ORIGINAL ARTICLE

Kinetics IgM and IgG SARS-CoV-2 in Recovery Patients with Negative Evaluation RT-PCR

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ABSTRACT

Background: Coronavirus is the major pathogens at human respiratory system. The antibody is a response infected patients largely not clearly. We need to understanding of antibody responses to great diagnosis and treatment studies.
Objective: In this article the objective is to describe that kinetics of IgM and IgG SARS-CoV-2 in recovery patients.
Method: Cohort study used a total 19 subjects who had negative evaluation RT-PCR after confirmed. IgG and IgM of SARS-CoV-2 were detected by IFA (Immuno fluorosence assay) used serum. Serum were collected three times after first, second and fourth months, secound and fourth months negative evaluation RT-PCR. We profiled the serological responses (IgM and IgG) to SARS-CoV-2.
Result: Majority the IgM SARS-CoV-2 post evaluation RT-PCR were very low after one, second and fourth months negative evaluation. IgG SAR-CoV-2 patients post negative evaluation RT-PCR decreased after 4 months. The level IgM and IgG level increase at first week and decrease after 12 weeks.
Conclusion: IgM level lower than IgG level overtime. Quantitative IgG and IgM detection could be point of diagnosis and manifestation.

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Introduction

Coronavirus disease (COVID-19) is one of major problem in human respiratory system. In late December 2019, a case of patients was admitted to hospital with initial symptoms like pneumonia.[1] The pathogenesis of COVID-19 infections is as follow.[2] In the last December 2019 five patients were hospitalized with acute respiratory syndrome and one of the patient died.[3] The China National Health Commission reported that first 17 deaths until January 2020. A total 1975 cases were confirmed COVID-19 infected in China with a total 56 deaths.[4] CDC reported first case human to human transmission of COVID-19 on January 30, 2020.[5]

COVID-19 is an infectious disease caused by the SARS-CoV-2 Virus. SARS-CoV-2 are a positive-sense, single stranded RNA (ssRNA) virus with genome length from 20-32 kb and about 125 nm in diameter that belongs to the Coronaviridae family.[6] The mortality rate of SARS-CoV-2 a novel beta coronavirus is about 3.4% but SARS-CoV-2 has potentially higher transmission than another Coronavirus.[7]

The diagnosis of COVID-19 is dependent on clinical symptoms, CT imaging and some

laboratory result. Although some clinical manifestations and laboratory result have indicated to lead as a COVID-19 disease, that not unique to SARS-CoV-2 infection. The rapid and accurate diagnostic of COVID-19 is needed to rapid and good management, accurate public surveillance, prevention and control disease. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) has been gold standard for diagnosis SARS-CoV-2.[8] Patients with COVID-19 infection have a mild disease and recover quickly after good clinical management. Some COVID-19 patients develop more severe disease, multiple organ failure and death in the short time.[7] Previous study reported that massive inflammatory responses induce the over-inflammation of T cells and be severe infection in SARS-CoV-2 pathogenesis.[9]

Serological examination of Covid 19 in survivors is not only helps identify affected cases but can provide important information regarding Covid-19 immunity to infected groups. In SARS-CoV-2 infection, specific IgG antibodies increase and reach a peak at 4 months after the onset of the disease and then decrease after 16 months.[10] Antibody level are generally regarded as protective, detrimental affected, as antibody dependent enhancement (ADE), hypothesis of SAR-CoV-2 infection.[11] It is still on investigation whether ADE play a role in pathogenesis of COVID-19. A study showed that patients in severity case with COVID-19 had higher total SARS-CoV-2 antibody titers compared mild patients SARS-CoV-2 infection.[12] However, weather higher neutralizing antibody (Nab) titers are associated with more lung manifestations to elucidated. Serological method another way to laboratory diagnostic, can diagnose disease by detecting antibody. Estimated decrease antibody to know antibodies circulated in the body. The aim this study to investigate the diagnostic value of serological method and the kinetic variance of IgG and IgM antibodies in patients with RT-PCR negative

Methods

Design and Subject Criteria

A total 19 patients were diagnosed by real-time PCR testing. After isolation the subject we informed to be subject in this study. Patients who agree with informed consent we included to this study. The patients were followed up level of IgG and IgM after negative result 1 month, 2 months and and 4 months after negative realtime-PCR testing. Demographic information and symptoms we got from patient interview. The diagnosis of COVID-19 and evaluation patients is based on report Health Office (Dinas Kesehatan Provinsi) Bengkulu Province, Indonesia. The study was ethical approved by Health Research Ethics Comitte of the Faculty of Medicine and Health Sciences Universitas Bengkulu, by numbers 189/UN30.14.9/LT/2020.

