AgriHealth
Journal of Agri-food, Nutrition and Public Health
ISSN 2722-0656 (Print) 2722-0648 (Online)

AgriHealth: Journal of Agri-food, Nutrition and Public Health. 3(2), 79-88, 2022
URL: https://jurnal.uns.ac.id/agrihealth/article/view/61315
DOI: http://dx.doi.org/10.20961/agrihealth.v3i2.61315

Trends in IgG Antibody Levels in Covid-19 Patients in Different Clinical Manifestations in Bengkulu City: Cohort Study

Debie Rizqoh[1*], Enny Nugraheni[1], Mulya Sundari[1,2], Dessy Triana[3], Mardhatillah Sariyanti[1] and Nikki Aldi Massardi[4]

[1]Department of Microbiology, Faculty of Medicine and Health Sciences, Bengkulu University, Bengkulu, Indonesia; [2]Department of Internal Medicine Specialist, M. Yunus Hospital Center, Bengkulu, Indonesia; [3]Department of Parasitology, Faculty of Medicine and Health Sciences, Bengkulu University, Bengkulu, Indonesia; [4]Department of Anatomical Pathology, Faculty of Medicine and Health Sciences, Bengkulu University, Bengkulu, Indonesia

Received: May 17, 2022; **Accepted**: June 17, 2022

Abstract

More than two years have lasted since the coronavirus disease 2019 (Covid-19) pandemic. The case of Covid-19 in Indonesia has spread to all provinces and areas such as Bengkulu City. This study aims to determine the profile of clinical manifestations and antibody dynamics in Covid-19 patients in Bengkulu City. This study used a cohort design on Covid-19 patients at two Bengkulu City referral hospitals, M. Yunus Hospital and Harapan dan Doa Hospital. Data on clinical manifestations were obtained from the medical records of Covid-19 patients. Meanwhile, to determine the level of IgM and IgG antibodies, patient serum samples were taken directly from respondents from the beginning of hospital admission three times on the 1st, 2nd and 3rd weeks. Then the samples were examined by immunofluorescent assay method. The results of this study indicate that, in general, there are no significant differences in anti-SARS-CoV-2 IgG levels in Covid-19 patients in the 1st, 2nd and 3rd weeks of Covid-19 in Bengkulu City. The Spearman correlation test also showed no correlation between IgG levels and the clinical manifestations of Covid-19. Although not statistically significant, there was a trend towards differences in antibody levels in patients with mild, moderate and severe clinical manifestations. The results of this study indicate that there are variations in antibody dynamics in Covid-19 patients in Bengkulu City.

Keywords: antibodies; clinical manifestations; Covid-19

INTRODUCTION

The coronavirus disease 2019 (Covid-19) pandemic is still ongoing worldwide. The number of confirmed cases of Covid-19 is increasing day by day, as are the victims who died. Covid-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), initially called novel coronavirus 2019 (2019-nCov), which first caused an outbreak in Wuhan, Hubei Province, China, in December 2019 (Wang et al., 2020). This virus infects humans by binding the spike glycoprotein component to the angiotensin-converting enzyme (ACE) receptor, the SARS-CoV virus that previously caused epidemics (Li et al., 2003; Hamming et al., 2004; del Rio and Malani, 2020).

Covid-19 is a species of the family Coronaviridae, genus Betacoronavirus (ICtV, 2020). Three species of coronavirus originating from animals have crossed species barriers, causing pneumonia that is deadly in humans

* ***Corresponding author***: debie.rizqoh@unib.ac.id

Cite this as: Rizqoh, D., Nugraheni, E., Sundari, M., Triana, D., Sariyanti, M., & Massardi, M. A. (2022). Trends in IgG Antibody Levels in Covid-19 Patients in Different Clinical Manifestations in Bengkulu City: Cohort Study. *AgriHealth: Journal of Agri-food, Nutrition and Public Health*, *3*(2), 79-88. doi: http://dx.doi.org/10.20961/agrihealth.v3i2.61315

in the 21st century, namely the SARS-CoV, MERS-CoV (Middle-East respiratory syndrome coronavirus) and SARS-CoV-2 (ICtV, 2020; Walls et al., 2020; Zhou et al., 2020). All of these viruses have their origin in zoonotic transmission from bats (Zhou et al., 2020).

Countries affected by the Covid-19 pandemic have made various efforts. Policies to implement social distancing protocols to lockdown have been implemented to prevent the transmission of the SARS-CoV virus. In addition, many studies related to Covid-19 have been carried out to develop diagnostic tests, therapies, vaccines and other research. The development of diagnostic tests is an essential component for screening and confirming Covid-19 patients (Nguyen et al., 2020).

