Oncology
and Radiotherapy©
18(8) 2024: 001-005 • RESEARCH ARTICLE

Evaluation of humoral response in COVID-19 Saudi patients

Nuha A. Alkhattabi, Nesrin I. Tarbiah

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT

Background: SARS-Cov-2 caused a viral pandemic that started in late December 2019. This study aimed to investigate the humoral immunity response against SARS-Cov-2 by evaluating the B cell counts and serum levels of IgG and IgM in different groups of patients.

Methods: A total of 74 samples were collected from different groups of confirmed COVID-19 patients to evaluate the concentrations of IgG and IgM in groups including control, mild, severe, and asymptomatic. In addition, 84 samples were collected for CD19+ and CD27+ B cells analysis in the same group.

Results: Levels of IgG and IgM were found to be significantly elevated in COVID-19 patients; the severe groups showed the highest level among the groups of patients. Furthermore, a significant decrease in B cell counts in patients was observed.

Conclusion: Peripheral B cells, IgG and IgM were shown to be related to the severity of the disease. While CD19+ and CD27+ B cells tended to decrease in groups of patients, antibodies' concentrations increased along with disease severity.

Keywords: COVID-19, SARS-CoV-2, CD19+ B cells, CD27+ B cells, IgG, IgM

Address for correspondence:

Nuha A. Alkhattabi and Nesrin I. Tarbiah

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

E-mail: naalkhattabi@kau.edu.sa; ntarabah@kau.edu.sa

Word count: 2951 **Tables:** 02 **Figures:** 04 **References:** 31

Received: 19 July, 2024, Manuscript No. OAR-24-142325
Editor Assigned: 23 July, 2024, Pre-QC No. OAR-24-142325(PQ)
Reviewed: 08 August, 2024, QC No. OAR-24-142325(Q)
Revised: 16 August, 2024, Manuscript No. OAR-24-142325(R)
Published: 24 August, 2024, Invoice No. J-142325

INTRODUCTION

In late December 2019, a viral pneumonia epidemic began in Wuhan, China caused by a novel coronavirus, which was later named the severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2), and the coronavirus disease 2019 (COVID-19) the official name of the disease caused by the new virus [1, 2]. COVID-19 characteristics and outcomes are highly variable ranging from asymptomatic or mild symptoms such as fever, cough, and fatigue to more severe symptoms with serious complications including multiple organ failure, Acute Respiratory Distress Syndrome (ARDS), and even death [3, 4].

The immune system responds to the SARS-Cov-2 infection by activating innate and adaptive immunity and their cellular and humoral components. B cells, CD8+ and CD4+ T cells (cytotoxic T cells and helper T cells) are three major components of adaptive immunity that work co-ordinately with innate immunity against viral infections [5]. B cells play a crucial role by producing antibodies to target virus and infected cells, which in turn protect against infection via various mechanisms including neutralization, complement activation, Antibody-Dependent Cellular Cytotoxicity (ADCC), and Antibody-Dependent Phagocytosis (ADP) [6].

Immunoglobulin G (IgG) is the most abundant antibody response for long-term immunity in viral infection, the serum IgG concentration maintained high for several weeks after infection. While IgM is the first antibody produces after pathogen invasion and its concentration decline earlier than IgG [7, 8].

B cells are additionally the key component in the management of COVID-19 spread as it is the main target of vaccine development. Vaccination is one of the most effective ways to control pandemics and reduce complications. As of 4 September 2023, approximately 13.5 billion vaccine doses have been administrated globally [2].

In this study the aim was to investigate the humoral immunity response against SARS-Cov-2 by evaluating the B cell counts and serum levels of IgG and IgM in different groups of patients.

MATERIALS AND METHODS

Patients

The Institutional Review Board (IRB) of the Research and Studies Department—Jeddah Health Affairs, registration number KACST, KSA: H-02-J-002 research number 1373 was obtained.