SARS-CoV-2 RNA Detection

A total of 19 patients were diagnosed with real-time PCR testing. Diagnosis of COVID-19 using the RT-qPCR method using a sore throat swab in the nasopharynx. The examination used primers and probes targeting NP and the SARS-CoV-2 open reading frame1b gene according to World Health Organization (WHO) guidelines.[13] Throat swab specimens were collected from all patients and samples were stored in viral transport media for laboratory testing.

Serology Testing

The specific IgG and IgM of SARS-CoV-2 were detected by IFA (Immuno fluorosence assay) used serum. The reagent used is COVID-19 IgG and IgM from (Frendcov, Nanoentech, Nanoentek America, Inc, USA). The catridge utilizas micro-fluicidics lateral flow technology where the analyte of interest in the sample forms immune complexes while moving through the fluidics pathway in the catridge. A well-mixed sample of 35 µL is transferred to the sample inlet of a single use frend COVID-19 total ab catridge. The catridge is then placed into the frend system, which is programmed to begin analysis once the sample has reacted with the reagents. IgM and IgG if present in the sample will bind to SARS-CoV-2 nucleocapsid fluorescent beads which then bind to the anti-IgM and anti-IgG in the test zone. All tests were performed according to the product manual.

Results

Demograpic and clinical characteristic patients

The study include total 19 subject with confirmed COVID-19 with RT-PCR diagnosis and were recovery. Among them 7 (36 %) subject is male and 12 (63,15 %) subject is female. The subject classified into two groups : mild (7 subjects, 36 %) and severe (12 subjects, 64%). The subject ages in the mild (28,85±4,3) and severe (34,25±4.9). There's np significant different age between mild and severe. But, the mean age of severe group more higher than mild group. The marriage status in mild 3 (15,78 %) subject and in severe group was 11 (57,9 %) subject. (Table 1)

Table 1. Demographic and clinical symptoms distribution

Paramaters	Mild N=7 (%)	Severe N=12 (%)
Gender		
Male	0 (0%)	4 (33.3)
Female	7 (100%)	8 (66.7)
Age	28.85±4.3	34.25±4.9
Marriage	3 (42.9)	11 (91.7)
Symptoms		
Febris	2 (28.6)	8 (66.7)
Cough	3 (42.9)	5 (41.7)
Headache	1 (14.3)	5 (41.7)
Anosmia	2 (28.6%)	7 (58.3)
Ageusia	2 (28.6)	7 (58.3)
Diarhea	1 (14.3)	2 (16.7)
Fatique	3 (42.9)	7 (58.3)
Dypsnoe	1 (14.3)	1 (8.3)
Comorbid	0 (0)	3 (25)

Most of subject had febris, cough, headache, anosmia, ageusia and fatique and dyspnoe of breath. Three subject in severe group had comorbidities. The comorbidities of 3 subject in severe group were diabetes mellitus. We investigated that severe group had higher percentages of clinical symptoms manifestation and comorbidities.

Most subject of mild group were isolated in isolation center (Bapelkes, Health office Bengkulu) (Figure 1). Most subject of severe group isolated at hospital. There are because of clinical symptoms of severe group subject need serious observation. There are no significant statistic different centralized isolation place

between mild group and severe group (Fisher exact, p>0.05)

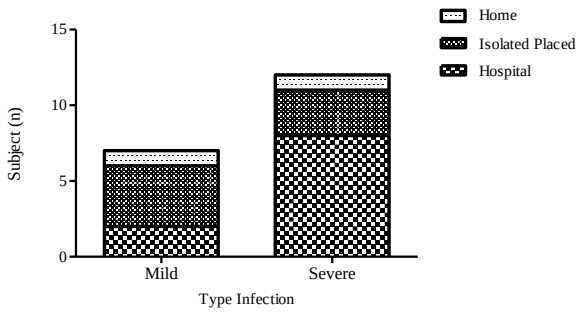

Figure 1. Distribution of centralized isolation

Recovery time between mild group and severe group not significant different (p>0.05, Mann-whitney test). Mean recovery time of severe group was longer than mild group. The maximum recovery time of severe group was 39 days. Maximum recovery time of mild group was 25 days. The minimum recovery time of severe group was 7 days and 10 days for severe group.