Clinical symptoms of Covid-19 infection are almost the same as symptoms of other respiratory diseases such as fever, cough, myalgia, dyspnoea and pneumonia (Huang et al., 2020). Therefore, supporting laboratory examinations are needed to confirm this Covid-19 disease. In this case, examination using the reverse transcription-polymerase chain reaction (RT-PCR) method is the gold standard in confirming Covid-19 (WHO, 2020). However, this molecular method is expensive and requires a long time and skilled experts, so it cannot be used for the need for rapid diagnosis (Cassaniti et al., 2020). Therefore, several countries have adopted the rapid detection test (RDT) method to screen Covid-19 patients, including Indonesia (Cassaniti et al., 2020; Djalante et al., 2020; Rashid et al., 2020).

T-cell and B-cell responses against SARS-CoV-2 were detected in the blood about one week after the onset of Covid-19 symptoms (Tay et al., 2020). IgG and IgM seroconversion can occur simultaneously or sequentially, and both reach a plateau within six days after seroconversion (Long et al., 2020). IgM seroconversion generally increases from the 9th day, while IgG increases on the 11th day after symptoms appear. Serological testing can help diagnose suspected patients with negative PCR results and identify asymptomatic patients.

The number of Covid-19 cases in Bengkulu City until June 16, 2022 has reached 29,115 confirmed cases (Ministry of Health, 2022). Nevertheless, clinical data and case studies of Covid-19 in Bengkulu City are still minimal. So, it is necessary to research to analyze cases of Covid-19 in Bengkulu City in the context of accelerating the prevention of Covid-19 in Bengkulu City. This study examines the clinical manifestations and analyses the levels of IgM and IgG antibodies in Covid-19 patients in Bengkulu City.

MATERIAL AND METHODS

Research design

The research method was cross-sectional descriptive by taking consecutive samples. The population of this study is suspected and confirmed Covid-19 patients in Bengkulu City. The sample size of this study was 20 people. The inclusion criteria of research subjects were patients who were confirmed positive on RT-PCR examination. Ethical committee has approved this research with ethical approval number 189/UN30.4.9/LT/2020.

Antibody detection

Data on antibody tests were carried out from suspected and confirmed Covid-19 patients treated at the Harapan dan Doa Hospital and M. Yunus Hospital in Bengkulu City. Both hospitals are the main referral hospital for Covid-19 patients in Bengkulu City. Serum samples were taken for ELISA (Enzyme-linked immunosorbent assay) examination on the 7th, 14th and 21st days after the onset of symptoms.

Anti-SARS-CoV-2 IgM and IgG antibodies were examined using the FREND™ COVID-19 IgG/IgM Duo kit (NanoEntek) with the principle of fluorescence immunoassay (FIA) on IgM and IgG examination (NanoEntek, 2020). The serological examination procedure follows the guidelines of the kit.

FREND™ COVID-19 IgG/IgM Duo is an *in vitro* medical diagnostic device to identify Covid-19. The stages in the qualitative detection of IgG and IgM antibodies use the FREND™ COVID-19 IgG/IgM Duo with the following examination technique. First, it is necessary to prepare the FREND™ COVID-19 IgG/IgM Duo kit and the specimen to be examined. The sample identity (ID) is written on the cartridge in the designated area. The specimen was put into a 35 µl sample dilution tube, mixed and then transferred to the sample inlet using a cartridge. Press the "test" button on the "play" screen on the FREND™ system. The system will automatically

switch to the patient ID screen, then input the patient ID and press enter to start the test, The system will start the reading process when the cartridge reaction is complete. When the measurement is complete the results will be displayed immediately and if the FREND™ system is connected to an optional printer, it can be printed immediately.

Data analysis

Statistical analysis was performed using SPSS software. Continuous variables which are normally distributed are analyzed by Repeated Measures ANOVA test, but if the distribution is not normal, it is analyzed by Friedman test, The independent variables were tested with the One-Way ANOVA test for normally distributed data or the Kruskal Wallis test for data that were not normally distributed. The correlation between variables was tested with the Spearman Correlation test, p-value < 0.05 was statistically significant.

RESULT AND DISCUSSION

Research subject recruitment

Research subjects have been recruited based on data from Covid-19 patients who confirmed negative on PCR examination. Subject recruitment has been running for one month. Meanwhile, as of October 22, 2020, research subjects who were successfully recruited and willing to take part in this study amounted to 57 Bengkulu Covid-19 patients (patients of Covid-19 in Bengkulu/PCB), consisting of 30 male patients (52.63%) and 27 female patients (Fig. 47.36%). However, not all samples of respondents can be examined. In this study, the number of samples was examined from 30 respondents. Some patients had mild (33%), moderate (50%) and severe (17%).

Antibody examination results

The antibody examination method was carried out using the immunofluorescent assay (IFA) method, which was carried out at the Prodia Bengkulu Laboratory. PCB antibody test results generally show reactive results on IgG and non-reactive on IgM. IgM antibody reactive results were only found in three patients in the 1st week of examination and one in the 2nd week of examination so that the IgM level data cannot be analyzed further.

