBD IgG Antibodies Till Eight Months after Recovery

In order to investigate the antibody response against SARS-CoV-2 over time, a total of 181 samples were obtained from symptomatic COVID-19 HCW collected one, three, five, and eight months post recovery. The serum samples were analyzed for IgG recognizing RBD of spike SARS-CoV-2 using a quantitative indirect ELISA. The mean values of anti-RBD IgG for one, three, five, and eight months were 4.86, 3.8, 4.48, and 4.35, respectively. Antibody titer was sustained at high levels for eight months post recovery. However, the mean values of serum SARS-CoV-2 IgG were not significantly different from those at post recovery (P>0.05) as shown in figure 7.

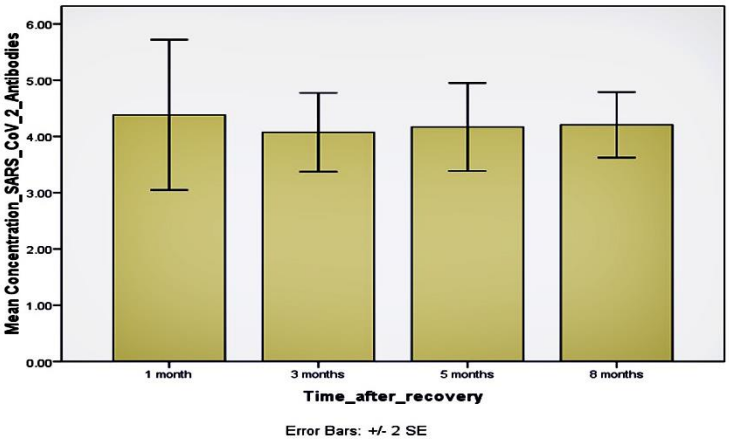

Figure 7. Anti-RBD antibody at one, three, five, and eight months of COVID-19 recovered health care workers

3.5. Severe Convalescent COVID-19 Health Care Workers Exhibited Persistent High Mean Anti-RBD IgG Antibody Levels and an Increasing Trend of Maximum and Third Quartile Levels

Antibody titer of a total of 79 severe convalescent HCW was analyzed in this study. The mean values of antibody titer for one, three, five, and eight months were 5.03, 4.47, 3.73, and 4.76, respectively. Intriguingly, post-recovery groups of severe convalescent subjects showed approximately similar levels of anti-RBD IgG as shown in figure 8. There was an insignificant difference regarding the mean level of anti-RBD IgG among one and three months (*P*=0.71), one and five months (*P*=0.36), as well as one and eight months (*P*=0.85). This suggests that antibody titers in severe HCW were not affected much by post-COVID-19 recovery time. However, the maximum and Q3 values of anti-RBD IgG were strangely found to have an increasing trend with post-recovery duration as these values were higher in eight and five months, compared to the earlier time intervals as shown in figure 9.