Patients or guardians were notified and given the chance to give informed permission prior to the collection of the specimen. Blood samples were collected at King Abdulaziz Hospital in Jeddah-Saudi Arabia, the collected samples were aliquoted and stored at (-80) till the investigation was done. All the patients were clinically and radiologically diagnosed with COVID-19 and confirmed by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The patients were clinically classified according to the National Health Commission of China's New Coronavirus Pneumonia Prevention and Control Program [9].

Patient groups were divided as follows:

- Group 1 (Control) included healthy individuals of similar age and sex, who had not been immunized with any dose of vaccine.
- Group 2 (Asymptomatic) included patients with no clinical symptoms and chest imaging findings, with positive SARS-CoV-2 RT-PCR testing.
- Group 3 (Mild group) included patients with mild clinical symptoms, and no signs of pneumonia on the imaging examination.
- Group 4 (Severe group) included patients meeting any of the following: oxygen saturation ≤ 93% at rest, shortness of breath with a respiratory rate of ≥ 30 times/min, or an arterial oxygen partial pressure (PaO_2)/oxygen concentration (FiO_2) ≤ 300 mmHg (1 mmHg = 0.133 kPa)

Exclusion criteria

Patients with chronic infections (HCV, HBV), cancers, any immunological disorders or patients on immunosuppressive drugs or chemotherapy, any underlying haematological disorder, and laboratory and clinical signs of other infections were exclude.

Measurement of IgG, and IgM

A total of 74 blood samples were collected in serum separator (SST) tube (3 ml) and classified into 4 groups as illustrated in patients' section (group 1 (n=18), group 2 (n=20), group 3 (n=18), group 4 (n=18). Serum IgG and IgM levels were measured using Human SARS-CoV-2 spike IgG and IgM ELISA kits (Thermo Fisher, Cat#: BMS2325, BMS2324 respectively) following the manufacturer's instructions. The concentrations were determined by reading optical density at 450 using The BioTek 800 TS absorbance reader.

Flow cytometry analysis of B cells

For flow cytometric analysis blood samples were collected in a Na-Heparin tube (3 mL) as follow: (group 1 (n=14), group 2 (n=22), group 3 (n=22), group 4 (n=26)). Each sample was diluted 1:1 in PBS. This was followed by adding the diluted blood sample on Histopaque to make up the total amount of 60% diluted sample. Next, buffy coats were collected after centrifugation and then washed twice with PBS. Samples were prepared for flow cytometry analysis by adding the target fluorescent antibodies at the recommended dilution by the manufacturers, as follows: anti-CD19-PECy5 (C7066, DAKO), anti-CD27-FITC (340424, BD). This was followed by incubation at room temperature in the dark for 30 min. Finally, samples were analysed using flow cytometry FACS Aria 3 from BD Company followed by data analysis using FACSDiva version 9 software (BD Biosciences, San Jose, CA, USA). Lymphocytes were gated according to light scatter parameters that reflect cell morphological characteristics. At least 10,000 events were assessed for each sample.

Statical analysis

Statical analyses were performed using Prism GraphPad software version 9.5.0 and presented as mean ± Standard Error of the Mean (SEM). One-way ANOVA test was used to compare the difference between normally distributed variables followed by Tukey's multiple comparison test to identify significant differences between groups, while Kruskal–Walli's test and post hoc test Dunn's multiple comparison test was used for parameters that are not normally distributed. p-value<0.05 was considered statistically significant.

RESULTS

Comparison of serum IgG and IgM levels in patient groups

A total of 74 serum samples were collected as shown in table 1. 36 of them were male (48.65%) while 38 of them were female (51.35%) with a mean age of (48.43 ± 14.71). Participants were classified into 4 groups: group 1 (n=18) included healthy controls, group 2 (n=20) included asymptomatic cases, group 3 (n=18) included clinically mild cases, group 4 (n=18) included clinically severe cases.