Anti-SARS-CoV-2 IgM antibodies were unreactive in almost all Covid-19 patients indicated by IgM level 1. It could occur because the testing method used is less sensitive in detecting IgM in Covid-19 patients or because the IgM concentration in Covid-19 patients is shallow. In another study, three types of seroconversion were found: concurrent IgG and IgM seroconversion, IgM seroconversion earlier than IgG seroconversion and IgM seroconversion slower than IgG seroconversion. (Long et al., 2020).

The dynamics of IgG antibody levels in Covid-19 patients in Bengkulu City are pretty diverse (Figure 1). Some patients continued to experience an increase in antibody levels in the 1st to the 3rd week, but some other patients also found an increase that only occurred until the 2nd week and a decrease in antibody levels in the 3rd week. Some samples of Covid-19 patients show different graphic trends. For example, PCB 01 and PCB 50 show an increasing trend from the 1st to the 2nd week, but there is a decrease in the 3rd week. In contrast to the PCB 05 case, the IgG level had decreased in the 2nd week but rose again in the 3rd week. Meanwhile, in the example, most of the patients continued to experience increased levels of IgG antibodies, such as on PCB 15, PCB 32, PCB 42 and PCB 48. However, it was found that patients experienced a decrease in antibody levels from the 1st to the 3rd week as in PCB 10.

IgM and IgG antibodies were detected positive on the 4th day after symptoms onset, cumulative IgM seroconversion increased rapidly from the 9th day, and IgG increased from the 11th day after the onset of symptoms. Both antibodies were seropositive in almost all patients in the course of the disease for more than 30 days (Long et al., 2020).

This study showed that each patient exhibited varying dynamics of IgG antibody levels. Even in the three groups of patients with mild, moderate and severe clinical manifestations, each showing a different trend. IgG levels continue to increase in patients with mild manifestations from the 1st to the 3rd week. It shows an excellent adaptive immune response in patients against SARS-CoV-2 virus infection so that it does not cause severe disease symptoms. Another study suggested that after SARS-CoV-2 infection, IgG levels are generated after one week, reach peak levels within three weeks and are maintained at high levels for a long time, even for 48 days (Hou et al., 2020).

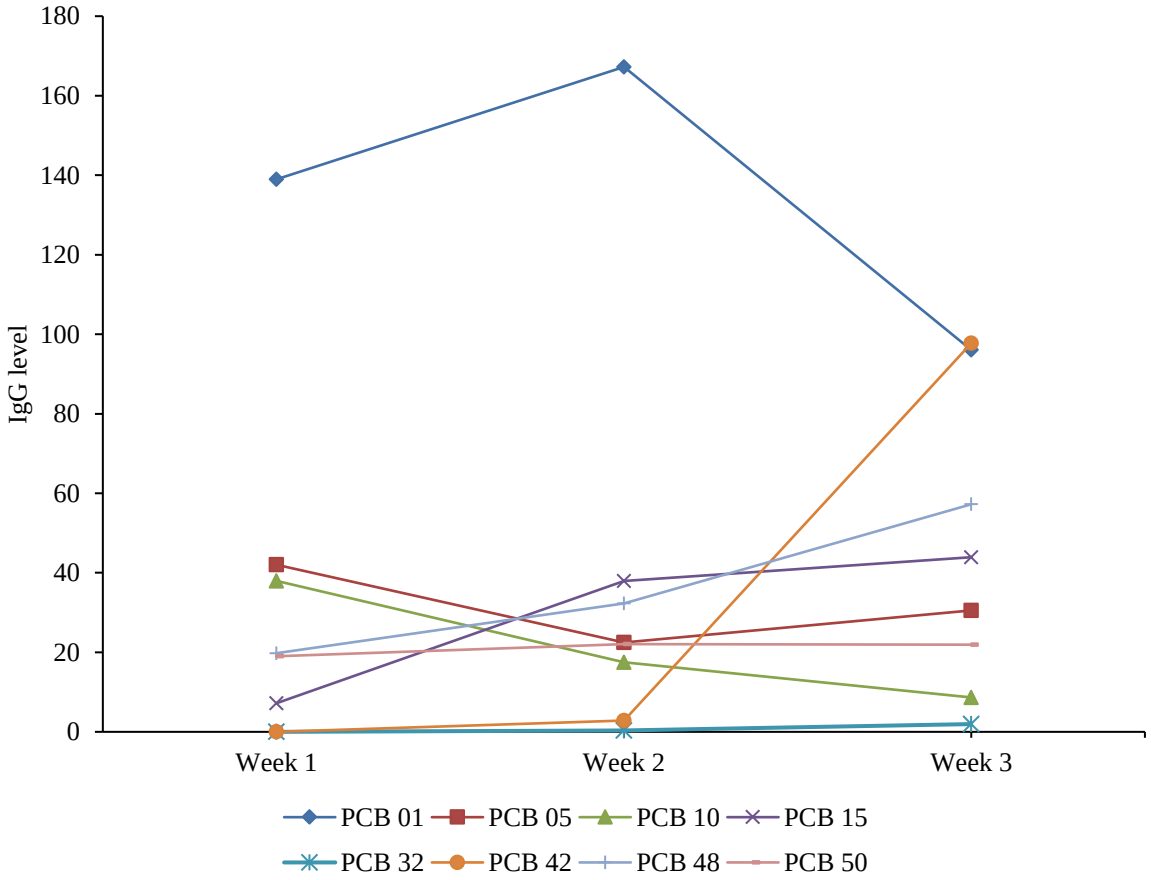
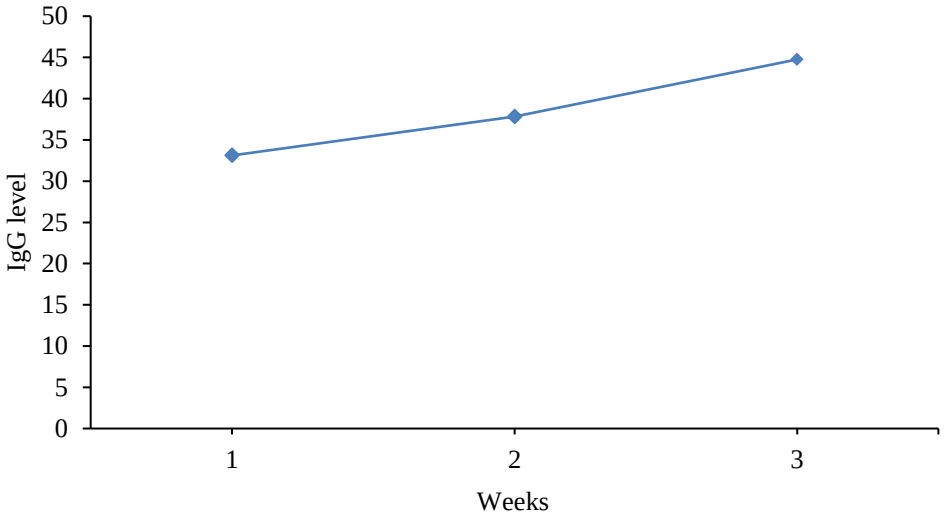


