The reduction of SARS-Cov-2 S1-RBD IgG after four months of covid-19 infection of the medical staff at the Neurosurgery Teaching in Baghdad

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Abstract
Background The SARS-CoV-2 pandemic continues to negatively impact the healthcare system globally despite the availability of vaccination since late 2020.Until July 2022, Iraq registered 2,438,101 million COVID-19 cases and 25,304 deaths putting Iraq in third place among the Eastern Mediterranean Sea countries. Methods This study included ninety volunteered medical staff whois working in the Neurosurgery Teaching Hospital and were diagnosed with COVID-19 by PCR and accepted to give 5 ml of their blood.The medical staff was categorized into two groups and every group containforty-five, we collected bloodfrom the first group after one month (30 days) from the day of COVID-19 infection diagnosis while the second group after 4 months (120 days).We used an ELISA kit (Diacino R: cat.No. DS 207704, china) which is an indirect ELISAto estimate Cov-19 –S1-RBD(anti-spike). Results the independent two-sample Mann–Whitney test was used which showed that there was a significant decrease ($P<0.05$) in the SARS Cov-2 S1-RBD serum levels of the volunteered medical staff after 4 months compared to the one month. Conclusion SARS – CoV-2 S1- RBD IgGsignificantly decreasedafter 4 months (120 days).
Keywords: COVID-19, SARS – CoV-2 S1- RBD IgG,Neurosurgery Teaching in Baghdad

1. Introduction

The SARS-CoV-2 pandemic continues to negatively impact the healthcare system globally spite the availability of vaccination since late 2020. In the fall of 2021, the worldwide health system experienced another exponential rise in case volume, which was attributed to several factors including a more virulent strain of SARS-CoV-2, B.1.617.2 (Delta), incomplete global vaccine uptake, and potentially waning vaccine-induced immunity. Healthcare workers have more risk of encountering infectious diseases such as COVID-19 than the normal population. (1).
Globally the WHO reported until July 2022, 572, 239,451 confirmed cases of COVID-19 including 6, 390, 401 deaths. In Iraq, COVID-19 infections began to appear in February 2020, specifically in Najaf province when an infected Iranian student who had entered Iraq before the prohibition by COVID-19 was announced there. Cumulatively and until July 2022, Iraq registered 2,438,101 million COVID-19 cases and 25,304 deaths putting Iraq in third place among the Eastern Mediterranean Sea countries (2).
The SARS-CoV-2 virus is made up of four major structural proteins which include the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. The S protein responsible for recognizing the host cellular receptor to initiate virus entry is divided into S1 and S2 domains. The S1 domain determines receptor recognition via the receptor binding domain (RBD) while the S2 protein plays a role in fusion and entry (3,4).
Antibodies are generated as part of an individual's immune response against SARS-CoV-2 and are produced by cells known as B cells. Antibody tests can detect these antibodies in the blood or saliva of individuals previously infected and/or vaccinated against SARS-CoV-2 (5). IgG antibodies can be reliably detected 14 days after a SARS-CoV-2 infection or vaccination but are longer lasting than IgM. Depending on the epitope and factors such as disease severity, duration of infection, age, genetic factors, co-morbidities, and the performance characteristics of the test used, IgG spike antibodies can be detected in most individuals for at least one year (6,7). In most individuals, antibody levels peak four to five weeks after infection and will decrease in subsequent months after the infection has cleared (8). In addition to antibodies produced by B cells, memory T cell responses are also likely to contribute to protection, particularly from severe disease. Furthermore, as serum antibodies decline over time, long-lived memory B cells activate and expand quickly upon re-exposure to SARS-CoV-2, rapidly generating antibodies to compensate for waning immunity (9).
Our aim of the study is to evaluate the SARS – CoV-2 S1- RBD IgGin the medical staff at the neurosurgery teaching in Baghdad who were infected with COVID-19 by measuring its serum level

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after 1 month (30 days) and 4 months(120days) of infection.

2. Materials and Methods

The research was approved by the Ethical Review Committee in the College of Medicine at Al-Iraqia University. This study included ninety volunteered medical staff who worked in the Neurosurgery Teaching Hospital and were diagnosed with COVID-19 by PCR and accepted to give 5 ml of their blood. Blood samples were collected from December 2021 to March 2022 in the virology laboratory inside the Neurosurgery Teaching Hospital and the demographic data have been collected by a questionnaire sheet. The medical staff was categorized into two groups and every group containforty-five, we collected bloodfrom the first group after one month (30 days) from the day of COVID-19 infection diagnosis while the second group after 4 months (120 days). The collected blood was put in a gel tube which was left at room temperature for about 10 minutes for clotting then centrifuged at 3600 Xg to gain serum. The collected serum was transferred into several Eppendorf tubes with 500 µL and kept at a frozen temperature-20 C° to be analyzed later to estimate Cov-19 –S1-RBD (anti-spike). We used an ELISA kit (Diacino R: cat.No. DS 207704, china) which is an indirect ELISA, and according manufactured the sensitivity of the quantification kit is less than 98.41% while the specificity is 98.02%.

The quantitative results have been studied according to the ratio of the extinction of the monitored or tested pattern to the extinction of the calibrator. The resulting data were counted by determination of the mean absorbance of each duplicated measurement. The mean calculation then was made by plotting the common logarithm of absorbance against concentration in DU/ml for each calibrator of the SARS-Cov2-S1- RBD IgG ELISA Kit.