Tab. 1. Demographic data of participants

		Age		Gender	
	n	Mean ± SD	Range	Male	Female
Group 1	18	40.56 ± 11.00	21-65	5 (27.8%)	13 (72.2%)
Group 2	20	39.05 ± 7.71	24-53	4 (20%)	16 (80%)
Group 3	18	53.50 ± 13.30	34-74	12 (66.7%)	6 (33.3%)
Group 4	18	61.67 ± 13.53	38-83	15 (83.3%)	3 (16.7%)

As illustrated in figure 1 the highest level of IgG showed in group 4 (severe). In addition, all patients' groups showed high significant difference with control group.

For IgM, Group 4 (severe) showed the highest level of IgM concentration compared to other groups (Figure 2). A significant difference was also recorded between controls and group 3 (mild).

Comparison of B cells indicators among different groups of COVID-19 patients

Table 2 represent the demographic data of 84 participants, 42 male (50%) and 42 (50%) female with the mean of ages 50.12 ± 15.08. group 1 (n=14) controls, group 2 (n=22) asymptomatic, group 3 (n=22) mild, group 4 (n=26) severe cases.

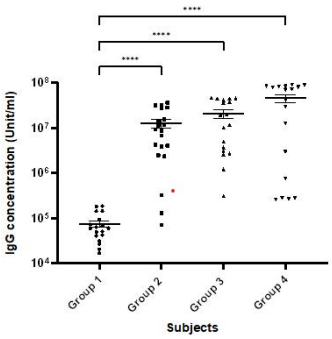

Fig. 1. Comparison of IgG concentration between control (group 1), asymptomatic (group 2), mild (group 3), severe (group 4). Data represent mean and SEM. * P-value<0.05 is considered significant, ** P-value < 0.01 is considered very significant, *** P-value<0.001, and **** P-value<0.0001 are considered extremely significant

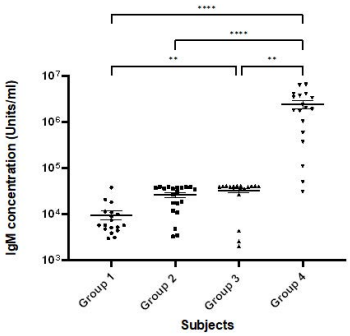

Fig. 2. Comparison of IgM concentration between control (group 1), asymptomatic (group 2), mild (group 3), severe (group 4). Data represent mean and SEM. * P-value<0.05 is considered significant, ** P-value<0.01 is considered very significant, *** P-value < 0.001, and **** P-value<0.0001 are considered extremely significant

Tab. 2. Demographic data of patients

		Age		Gender	
	n	Mean ± SD	Range	Male	Female
Group 1	14	41.79 ± 10.01	31-65	3 (21.43%)	11 (78.57%)
Group 2	22	39.09 ± 7.35	24-53	5 (22.73%)	17 (77.27%)
Group 3	22	53.64 ± 14.69	18-74	12 (54.55%)	10 (45.45%)
Group 4	26	60.96 ± 14.02	38-84	22 (84.62%)	4 (15.38%)

CD19+ B cells percentage showed decrease in the three groups of patients when compared to control group with a significant difference in their absolute numbers (Figure 3). Group 2 and group 3 showed a significant decrease (p-value<0.05) compared to control group, while high significant decrease showed in group 4 (p-value<0.01).

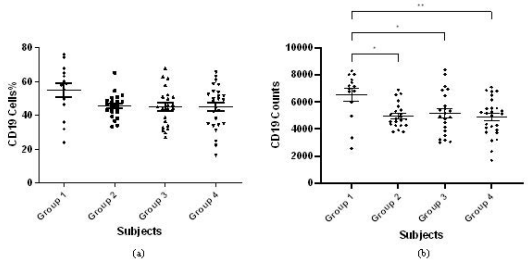

Fig. 3. Comparison of percentages (a) and absolute counts (b) of total CD19+ B Cells in different COVID-19 patients, Control (group 1), asymptomatic (group 2), mild (group 3), severe (group 4). Data represent mean and SEM. * P-value<0.05 is considered significant, ** P-value<0.01 is considered very significant, *** P-value<0.001, and **** P-value<0.0001 are considered extremely significant

On the other hand, CD27+ B cells showed a significant decrease in their percentage and absolute numbers in the 3 groups of patients when compared to control group (Figure 4). Group 2 showed high significant decrease (p-value<0.01) in CD27+ compared to control group, while both group 3 and group 4 showed an extremely significant decrease (p-value<0.0001), compared to control group.