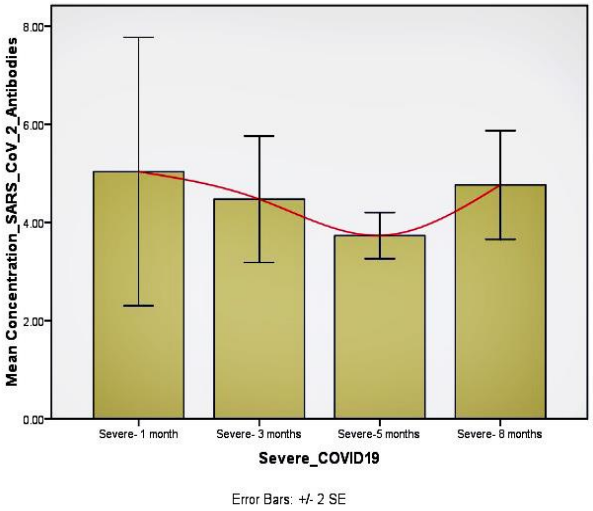

Figure 8. Anti-RBD IgG titer of severe convalescent COVID-19 health care workers

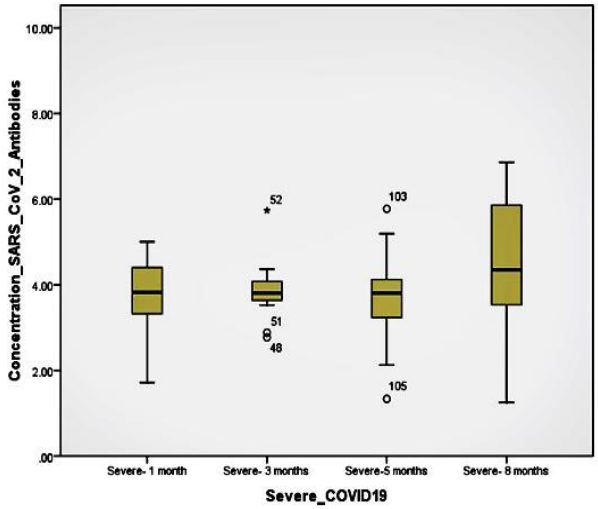

Figure 9. Anti-RBD IgG titer of severe convalescent health care workers using a boxplot

3.6. Mild-Moderate Convalescent Health Care Workers Exhibited Persistent Moderate Mean Values of Anti-RBD IgG Antibodies with Comparable Maximum and Third Quartile Values

Antibody titer of a total of 102 convalescent HCW was analyzed in this study. Intriguingly, mild groups showed an approximately similar amount of anti-RBD IgG. The mean values of antibody titer were 3.7925, 3.7265, 4.4353, and 3.7785 for one, three, five, and eight months, respectively as shown in figure 10. There was an insignificant difference among one and three months (*P*=0.88), one and five months (*P*=0.35), as well as one and eight months (*P*=0.91). For the boxplot analysis, again the median, maximum and Q3 values were close to each other over the time intervals from one to eight months post recovery as shown in figure 11. These findings indicate that humoral immunity in mild-moderate convalescent HCW was not waning quickly along with post-COVID-19 recovery time.

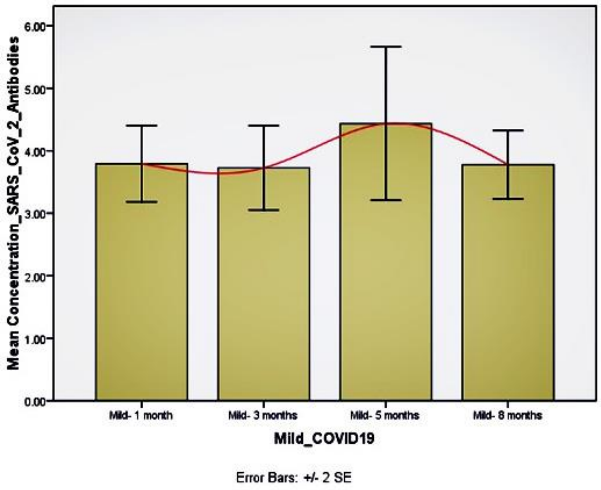

Figure 10. Anti-RBD IgG titer of mild-moderate convalescent COVID-19 health care workers

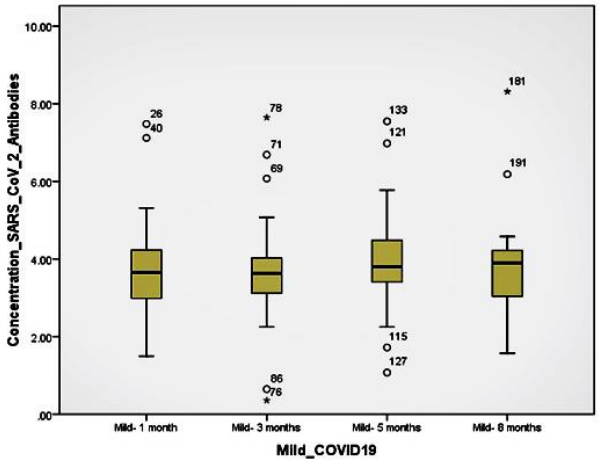

Figure 11. Anti-RBD IgG titer of mild-moderate convalescent COVID-19 health care workers using a boxplot