Figure 1. Dynamics of IgG antibody levels in Covid-19 patients in Bengkulu City. The value of p ($\alpha = 0.05$) = 0.417 after the Friedman test, PCB = Patient of Covid-19 in Bengkulu

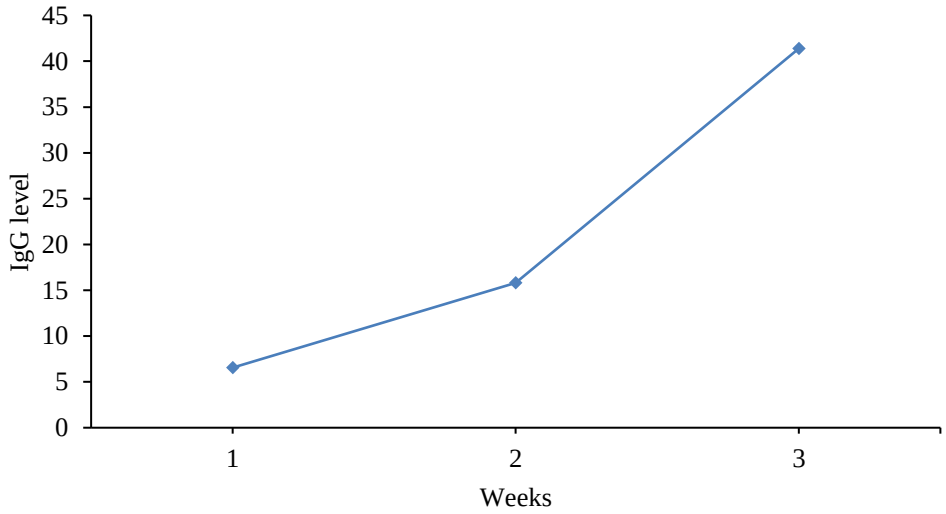
In contrast to the trend of IgG levels in moderate and severe cases, there is a tendency to decrease IgG levels, especially in patients with severe clinical manifestations. It may be due to high disease activity or impaired immune response in more severe cases (Hou et al., 2020).

Based on statistical analysis, the average IgG level in the 1st week was 33.11 ± 45.58, the 2nd week was 37.84 ± 53.85 and the 3rd week was 44.81 ± 36.84. The distribution of the data obtained in this study was included in the abnormal category so that it was further analyzed by non-parametric statistical tests, namely the Friedman test, The hypotheses tested are, H_0: there is no difference in anti-SARS-CoV-2 IgG levels in Covid-19 patients in the 1st, 2nd and 3rd weeks; H_1: there are differences in anti-SARS-CoV-2 IgG levels in Covid-19 patients during the 1st, 2nd and 3rd weeks. With $\alpha = 0.05$, the test results show a significance value of 0.417. So from the results of the statistical test, it was explained that there was no significant difference in anti-SARS-CoV-2 IgG levels in Covid-19 patients in the 1st, 2nd and 3rd weeks.