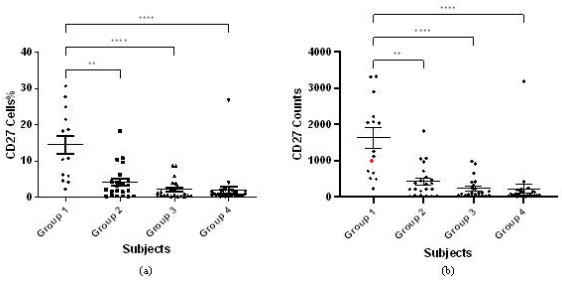

Fig. 4. Comparison of percentages (a) and absolute counts (b) of total CD27+ B Cells in different COVID-19 patients (control (group 1), asymptomatic (group 2), mild (group 3), severe (group 4)). Data represent mean and SEM. * P-value < 0.05 is considered significant, ** P-value < 0.01 is considered very significant, *** P-value < 0.001, and **** P-value < 0.0001 are considered extremely significant

DISCUSSION

Humoral immunity response provides effective mechanisms to restrict the infection and prevention of reinfection caused by SARS-Cov-2 like other viruses. During the infection, B cells play an important role in producing cytokines, presenting antigens, and secreting antibodies [10]. Lymphopenia is one of the most common risk factors has been associated with the severity of the disease [11-13]. Although the reduction of absolute T cell counts is the key factor in the development of lymphopenia, the role of B cells remains debatable. The study of Liu et al. (2020) found that the absolute counts of B cells in severe patients were within the normal range [14]. In contrast, other studies found a significant reduction in the absolute counts of B cells in patients compared to healthy control current results revealed that COVID-19 patients had their absolute CD19+ and CD27+ B cell counts significantly lower than control, these findings are in agreement with previous studies and wang et al. (2020) who also observed a significant decrease in CD19+ B cells in severe compared to mild cases [15-17]. B lymphopenia in COVID-19 patients might be caused by the direct cytopathic effect of the virus or indirectly due to virus-induced immunosuppression or uncontrolled inflammatory response [18, 19]. However, other studies proved increase in B cells of COVID-19 patient's samples with a significant increase in severe compared to mild cases [20, 21]. The heterogeneity of B cells response in the previous studies could be a result of different sample sizes and different sampling times after symptoms onset [22].

Current results demonstrate that IgG and IgM were generally increased in patient groups in comparison to healthy control. These findings were similar in alignment with previous studies [23-25]. In addition, Liu et al. (2020), Ma et al. (2020), and Zhao et al. (2020) have observed an association between higher IgG and IgM titers with disease severity, their findings were in line with current findings, whereas the antibodies found to be significantly increased in the severe group compared to control, thus high antibodies titer suggested to be an independent risk factor for the COVID-19 severity [26-28]. In severely COVID-19 patients the formation of Germinal Centre (GC) has been observed to be failed due to the significant decrease in the T follicular helper cells (Tfh), resulting in the robust extrafollicular response rather than GC response that could be explain the elevated antibodies titer in severe cases [10, 29].

Among current study, groups' gender distribution was undistributed. It had been demonstrated that females were less susceptible to infections than males due to biological gender differences in immune system and receptors. This might be related to sex hormones (including estrogens, progesterone, and androgens), and immune-regulatory genes on X-chromosome. Additionally, these differences suggested to affect the infection outcomes concerning infection severity, viral load and others comorbidities [30, 31].

STUDY LIMITATIONS

It is important to consider the limitations of this study when evaluating the findings as the sample size and gender distribution isn't typical of the larger population. This was a result of the high restrictions for sample collection and quarantine at the start of the pandemic. As a result, care should be taken when extrapolating the findings to different groups or situations.

