

DETEKSI MUTASI EKSON 1 GEN β -GLOBIN PADA CARRIER HEMOGLOBIN E DENGAN METODE RFLP (RESTRICTION FRAGMENT LENGTH POLYMORPHISM)

Farida Noor Irfani
11/316218/BI/08762

INTISARI

Hemoglobin E (HbE) merupakan salah satu kelainan struktural hemoglobin yang disebabkan oleh substitusi G \rightarrow A pada kodon ke-26 ekson 1 gen HBB, sehingga mengakibatkan perubahan hasil translasi dari asam glutamat menjadi lisin. HbE homozigot maupun heterozigot bersifat *asymptomatic*, tetapi dicirikan oleh eritrosit mikrositik dan hipokromik serta konsentrasi HbA₂ melebihi 13%. Persebaran *carrier* HbE cukup tinggi di wilayah Asia Tenggara, termasuk Indonesia sehingga diperlukan suatu upaya pencegahan berupa deteksi sederhana, cepat, dan murah. NESTROFT (*Naked Eye Single Tube Red Cell Osmotic Fragility Test*) sebagai metode deteksi pada *carrier* β -thalassemia dapat pula memberikan hasil positif pada jenis thalassemia dan varian Hb lain, termasuk HbE. Tujuan penelitian ini adalah mengetahui validasi NESTROFT untuk deteksi *carrier* HbE dengan analisis hematologis dan teknik molekular PCR-RFLP.

Spesimen penelitian ini berupa *frozen blood* sebelas individu yang berdasarkan uji NESTROFT dan analisis hematologis sebagai *carrier* HbE serta satu individu normal sebagai pembanding. DNA sampel darah diisolasi lalu diamplifikasi dengan sepasang primer spesifik menghasilkan ampikon berukuran 335 bp. Selanjutnya, produk PCR didigesti menggunakan enzim endonuklease restriksi *Mnl*I. Interpretasi data dilakukan dengan membandingkan perbedaan ukuran dan jumlah fragmen digesti normal dengan terduga *carrier* HbE. Hasil penelitian menunjukkan 5 dari 11 individu positif NESTROFT terkonfirmasi sebagai *carrier* HbE melalui analisis hematologis (45,5%). Selanjutnya, kelima individu tersebut (positif NESTROFT dan analisis hematologis) juga terkonfirmasi sebagai *carrier* HbE dengan teknik PCR-RFLP (100%). Teknik molekular PCR-RFLP dengan enzim endonuklease restriksi *Mnl*I dapat mengkonfirmasi terduga *carrier* HbE pada hasil positif uji NESTROFT dan analisis hematologis.

Kata Kunci : HbE, RFLP, mutasi, NESTROFT, analisis hematologis

**DETECTION OF EXON 1 MUTATION IN β -GLOBIN GENE ON
HEMOGLOBIN E CARRIER WITH RFLP METHOD
(RESTRICTION FRAGMENT LENGTH POLYMORPHISM)**

Farida Noor Irfani
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ABSTRACT

Hemoglobin E (HbE) is one of structural abnormality in hemoglobin that caused by substitution G \rightarrow A in exon 1 codon 26 HBB gene, that can change the translation process from glutamate acid into lysine. Both HbE homozygote and heterozygote are asymptomatic, which is marked by erythrocyte microcytic, hypochromic, and also the concentration of HbA₂ gets over 13%. The distribution of HbE carrier is high enough in South East Asia, including Indonesia. A simple, fast, and cheap detection is needed in order to prevent this disease. NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) can be used to detect β -thalassemia carrier and also give positive results for another thalassemia and Hb variation including HbE. The aim of this result is to validate NESTROFT that used to detect HbE carrier with hematologist analysis and molecular technique PCR-RFLP.

Specimen of this research is frozen blood of 11 individuals that passed NESTROFT test and hematologist analysis as HbE carrier and also one normal individuals as a comparison. DNA sample of the blood is isolated and amplified with a specific primer with amplicon size 335 bp. The PCR product is being digested using endonuclease restriction enzyme *Mnl*I. Visualization can be done by compare the differences between the size and amount of normal digestion fragment suspected as HbE carrier. The result showed that 5 from 11 individual positive NESTROFT confirmed as HbE carrier using hematologist analysis (45,5%) also confirmed as HbE carrier with PCR-RFLP technique (100%). Molecular technique PCR-RFLP with endonuclease restriction enzyme *Mnl*I can be used to confirm HbE carrier suspect in the positive result in NESTROFT test and hematological analysis.

Kata Kunci : HbE, RFLP, mutation, NESTROFT, hematological analysis.