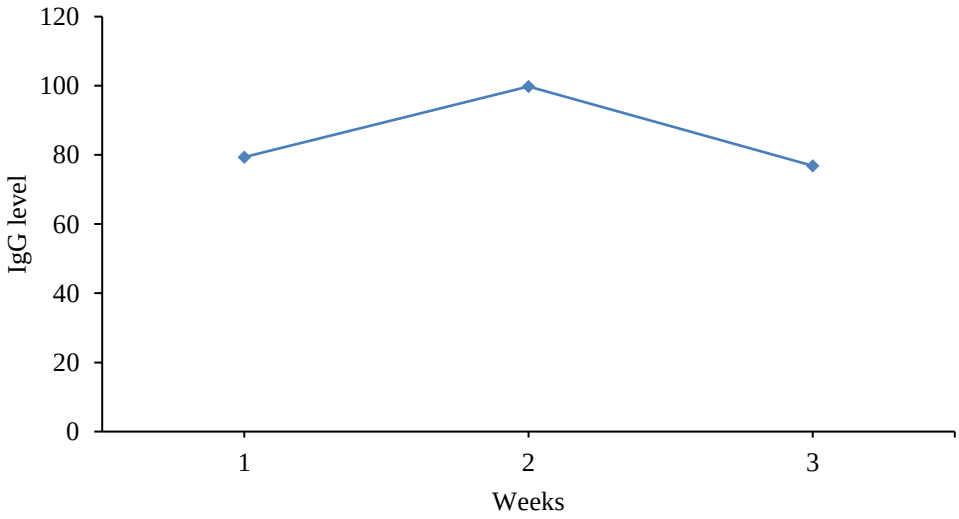
Although not statistically significant, the average IgG level in all patient samples showed an increasing trend from the 1st to the 3rd week (Figure 2a). In patients with mild clinical manifestations, antibody levels significantly increased from the 1st to the 3rd week (Figure 2b). In patients with moderate clinical manifestations, the mean antibody level showed an increasing trend from the 1st to the 2nd week, but decreased at the 3rd week (Figure 2c). In patients with severe clinical manifestations, the trend of antibody levels showed a decrease in levels from the 1st to the 3rd week (Figure 2d).



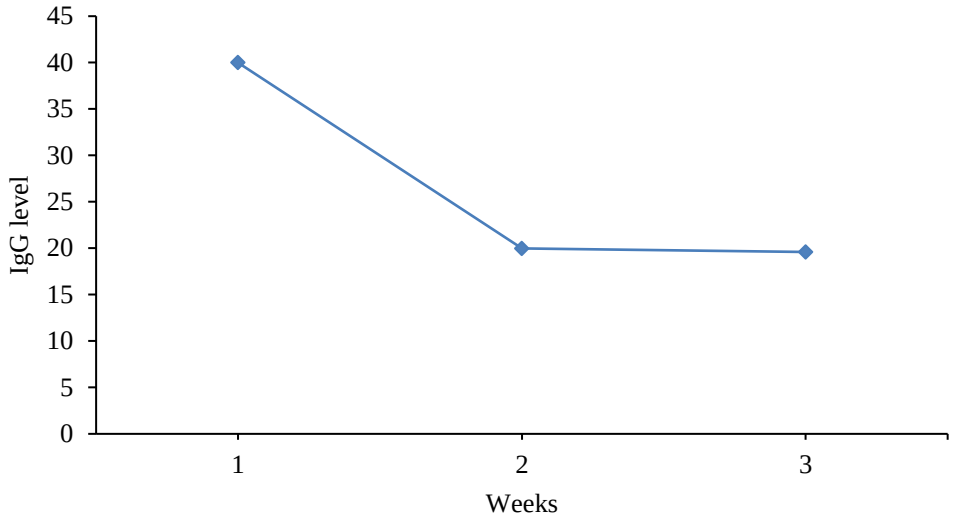
a.



b.



c.



d.