3.7. Interplay between Disease Severity and Cell-Mediated Immunity

SARS-CoV-2 CMI in a total of 50 severe and 72 mild-moderate cases was analyzed in this study. PI was compared between mild-moderate and severe cases over the post-recovery time intervals using the ANOVA test. The findings showed that lymphocytes PI was insignificantly correlated with disease severity (*P*=0.36) as demonstrated in figure 12.

Furthermore, the CMI of mild-moderate and severe cases in each time of the study was analyzed using the t-test. In the one-month group (11 severe and 17 mild-moderate cases), the results showed that the mean values of PI for severe cases (55.53) were significantly higher than those in the mild-moderate group (17.25) (*P*=0.05) as shown in figure 13. In the three-month group (15 mild-moderate and 9 severe cases), the results showed that the severe cases had insignificantly higher lymphocytes PI (*P*=0.27) as noticed in figure 14. In the five-month group (21 mild-moderate and 10 severe cases), the results showed that severe cases had insignificantly higher lymphocytes PI, compared to the mild cases (*P*=0.17) as shown in figure 15. In the eight-month group (20 severe and 10 mild-moderate cases), the results showed that the severe cases had insignificantly lower PI with S1 protein (*P*=0.68) as shown in figure 16.

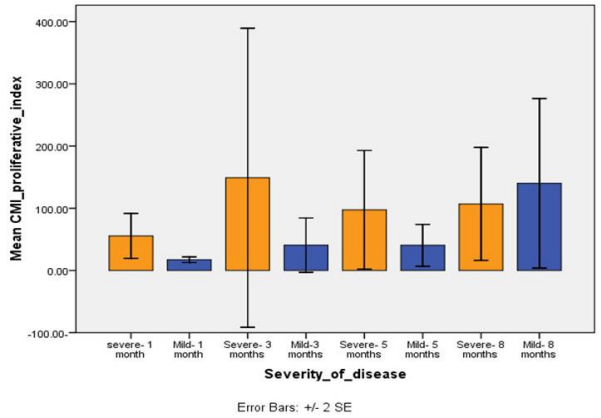

Figure 12. Proliferation index with S1 protein of mild-moderate and severe convalescent COVID-19 health care workers

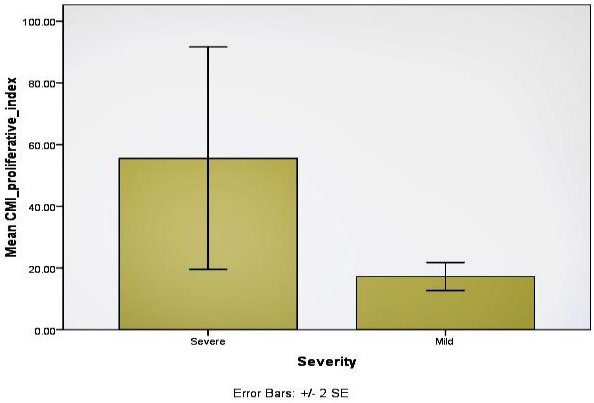

Figure 13. Proliferation index with S1 protein of mild-moderate and severe convalescent health care workers one month post-COVID-19 recovery

