

INTISARI

ANALISIS URUTAN BASA DAN MUTASI GEN PENGKODE PROTEIN SPIKE SARS-CoV-2 ASAL INDONESIA SERTA PENGEMBANGAN METODE *qPCR-HRM* UNTUK DETEKSI MUTASI D614G

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Laju transmisi SARS-CoV-2 yang tinggi menyebabkan munculnya beragam mutasi. Salah satu mutasi penting yang terdapat di protein *Spike* adalah mutasi D614G. Mutasi tersebut menyebabkan SARS-CoV-2 menjadi lebih infeksius. Oleh karena itu, varian G614 hingga saat ini masih memerlukan perhatian khusus karena telah terbukti dapat memunculkan berbagai varian dengan mutasi yang lebih mengkhawatirkan. Penelitian ini dilakukan untuk mengetahui karakter sekuens gen *S* dari SARS-CoV-2 asal Indonesia khususnya Yogyakarta, mengetahui kekerabatannya terhadap varian global, menganalisis laju mutasi region yang terdapat pada protein *Spike*, mempelajari letak daerah pusat mutasi pada protein *Spike*, serta mempelajari metode analisis *HRM* untuk membedakan varian D614 dan G614. Penelitian ini dilakukan dengan tiga pendekatan, yakni metode *nested-PCR-primer walking* untuk proses identifikasi dan karakterisasi *full-length* gen *S*, metode PCR untuk proses amplifikasi region *D614G Hotspot-FCS* pada gen *S*, dan metode *two-step RT-qPCR-HRM* untuk mendeteksi mutasi D614G. Hasil penelitian ini menunjukkan bahwa tingkat konservasi gen *S* SARS-CoV-2 asal Yogyakarta (Isolat 2.13) adalah sebesar 99,9% dengan hanya terdapat satu mutasi *missense*, yakni D614G. Terdapat sebanyak 21 mutasi *missense* dan tujuh mutasi *silent* pada region *D614G Hotspot-FCS* SARS-CoV-2 asal Indonesia yang didasarkan pada data sampel penelitian dan data GISAID. Analisis filogenetik *Maximum Likelihood* (ML) berbasis gen *S* menunjukkan bahwa SARS-CoV-2 asal Yogyakarta (Isolat 2.13) berkerabat dekat dengan varian G614 yang berasal dari berbagai negara. Berdasarkan analisis data sampel penelitian dan data GISAID, laju mutasi region-region protein *Spike* berkisar $1,61 \times 10^{-3}$ hingga $1,34 \times 10^{-2}$ substitusi per situs per tahun, serta region *D614G Hotspot-FCS* diketahui sebagai daerah pusat mutasi pada protein *Spike*. Analisis *HRM* dapat mendeteksi mutasi D614G, sehingga dapat digunakan untuk surveilans sirkulasi varian G614 pada suatu daerah. Hasil penelitian ini diharapkan dapat digunakan sebagai acuan untuk melakukan pengembangan penelitian lain yang berkaitan dengan deteksi cepat varian SARS-CoV-2 dan pengembangan vaksin yang berbasis sekuens gen *S* SARS-CoV-2 asal Indonesia.

Kata kunci : SARS-CoV-2, Spike, FCS, D614G, qPCR-HRM

ABSTRACT

ANALYSIS OF SEQUENCE AND MUTATION OF THE *SPIKE* PROTEIN CODING GENE FROM INDONESIAN ORIGIN SARS-CoV-2 AND THE DEVELOPMENT OF *qPCR-HRM* METHOD FOR THE D614G MUTATION DETECTION

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The high transmission rate of SARS-CoV-2 has caused the occurrence of various of mutations. One of the pivotal mutation located in the *Spike* protein is the D614G mutation. This mutation enforce SARS-CoV-2 to be more infectious. Therefore, G614 variant still needs a special concern until now because its attribute to generate various variants with the more concerning mutations. This research aims to know the sequence character of *S* gene from Indonesian origin SARS-CoV-2 especially Yogyakarta origin, to observe the phylogenetic relationship with the global circulating variant, to analyze the mutation rate of the regions within the *Spike* protein, to determine the location of mutation hotspot region within the *Spike*, and to evaluate the ability of *qPCR-HRM* assay on discriminating D614 and G614 variants. This research was done following three approaches: nested-PCR-primer walking method for identification and characterization of the full length of *S* gene, PCR method for amplification of the *D614G Hotspot-FCS* region of the *Spike*, and a two-step *RT-qPCR-HRM* method for D614G mutation detection. This research showed that the conservation level of *S* gene from Indonesia origin SARS-CoV-2 (Isolate 2.13) was 99.9% with only single *missense* mutation, the D614G mutation. There were 21 *missense* mutations and seven *silent* mutations found within the *D614G Hotspot-FCS* region from the sequences of Indonesia origin SARS-CoV-2, based of the research sample and GISAID data. *Maximum Likelihood* (ML) phylogenetic analysis which based on the *S* gene showed that Yogyakarta origin SARS-CoV-2 (Isolate 2.13) was closely related with other G614 variants originating from various countries. Based on the analysis of research sample and GISAID data, the mutation rate of each region of *Spike* were hanging from 1.61×10^{-3} to 1.34×10^{-2} subs per site per year, whereas the *D614G Hotspot-FCS* region was determined as the mutation hotspot region within the *Spike* protein. The *qPCR-HRM* assay had been shown to reliably detect the D614G mutation, therefore might be useful for G614 variant surveillance in a particular location. This research was expected to give insights for the development of other kind of research related to the rapid detection of SARS-CoV-2 variants as well as the development of vaccine which refers to the *S* gene sequence of Indonesian origin SARS-CoV-2.

Keywords : SARS-CoV-2, *Spike*, *FCS*, D614G, *qPCR-HRM*