CONCLUSION

This study provides valuable data on humoral immunity response to SARS-Cov-2. Peripheral B cells, IgG and IgM were shown to be related to the severity of the disease, CD19+ and CD27+ B cells tended to decrease in groups of patients, while antibodies concentrations increased along with disease severity.

REFERENCES

1. Wan S, Yi Q, Fan S, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. Br J Haematol. 2020;189(3):428-437.
2. World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. 2023 Sep 12 [cited 2023 Sep 12].
3. Grasselli G, Zangrillo A, Zanella A. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. JAMA. 2020;323:1574-1581.
4. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239-1242.
5. Moderbacher CR, Ramirez SI, Dan JM. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell. 2020;183:996-1012.
6. Yaugel-Novoa M, Bourlet T, Paul S. Role of the humoral immune response during COVID-19: guilty or not guilty? Mucosal Immunol. 2022;15:1170-1180.
7. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.
8. Keyt BA, Foote J, Finn OJ. Structure, function, and therapeutic use of IgM antibodies. Antibodies (Basel). 2020;9:53.
9. National Health Commission of China. New Coronavirus Pneumonia Prevention and Control Program. 2020 Jul 5 [cited 2020 Jul 5].
10. Chen S, Yuan J, Hu W. The role of B cells in COVID-19 infection and vaccination. Front Immunol. 2022;13:988536.
11. Gao YD, Ding M, Dong X. Risk factors for severe and critically ill COVID-19 patients: a review. Allergy. 2021;76:428-455.
12. Malik P, Patel U, Mehta D. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ Evid Based Med. 2021;26:107-108.
13. Ponti G, Maccaferri M, Ruini C. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020;57:389-399.
14. Liu J, Li S, Liu J, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020;55:102763.
15. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis. 2020;221:1762-1769.
16. Mazzoni A, Salvati L, Maggi L. Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest. 2020;130:4694-4703.
17. De Biasi S, Meschiari M, Gibellini L. Expansion of plasmablasts and loss of memory B cells in peripheral blood from COVID-19 patients with pneumonia. Eur J Immunol. 2020;50:1283-1294.
18. Wong RS. Inflammation in COVID-19: from pathogenesis to treatment. Int J Clin Exp Pathol. 2021;14:831-836.
19. Çölkesen F, Gazi H, Çelikbaş A, et al. Memory B cells and serum immunoglobulins are associated with disease severity and mortality in patients with COVID-19. Postgrad Med J. 2022;98:765-771.
20. Sosa-Hernandez VA, Torres-Ruiz J, Romero-Ramirez S. B cell subsets as severity-associated signatures in COVID-19 patients. Front Immunol. 2020;11:611004.
21. Chen G, Wu D, Guo W. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020;130:2620-2629.
22. Widjaja G, Nawangsih EN, Yazid SF. Humoral immune mechanisms involved in protective and pathological immunity during COVID-19. Hum Immunol. 2021;82:733-745.
23. Liu X, Wang J, Xu X. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect. 2020;9:1269-1274.
24. Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. Clin Transl Immunol. 2020;9.
25. Narasimhan M, Mahimainathan L, Araj E. Clinical evaluation of the Abbott Alinity SARS-CoV-2 spike-specific quantitative IgG and IgM assays among infected, recovered, and vaccinated groups. J Clin Microbiol. 2021;59.
26. Zhao J, Yuan Q, Wang H. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis. 2020;71:2027-2034.
27. Liu ZL, Liu Y, Wan LG. Antibody profiles in mild and severe cases of COVID-19. Clin Chem. 2020;66:1102-1104.
28. Ma H, Zeng W, He H. Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol. 2020;17:773-775.
29. Kaneko N, Kuo HH, Boucau J. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. Cell. 2020;183:143-157.
30. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. J Biol Regul Homeost Agents. 2020;34:339-343.
31. Sharma G, Volgman AS, Michos ED. Sex differences in mortality from COVID-19 pandemic: are men vulnerable and women protected? Case Rep. 2020;2:1407-1410.