Statistical analysis was made by STATISTICA version 12 in addition to SPSS statistical software v.26. The distribution standard was analyzed preliminarily by Kolmogorov–Smirnov, and Shapiro–Wilk tests. Categorical values havebeen expressed as absolute and relative frequencies. The distinction between the groups for persistent as well as categorical variables was made by the non-parametric Kruskal–Wallis test which deals with more than two groups and by the Mann–Whitney U-test which deals with Bonferroni's correction when necessary, to find out the differences between 1 and 4 months of SARS anti-spike IgG plasma levels (P•0.05).

3. Results

The demographic data like the age and gender of the ninety volunteered in 1 month and 4 months were studied and presented in table 1 and table 2.

Table 1. The Frequency and Percentage of Gender after one month

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Female	11	24.4	24.4	24.4
Male	34	75.6	75.6	100.0
Total	45	100.0	100.0	

Table 2. The Frequency and Percentage of Gender after four months

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Female	16	35.6	35.6	35.6
Male	29	64.4	64.4	100.0
Total	45	100.0	100.0	

After obtaining the ELISA results of SARS-Cov-2 S1-RBD IgG serum levels (1months) and (4 months), the data were analyzed according to the Kolmogorov–Smirnov, and Shapiro–Wilk tests as they were non-parametric.

Accordingly, the independent two-sample Mann–Whitney test was used which showed that there was a significant decrease (P<0.05) in the SARS Cov-2 S1-RBD serum levels of the volunteered medical staff after 4 months compared to the one month as shown in table 3 and 4.

Table 3. The normality Test for Covid-19 S1 serum level after 30 and 120 days

Variable		COVID-19 (1 month)	CCOVID-19 (4 months)
N		45	45
Normal Parametersa,b	Mean	1.1434	.8106
	Std. Deviation	.78116	.75053
Most Extreme Differences	Absolute	.147	.180
	Positive	.147	.180
	Negative	-.093	-.162
Test Statistic		.147	.180
P (2-tailed)		.015c	.001c

Table 4. Independent two-sample Mann-Whitney test for Covid -19 S1 serum levels after 1 and 4 months

Variable	The Medical Staff infected with COVID-19	Mean Rank	Z	P-value. (2-tailed)
Covid-19- S1 IgG serum level	1 Month	51.72	-2.261	.024
	4 Months	39.28		

4. Discussion

COVID-19 infections can effectively induce the immune system and produce potent neutralizing immunoglobulins that have the ability to distinguish viral antigens and block the infectivity of the virus. As a result, most SARS-CoV-2 vaccines are designed for

stimulation of antibody generation against the spike SARS-CoV2 protein. Thus, measuring the prevalence of anti-S1-RBD IgG levels could supply valuable insights into acquired immunity to SARS-CoV-2(10).

There is a strong debate concerning the nature, stability, and durability of antibody responses over time in COVID-19 patients, with several studies reporting rapidly waning antibody immunity, or late appearance with low antibody levels, and/or complete lack of long-lasting antibodies (11, 12) which is similar to our study results that showed a significant decrease by time (120days) after the COVID-19 infection diagnosis compared to (30 days).

It was found that impaired cell counts of peripheral lymphocytes including CD3+, CD8+, and CD4+ T cells were the notable features of SARS-CoV-2 patients in the early time of infection, which is similar to HIV infection.Together with the restoration of periphery lymphocyte pools, the anti-S IgG and IgM increased and reached a certain level after treatments(13).

Therefore, the recovery of lymphocyte numbers might be a significant event associated with the induction of viral-specific anti-IgG and IgM antibodies. This is entirely rational. It is well known that CD4+ T cells play an essential role in mediating adaptive humoral immunity. Upon viral infection, host CD4+ T cells are activated and differentiated into functional subsets, including type 2 help T cells (Th2) to assist B cell activation and trigger humoral immunity. In fact, our previous study reported that CD4+ T lymphocyte count at admission was inclined to predict the duration of SARS-CoV-2 viral RNA detection (14), total lymphocyte and CD4+ T cell counts and the CD4/CD8 ratio increased in almost all patients, with the clearance of SARS-CoV-2 at discharge. Interestingly, we also observed that the hospital stay was negatively correlated with the anti-S IgG titer, suggesting that anti-S IgG might be protective in COVID-19 patients. So, the recovery of T cell homeostasis with antibody induction might be indicative of the clearance of the virus in the rehabilitation of COVID-19 patients. Being a newly emerging virus, the generation kinetics of SARS- CoV-2-specific IgM and IgG is very important for diagnosis and treatment. According to the WHO's recommendation, the estimated latent period for COVID-19 infection is suggested to be around five days ranging from 1–14 days (WHO). We have observed that the durations to induce high levels of both anti-S IgG and IgM upon SARS-CoV-2 infection were 14 days and ten days, respectively. Another interesting issue is whether viral-specific IgA levels are generated earlier due to the airborne infection, which can be investigated (15).

A cohort study of 56 convalescent patients with SARS-CoV has shown that virus antigen-specific IgG antibodies peaked at month four after the onset of the disease but decreased after that (16,17).

Conclusion:

SARS – CoV-2 S1- RBD IgGsignificantly decreasedafter 4 months (120 days).

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