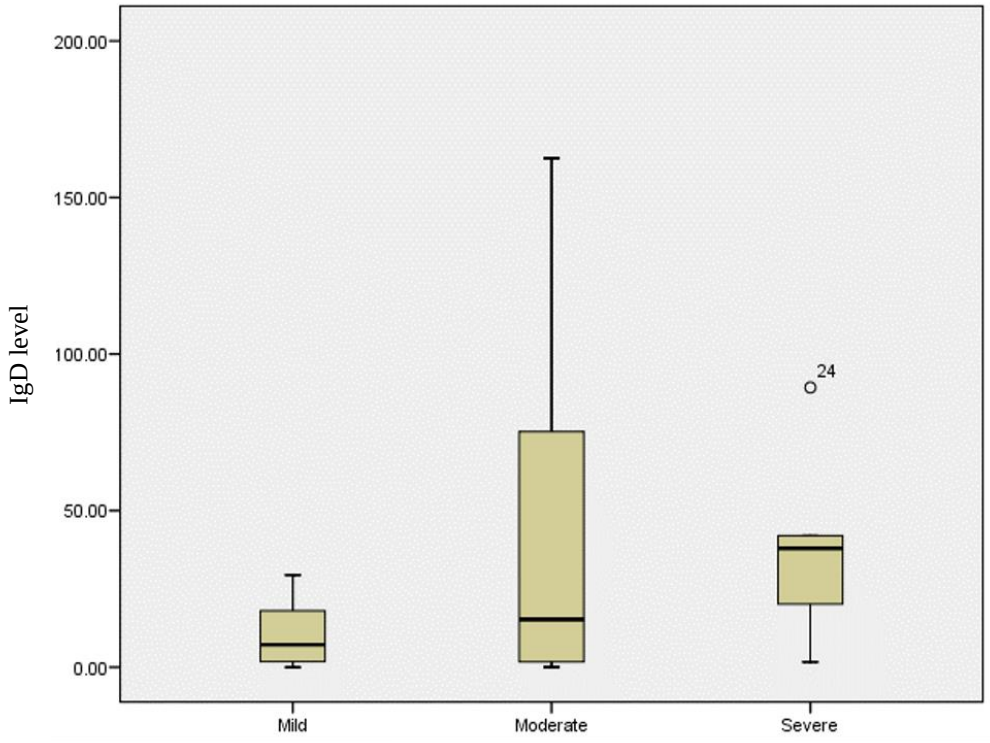
Figure 2. Trends in IgG levels in Covid-19 patients in Bengkulu City, a) average IgG levels in all samples of Covid-19 patients in Bengkulu City; b) mean IgG level in patients with mild clinical manifestations category; c) mean IgG level in patients with moderate clinical manifestation category; d) mean IgG level in patients with severe clinical manifestations category. After Friedman's test, p-value > 0.05

In addition, the results of statistical analysis showed that at the 1st, 2nd and 3rd week, the difference in IgG antibody levels in mild, moderate and severe cases of Covid-19 was not significantly different. Furthermore, the Spearman correlation test also showed no correlation between IgG levels and the clinical manifestations of Covid-19 (Figure 3).

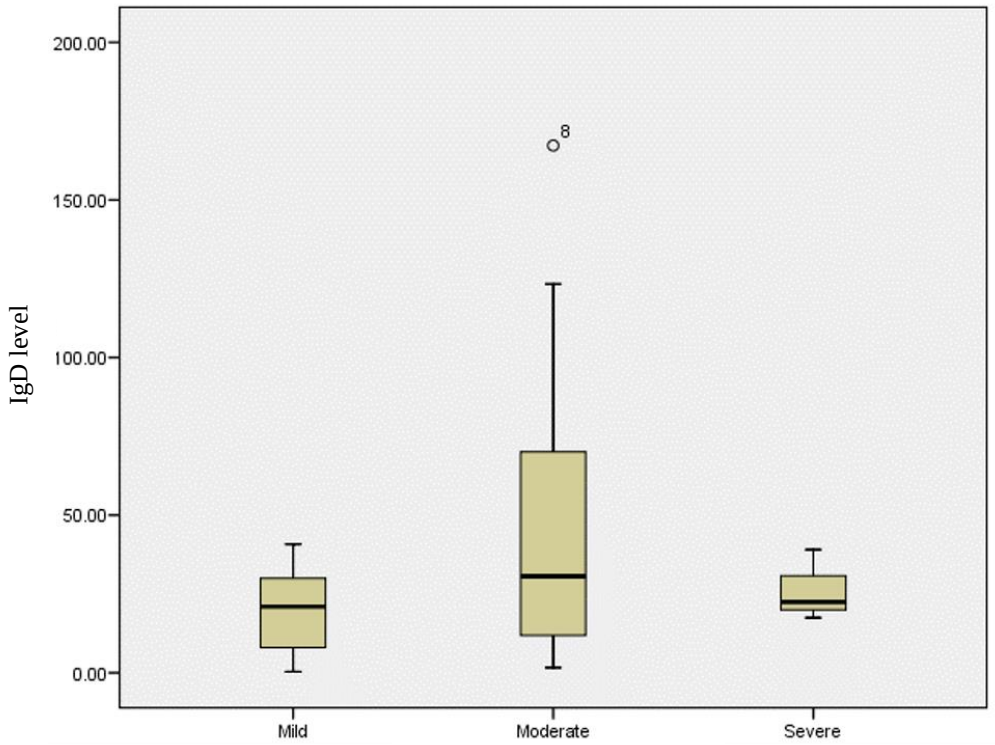
Although not statistically significant, in the 1st week, it was seen that the more severe the patient's illness, the higher the patient's IgG level. It is in line with the results of another study which reported that seriously ill Covid-19 patients had the highest levels of anti-RBD (receptor binding domain) and anti-spike antibodies (Garcia-Beltran et al., 2021).

