DETEKSI MUTASI IVSI-5 (G→C) DAN CD 35 (DEL C) GEN PENGKODE β-GLOBIN PADA *CARRIER* β-THALASSEMIA DENGAN *HIGH-RESOLUTION MELTING ANALYSIS*

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INTISARI

Thalassemia adalah suatu kelainan genetik yang disebabkan oleh mutasi gen yang berperan dalam sintesis rantai globin dan bersifat autosomal resesif. Prevalensi *carrier* β-thalassemia di Indonesia cukup tinggi, yaitu 3-10%. Salah satu upaya pengendalian jumlah penderita β-thalassemia adalah dengan skrining *carrier* β-thalassemia dan deteksi mutasi. Beberapa metode seperti PCR-SSCP, ARMS, dan RFLP telah digunakan untuk deteksi mutasi, namun metode tersebut membutuhkan proses lanjut pasca-PCR. *High-Resolution Melting Analysis* (HRMA) adalah suatu metode deteksi mutasi yang tidak membutuhkan proses lanjut pasca-PCR. Penelitian ini bertujuan untuk mendeteksi mutasi IVSI-5 (G→C) dan Cd 35 (del C) pada gen pengkode β-globin *carrier* β-thalassemia menggunakan HRMA. Sampel DNA diisolasi dari koleksi darah *carrier* β-thalassemia. Analisis DNA dilakukan dengan HRMA, PCR pada sebagian ekson 1 sampai dengan sebagian ekson 2 gen pengkode β-globin, dan sekuensing hasil PCR. *High-Resolution Melt Software* v3.1 digunakan untuk analisis data HRMA. Hasil sekuensing dianalisis menggunakan *software* Chromas dan dilakukan *alignment* dengan urutan DNA gen pengkode β-globin normal dari *database* NCBI NC_000011.10 menggunakan Clustal Omega. Deteksi mutasi oleh HRMA dikonfirmasi dengan hasil sekuensing. Hasil penelitian menunjukkan bahwa HRMA dapat mendeteksi mutasi IVSI-5 (G→C) dan Cd 35 (del C). Dari sepuluh sampel, mutasi IVSI-5 (G→C) ditemukan sebanyak 50%, sedangkan mutasi Cd 35 (del C) ditemukan sebanyak 30%. Terdapat 20% sampel yang tidak teridentifikasi memiliki kedua jenis mutasi tersebut. Hasil deteksi mutasi dengan HRMA pada gen pengkode β-globin *carrier* β-thalassemia dapat dikonfirmasi dengan hasil sekuensing dan menunjukkan 100% kesesuaian.

Kata kunci: β-thalassemia, deteksi mutasi, *High-Resolution Melting Analysis*, mutasi IVSI-5 (G→C), mutasi Cd 35 (del C)

DETECTION OF IVSI-5 (G→C) AND CD 35 (DEL C) MUTATIONS IN THE β-GLOBIN CODING GENE OF β-THALASSEMIA CARRIERS USING HIGH-RESOLUTION MELTING ANALYSIS

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ABSTRACT

Thalassemia is an autosomal recessive genetic disorder caused by gene mutation involved in the globin chains synthesis. The prevalence of β-thalassemia in Indonesia is quite high, which is 3-10%. Screening for β-thalassemia carriers and mutation detection can be useful to control the number of β-thalassemia patients. There are many methods to detect the mutation, such as PCR-SSCP, ARMS, and RFLP, but these methods require post-PCR processing. High-Resolution Melting Analysis (HRMA) is a method that does not require post-PCR processing. The aim of this research was to detect IVSI-5 (G→C) and Cd 35 (del C) mutations in the β-globin gene of β-thalassemia carriers using HRMA. DNA was isolated from blood collection of β-thalassemia carriers. DNA analysis was carried out by HRMA, PCR in part of 1st to 2nd exon of the β-globin gene, and sequencing of the PCR product. High-Resolution Melt Software v3.1 was used to analyze HRMA results. Sequencing results were analyzed using Chromas software. The nucleotides sequence of the sequencing results were aligned with the normal sequence in NCBI database NC_000011.10 using Clustal Omega. Detection of mutation by HRMA was confirmed by sequencing results. The results showed that IVSI-5 (G→C) and Cd 35 (del C) mutations can be detected by HRMA. From ten samples, IVSI-5 (G→C) mutation found in 50% samples, while Cd 35 (del C) mutation found in 30% samples. Twenty percent of samples revealed neither IVSI-5 (G→C) nor Cd 35 (del C) mutation. Mutation detection results by HRMA can be confirmed with sequencing results and showed 100% agreement.

Keywords: β-thalassemia, mutation detection, High-Resolution Melting Analysis, IVSI-5 (G→C) mutation, Cd 35 (del C) mutation