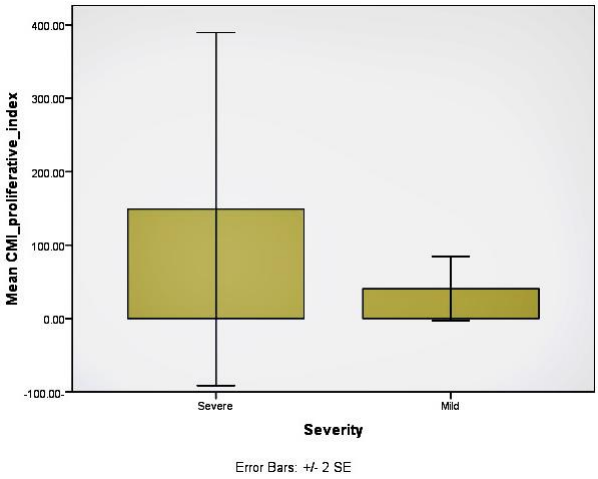

Figure 14. Proliferation index with S1 protein of mild-moderate and severe convalescent health care workers three months post-COVID-19 recovery

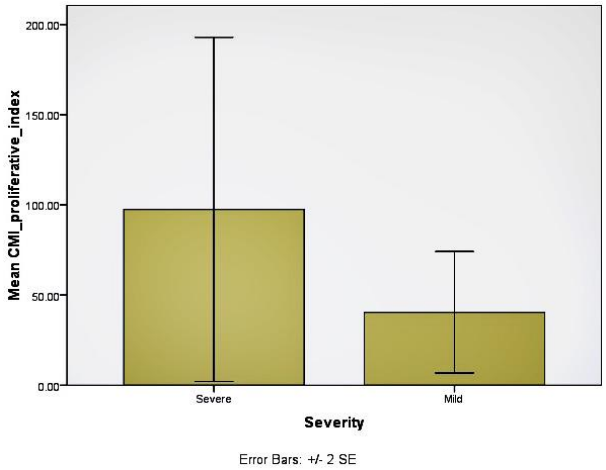

Figure 15. Proliferation index with S1 protein of mild-moderate and severe convalescent health care workers five months post-COVID-19 recovery

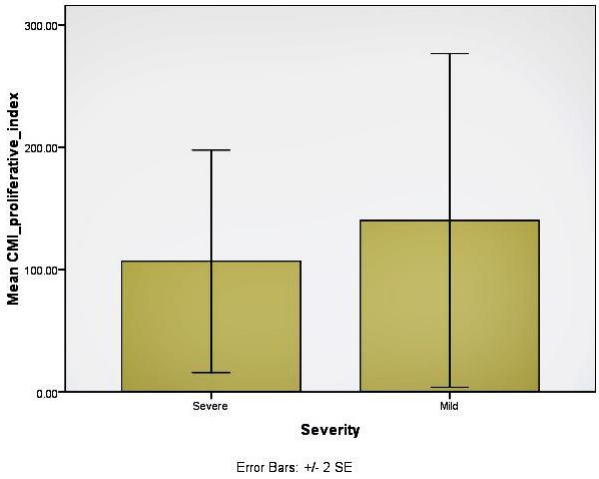

Figure 16. Proliferation index with S1 protein of mild-moderate and severe convalescent health care workers eight months post-COVID-19 recovery

3.8. Interplay between Disease Severity and the Titer of SARS-CoV-2 Anti-RBD IgG

Antibody titers of 102 mild-moderate cases and 79 severe cases were compared in this study. These cases based on the time of recovery were divided into four groups of one-month post recovery (22 non-severe and 20 severe cases), three-month post recovery (23 non-severe and 20 severe cases), five-month post recovery (31 non-severe and 19 severe cases), and eight-month post recovery (26 non-severe and 20 severe cases). Antibody titers of mild-moderate and severe cases were compared in each group using a t-test. There was no significant higher antibody response in severe cases at one (P=0.36), three (P=0.29), five (P=0.3), and eight months (P=0.09) as shown in figures 17, 18, 19, and 20, respectively.