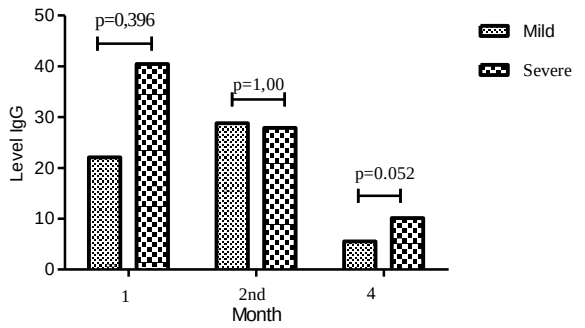

Figure 2. Recovery time based infection type

Analyzed cohort the IgM and IgG level antibodies in COVID-19 with RT-PCR negative result. The average of IgM and IgG level detection after 1st, 2nd and 4th months the RT-PCR was negative. After 1st month RT-PCR test negative the level of IgG in mild group lower than severe group. The result comparison IgG level between two group with Mann-Whitney test was not significant (0,396). After 2nd recovery the IgG level antibody in mild group same with severe group. There is not significant different IgG level between mild group and severe group (p=0.001). After 4th recovery the statistic analyzed with Mann-Whitney test was not significanat (p=0.052), but the mean IgG level antibody severity group higher than mild group. (Figure 3).

The figure 3a and 3b showed that the IgM level rate increase slightly at first and than decrease after the number of week form serological detected. In contrast, IgG level increase were higher than IgM overtimes.

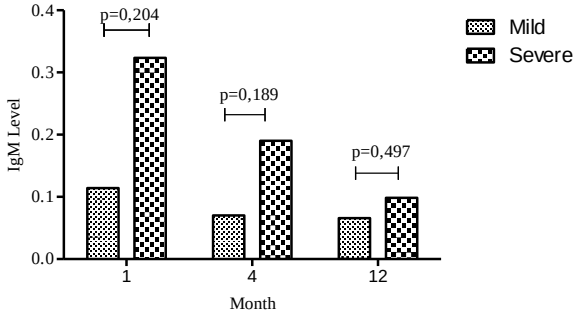

Figure 3. Comparison Kinetic (a) IgG (b) IgM with severity COVID-19

Discussion

Detection IgM and IgG SARS-CoV-2 during SARS epidemic allowed for serological diagnosis.[14] Identic antibody responses have been observed in SARS-CoV-2 infection patients, and the pattern is similar with acute viral infection.[15] Antibody diagnostic COVID-19 was rapid and sensitive for the diagnosis SARS-CoV-2 infection. Several detection test for antibody such as lateral flow immunoassay, CLIA, IFA and ELISA are currently available. In this study, the antibody respons were level IgM and IgG antibodies.We were analyzed at recovery COVID-19 subject and negative RT-PCR test.

Viral infection process with SARS-CoV-2, antibodies specific produced always consistent in

patients, except patients with immunodeficient. IgM antibody can detected after 3 days infection and first defense in humoral immunity, and IgG antibody are initiated and play a key role in long immune respond memory.[16] We investigated the IgM level subject. IgM level of subject were lower. Some subject not detect IgM in the serum. It was similar with 4 patients in previous study that the level IgM are very low.[17]

Data in this study showed that IgG was increased in 2 month after negative PCR resulted and than decrease after 4 month after negative PCR resulted. The kinetics of SARS-CoV-2 RBD IgGs and NtAb followed a predictable course[9], with antibody levels in both assays showing a consistent increase over time, and reaching a peak within the second and third week after onset symptoms for NtAb or slightly later for RBD-specific IgGs.[18]

Disregulated synthesis and release of pro-inflammatory cytokines is thought to be a pathogenetic hallmart of most severe forms of COVID-19.[19] In this study, the mean level IgM and IgG antibodies in mild group and severity group are different. Mean level IgM and IgG severe group higher than mean level IgM and IgG mild group. Quantitave result antibody detection are associated with the severity of COVID-19 and have potential value for use in predicting the disease prognosis.[17]

The limitations this study should be mentioned. Result this study need to be further the validated by studies in a lerge data of subject. The antibodies study need more sensitive method for detection comprehensive antibodies level.

Conclusion

The level IgM and IgG level increase at first week and decrease after 12 weeks. IgM level lower than IgG level overtime. Quantitative IgG and IgM detection could be point of diagnosis and manifestation.

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Conflict of Interest

No conflict of interest in this study

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