In contrast, the mean antibody levels at the 2nd and 3rd weeks showed the highest antibody levels in patients with moderate clinical manifestations and decreased in severe cases. Another study reported that anti-spike IgA, IgM and IgG antibody titers were positively correlated with neutralizing antibody (NAb) titers in mild/moderate and severe cases. However, IgM and IgG antibody titers against RBD protein positively correlated with NAbs titers only in mild/moderate cases but not in severe cases (Ren et al., 2020).

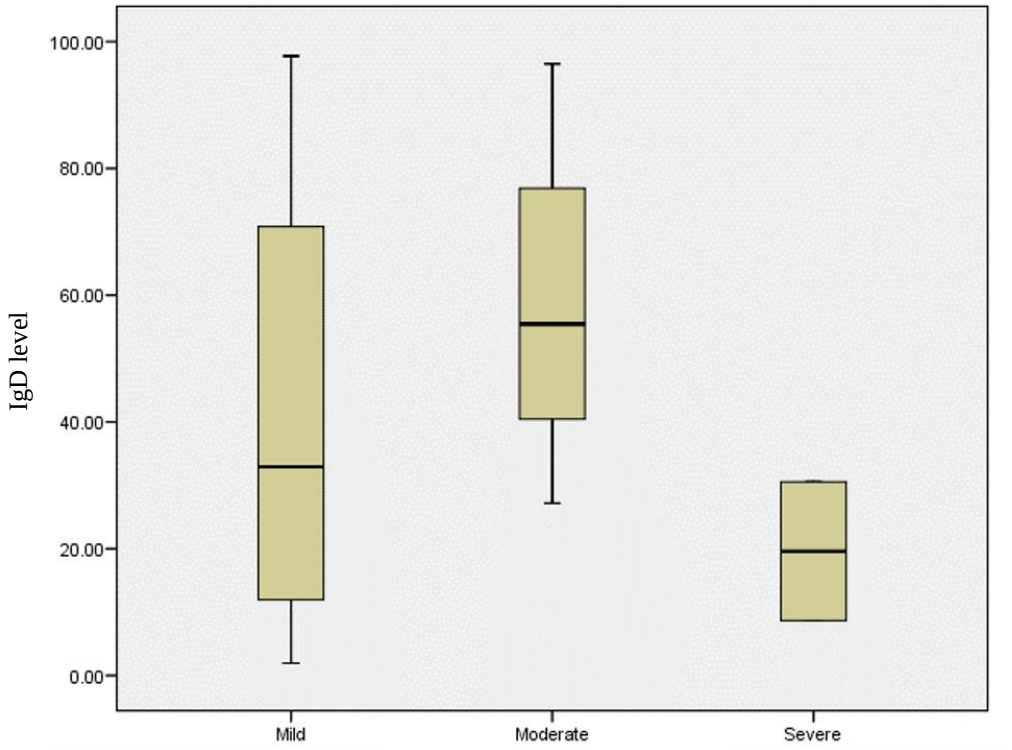
This study has several limitations. First, the study only used commercial FIA methods for antibody detection; thus, the antibody response pattern may be specific for the assay. The absence of seroconversion in some patients might be due to the test conditions and the antigen used in the FIA in this study. Second, the onset of symptoms was determined based on self-reported symptoms. Since Covid-19 symptoms are non-specific, this may have resulted in the variability of optical density (OD) values at similar time points since symptom onset among patients. Third, the sampling time is limited to only three times in 3 weeks so that it cannot describe the conditions when the sample was not taken. Fourth, the number of research subjects is small, so it can cause bias in the statistical analysis. Further large-scale investigations are required to verify antibody response patterns using several serological methods with larger sample sizes.



a.



b.



c.

Figure 3. Box plot the difference in the average weekly IgG antibody levels in mild, moderate and severe clinical manifestations cases, a) differences in mean IgG levels at 1st week; b) difference in mean IgG levels at 2nd week; c) difference in mean IgG levels at 3rd week. p-value > 0.05 on graphs a, b and c after the Kruskal Wallis test continued with the Spearman correlation test

CONCLUSION

The level of anti-SARS-CoV-2 IgG antibodies in the patient's body has various trend patterns in Covid-19 patients. IgG levels in mild, moderate and severe cases have different trend patterns, but statistical results show changes in IgG levels are not significantly different in Covid-19 patients at 1st, 2nd and 3rd weeks.

ACKNOWLEDGEMENT

This research is supported by a PNBP Faculty of Medicine and Health Sciences grant, Bengkulu University. Thank you for the support.