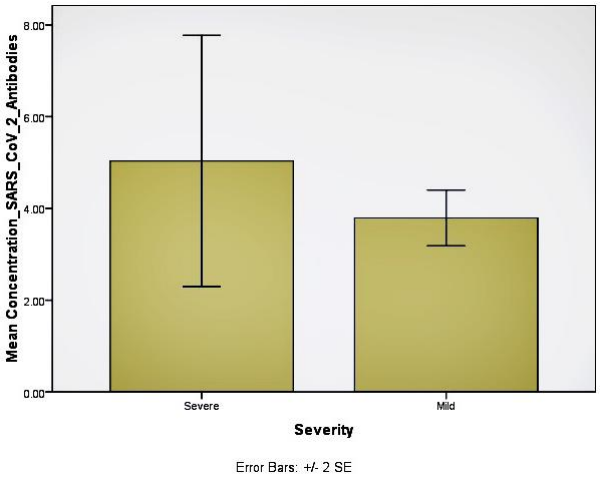

Figure 17. Antibody titers of mild-moderate and severe cases one month post-COVID-19 recovery

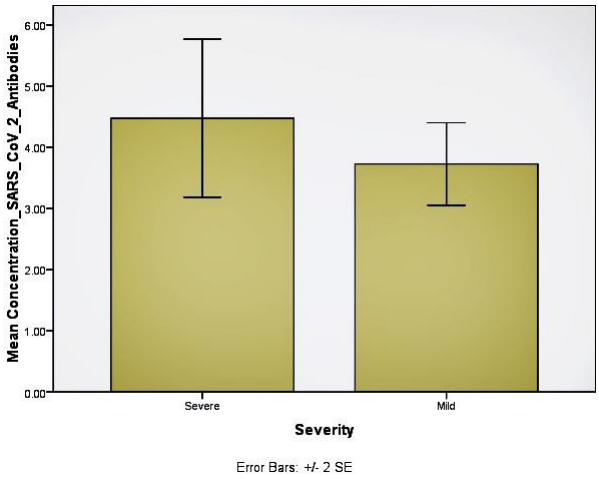

Figure 18. Antibody titer of mild-moderate and severe cases three months post-COVID-19 recovery

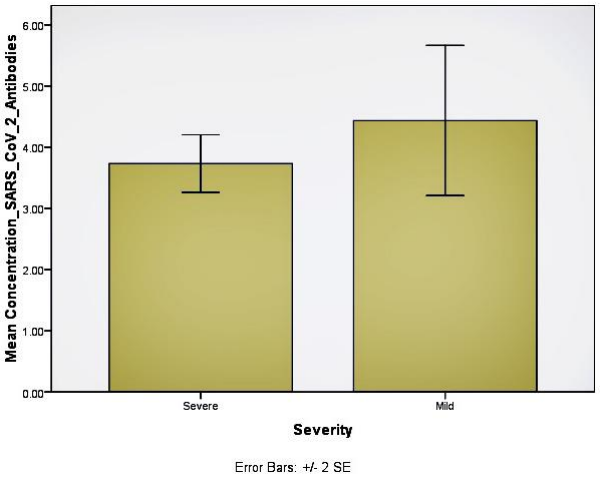

Figure 19. Antibody titer of mild-moderate and severe cases five months post-COVID-19 recovery

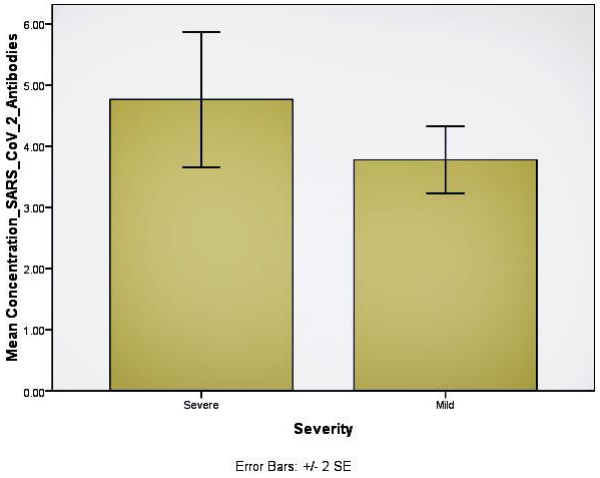

Figure 20. Antibody titer of mild-moderate and severe cases eight months post-COVID-19 recovery

4. Discussion

SARS-CoV-2 is globally spread, and in the absence of effective treatment, it is critical to understand the key features of adaptive immunity to develop effective strategies to control this pandemic. The knowledge about adaptive immunity remains limited. This study reported two branches of adaptive immunity, namely CMI via MTT assay and humoral immunity by indirect ELISA in convalescent HCW after mild, moderate, and severe COVID-19. It was observed that PBMC proliferation with S1 protein was at higher levels, compared to the PBMC proliferation without S1 protein. These findings suggest that S1 protein is a strong stimulator to immune cells. A previous report stated that T cells produce abundant levels of interferon-gamma (IFN-y) once exposed to SARS-CoV-2 RBD (26). Therefore, the S1 protein is suited to detect and analyze the SARS-CoV-2 immune response. The SARS-CoV-2-specific CMI was remarkably primed after one month from recovery. Data from previous research found that SARS-CoV-2-specific T cells were detected in the blood of COVID-19 patients and in recovered patients even in the absence of SARS-CoV-2-specific circulating antibodies (27, 28). These

data are consistent with our results suggesting that these cells most likely have a pivotal role in the protection and recovery from COVID-19.

The current study indicated a significant proliferation of peripheral blood lymphocytes with S1 protein, compared to lymphocytes PI without S1 protein in convalescent subjects after eight months from recovery. This suggests the potential of SARS-CoV-2 infection in generating long-term immunity against the virus. The difference in lymphocytes proliferation with S1 protein among study intervals (one, three, five, and eight months post recovery) in mild-moderate convalescent HCW was insignificant; however, an increasing trend in the level of SARS-CoV-2-specific lymphocytes was evident. Similar to mild-moderate cases, the severe convalescent groups showed consistently high with an increasing trend of cellular immunity specific to SARS-CoV-2 cells. In line with other studies, T cells were found to be generated in asymptomatic as well as severe COVID-19 cases with the production of high levels of antiviral cytokines and cytotoxic activities (29, 30). Moreover, high levels of T cells were demonstrated in the peripheral blood of severe COVID-19 patients with ARDS along with the production of remarkable levels of IFN-y and tumor necrosis factor-α in response to viral antigen (31). Altogether, the findings of the current study prove that convalescent HCW were in continuous re-exposure to the virus without the necessary development of symptoms. This explains why the cellular immunity in convalescent HCW was increasing instead of waning with time after infection. This also shows that SARS-CoV-2 memory cells have a role in preventing symptomatic re-infection/lowering the severity of disease for at least eight months after primary symptomatic infection.

In one month, severe cases showed significantly higher lymphocyte PI, compared to mild-moderate cases (P=0.05). In three-, five-, and eight-month intervals, lymphocyte PI of severe cases was a bit higher than that in mild-moderate cases; however, this difference was not statistically significant. These findings indicate that cellular immunity has a role in COVID-19 severity, and the severity of the disease affects as well the course of activation of the cellular immunity. It was found that the viral antigens of SARS-CoV-2 proportionally activate alveolar macrophages that trigger the production of a wave of pro-inflammatory cytokines and chemokines, such as IL-6 and IFN-y that in turn activate T cells to destroy viral infected cells (32). However, the difference in the activation level of cellular immunity between severe and mild-moderate groups was not remarkable. This might be attributed to the fact that most severe cases that participated in this study were not critical cases, and many of them had an oxygen saturation of above 80% that may mount the immune response similar to that in moderate cases.