REFERENCES

Cassaniti, I., Novazzi, F., Giardina, F., Salinaro, F., Sachs, M., Perlini, S., Bruno, R., Mojoli, F., & Baldanti, F. (2020). Performance of VivaDiag Covid-19 IgM/IgG rapid test is inadequate for diagnosis of Covid-19 in acute patients referring to emergency room department. *Journal of Medical Virology*, *92*(10), 1724–1727. https://doi.org/10.1002/jmv.25800

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses [ICtV]. (2020). The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*, *5*, 536–544. https://doi.org/10.1038/s41564-020-0695-z

del Rio, C., & Malani, P. N. (2020). Covid-19–New insights on a rapidly changing epidemic. *Journal of American Medical Association*, *323*(14), 1339–1340. https://doi.org/10.1001/jama.2020.3072

Djalante, R., Lassa, J., Setiamarga, D., Sudjatma, A., Indrawan, M., Haryanto, B., Mahfud, C.,

Sinapoy, M. S., Djalante, S., Rafliana, I., Gunawan, L. A., Surtiari, G. A. K., & Warsilah, H. (2020). Review and analysis of current responses to Covid-19 in Indonesia: Period of January to March 2020. *Progress in Disaster Science*, *6*, 100091. https://doi.org/10.1016/j.pdisas.2020.100091

Garcia-Beltran, W. F., Lam, E. C., Astudillo, M. G., Yang, D., Miller, T. E., Feldman, J., Hauser, B. M., Caradonna, T. M., Clayton, K. L., Nitido, A. D., Murali, M. R., Alter, G., Charles, R. C., Dighe, A., Branda, J. A., Lennerz, J. K., Lingwood, D., Schmidt, A. G., Iafrate, A. J., & Balazs, A. B. (2021). Covid-19-neutralizing antibodies predict disease severity and survival. *Cell*, *184*(2), 476–488.e11. https://doi.org/10.1016/j.cell.2020.12.015

Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *Journal of Pathology*, *203*(2), 631–637. https://doi.org/10.1002/path.1570

Hou, H., Wang, T., Zhang, B., Luo, Y., Mao, L., Wang, F., Wu, S., & Sun, Z. (2020). Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clinical and Translational Immunology*, *9*(5), e1136. https://doi.org/10.1002/cti2.1136

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., & Gu, X. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, *395*, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., Somasundaran, M., Sullivan, J. L., Luzuriaga, K., Greenough, T. C., Choe, H., & Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*, *426*(6965), 450–454. https://doi.org/10.1038/nature02145

Long, Q., Liu, B., Deng, H., Wu, G., Deng, K., Chen, Y., Liao, P., Qiu, J., Lin, Y., Cai, X., Wang, D., Hu, Y., Ren, J., Tang, N., Xu, Y., Yu, L., Mo, Z., Gong, F., Zhang, X., … & Niu, C. (2020). Antibody responses to SARS-CoV-2 in patients with Covid-19. *Nature Medicine*, *26*, 845–848. https://doi.org/10.1038/s41591-020-0897-1

Ministry of Health. (2022). *Situasi terkini perkembangan coronavirus disease* (Covid-19). Jakarta: Ministry of Health Republic of Indonesia. Retrieved https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200423-sitrep-94-covid-19.pdf

NanoEntek. (2020). *FREND COVID-19 IgG/IgM Duo*. Retrieved from http://www.nanoentek.com/theme/nanont2_en/shop/02/product01_view.php?it_id=1585699547

Nguyen, T., Bang, D. D., & Wol, A. (2020). 2019 novel coronavirus disease (Covid-19): Paving the road for rapid detection and point-of-care diagnostics. *Micromachines*, *11*(3), 306. https://doi.org/10.3390/mi11030306

Rashid, Z. Z., Othman, S. N., Samat, M. N. A., Ali, U. K., & Wong, K. K. (2020). Diagnostic performance of Covid-19 serology assays. *Malaysian Journal of Pathology*, *42*(1), 13–21. Retrieved from https://pubmed.ncbi.nlm.nih.gov/32342927/

Ren, L., Zhang, L., Chang, D., Wang, J., Hu, Y., Chen, H., Guo, L., Wu, C., Wang, C., Wang, Y., Wang, Y., Wang, G., Yang, S., Dela Cruz, C. S., Sharma, L., Wang, L., Zhang, D., & Wang, J. (2020). The kinetics of humoral response and its relationship with the disease severity in Covid-19. *Communications Biology*, *3*(1), 1–7. https://doi.org/10.1038/s42003-020-01526-8

Tay, M. Z., Poh, C. M., Rénia, L., Macary, P. A., & Ng, L. F. P. (2020). The trinity of Covid-19: immunity, inflammation and intervention 1,2. *Nature Reviews Immunology*, *20*, 363–374 https://doi.org/10.1038/s41577-020-0311-8

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, *181*(2), 281–292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. *Lancet*, *395*(10223),

470–473. https://doi.org/10.1016/S0140-6736(20)30185-9

WHO. (2020). *Laboratory testing strategy recommendations for COVID-19: Interim guidance*. World Health Organization. Retrieved from https://apps.who.int/iris/handle/10665/331509

Zhou, P., Yang, X., Wang, X., Hu, B., Zhang, L., Zhang, W., Guo, H., Jiang, R., Liu, M., Chen, Y., Shen, X., Wang, X., Zhan, F., Wang, Y., Xiao, G., & Shi, Z. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*, 270–273. https://doi.org/10.1038/s41586-020-2012-7