Anti-spike SARS-CoV-2 antibody was detected in COVID-19 recovered patients (33). These antibodies may block the binding of RBD to ACE2 receptor and prevent the entry of virus to the cells suggesting that these antibodies have a role in recovery from disease and exert a neutralizing effect. It is crucial to measure the level and duration of anti-RBD antibodies in recovered COVID-19 patients.

Recent research to understand the longevity of humoral immunity observed that anti-RBD IgG was detected in convalescent subjects after eight months post-onset of symptoms, and the level of these antibodies decrease over time (34). In our study, anti-RBD IgG antibodies in convalescent HCW were at high levels after one, three, five, and eight months post recovery from symptomatic COVID-19. The level of anti-RBD IgG antibodies among these different post-recovery time groups was not significantly different (P>0.05). In mild-moderate convalescent subjects, it was observed that the level of anti-RBD IgG antibodies was approximately consistent and similar from one to eight months post recovery. The results obtained from the analysis by boxplot showed that the median, Q3, and maximum values were close to each other in the studied recovery groups. Furthermore, in the analysis of the results the anti-RBD IgG antibody levels in convalescent severe cases, antibody level was found to

be consistently high with an insignificant difference among one-, three-, five-, and eight-month groups; nevertheless, an increasing trend of maximum and Q3 values was found at five and eight months when compared to one- and three-month groups

Consistent with the findings of other research, the duration of humoral immune response in mild-moderate COVID-19 subjects was found to persist 4-8 months post-infection. This is an important finding as anti-RBD IgG antibodies were found to be correlated strongly with neutralizing antibodies (35, 36), and the current study results indicate that the studied recovered HCW subjects maintained high levels of anti-RBD IgG antibodies for at least eight months which is a long time in terms of Cornoaviridae-triggered humeral immune response. This can be attributed to the fact that recovered HCW are in continuous exposure to re-infection inside hospitals setting and multiple asymptomatic reinfections might be the reason behind the persistence of high anti-RBD IgG antibodies levels over eight months after recovery. However, these results might not fit with the findings obtained from the recovered COVID-19 patients other than those HCW working in hospitals.

Both humoral and cellular immunity screened in COVID-19 recovered HCW were found to persist at high levels for at least eight months post recovery. This might not apply to people who do not work in the health care sector. The findings of the current study indicate that COVID-19 recovered or vaccinated HCW might not need additional doses of vaccination as like as people outside of the health care sector. This actually needs further research to prove or refute this conclusion.

There is a notion that severe COVID-19 has higher viral titer and higher viral antigens leading to stronger inflammation and stronger humoral immune response, especially in patients who need hospitalization (37, 38). In the current study, this notion was not clear may be due to the fact that most of the involved severe HCW had short hospitalization and no critical condition. In addition, the number of participants may not be as high as needed to reach a statistical significance difference of IgG antibodies between mild and severe cases. Overall, this study provided evidence that mild, moderate, and severe cases of recovered HCW exhibited good immunity for relatively long-time post recovery. Taken together, the findings of the current study reveal that the convalescent HCW with mild, moderate, and severe disease exhibit a good with relatively long-term humoral and cellular immunity to SARS-CoV-2. This immunity could last more than eight months after recovery. This indicates a robust adaptive immunity that is developed by the natural infection.

Authors' Contribution

Study concept and design: I. H. A.
Acquisition of data: A. S. A.
Analysis and interpretation of data: I. H. A.
Drafting of the manuscript: A. S. A.
Critical revision of the manuscript for important intellectual content: I. H. A.
Statistical analysis: A. S. A.
Administrative, technical, and material support: I. H. A.

Ethics

All the procedures were approved by the Ethics Committee at the Al-Nahrain University College of Medicine, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

None

Acknowledgment

The authors would like to thank all convalescent health care workers for their donation to participate in this study

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