ANALISIS EPITOP DARI PROTEIN *NON-STRUCTURAL* 1 (NS-1) VIRUS DENGUE MENGGUNAKAN ANTIBODI MONOKLONAL

INTISARI

Latar Belakang. Dengue mempengaruhi 50–100 juta orang setiap tahun, terutama di wilayah Asia-Pasifik yang memiliki tingkat endemisitas tinggi dan sirkulasi keempat serotipe DENV. *Rapid Diagnostic Tests* (RDT) memberikan solusi praktis tetapi memiliki sensitivitas dan spesifisitas terbatas, terutama pada fase akut. Protein NS1 merupakan biomarker kunci untuk deteksi dini, meskipun konservasi sebesar 79% antar serotipe dan reaktivitas silang dengan flavivirus seperti Zika menimbulkan tantangan. Pengembangan RDT berbasis NS1 menggunakan antibodi monoklonal untuk mendeteksi semua serotipe DENV di Indonesia sangat penting untuk meningkatkan akurasi diagnostik.
Metode. Penelitian ini merupakan kuasi ekperimental dengan pendekatan in vivo, in vitro, dan in silico. Variabel penelitian mencakup protein rekombinan NS1, antibodi monoklonal spesifik NS1, isotipe, konsentrasi antibodi, dan prediksi epitop NS1. Data dianalisis secara deskriptif.
Hasil. Ditemukan delapan wilayah *conserved* pada NS1 di seluruh serotipe DENV. Semua wilayah *conserved* bersifat antigenik, dengan HTWTEQYKFQ dan SQHNYRPGY digunakan untuk pembuatan peptida sintetik. Antibodi monoklonal WRD12, WRD11, WRD10, dan WRD8 menunjukkan reaktivitas terhadap protein rekombinan NS1 DENV-3 hingga konsentrasi 1:1000 dalam dot blotting, dengan WRD8 spesifik terhadap peptida sintetik β-roll kecil di N-terminal NS1 (HTWTEQYKFQ) pada konsentrasi 1:10. Sebaliknya, WRD9 menunjukkan sinyal lemah, mengindikasikan afinitas rendah. Hasil ELISA menunjukkan WRD12 dan WRD11 memiliki reaktivitas tertinggi terhadap NS1 dengan nilai absorbansi 0,37 dan 0,32, sementara WRD10 sedang (0,25), dan WRD8 serta WRD9 rendah (0,18 dan 0,15). Validasi Western blotting menunjukkan WRD12, WRD11, dan WRD10 mendeteksi NS1 dalam bentuk monomer (~55 kDa), dimer (~80–109 kDa), dan SUMO-NS1 (~68 kDa dan ~120 kDa) dengan reaktivitas kuat. WRD8 memiliki afinitas rendah terhadap bentuk monomer dan dimer NS1, sementara WRD9 memiliki kemampuan moderat tetapi terbatas untuk SUMO-NS1.
Kesimpulan. Prediksi in silico mengidentifikasi delapan wilayah *conserved* dengan dua epitop antigenik utama, HTWTEQYKFQ dan SQHNYRPGY. Imunisasi mencit menghasilkan lima antibodi monoklonal, dengan WRD12, WRD11, dan WRD10 memiliki potensi diagnostik tertinggi. Uji **in vitro** menunjukkan WRD12, WRD11, dan WRD10 mengenali NS1 dalam ketiga uji (ELISA, dot blotting, dan Western blot), WRD9 pada dua uji, dan WRD8 hanya dalam dot blotting dengan spesifisitas tinggi terhadap epitop sekuensial.

Kata Kunci: Virus Dengue, NS1 protein, antibodi monoklonal, rapid diagnostic test, epitop sekuensial, deteksi dini

EPITOPE ANALYSIS OF DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 (NS-1) USING MONOCLONAL ANTIBODIES

ABSTRACT

Background. *Dengue affects 50–100 million people annually, primarily in the Asia-Pacific region with high endemicity and circulation of all four DENV serotypes. Rapid Diagnostic Tests (RDTs) provide practical solutions but have limited sensitivity and specificity, particularly during the acute phase. The NS1 protein is a key biomarker for early detection, though its 79% conservation across serotypes and cross-reactivity with flaviviruses like Zika pose challenges. Developing NS1-based RDTs using monoclonal antibodies to detect all DENV serotypes in Indonesia is vital for improving diagnostic accuracy.*
Methods. *This study is quasy experimental employing in vivo, in vitro, and in silico approaches. The research variables include recombinant NS1 protein, NS1-specific monoclonal antibodies, antibody subclasses, antibody concentrations, and NS1 epitope predictions. Data were analyzed descriptively.*
Results. *Eight conserved regions were identified within the NS1 protein across all DENV serotypes. All conserved regions exhibited antigenic properties, with HTWTEQYKFQ and SQHNYRPGY selected for synthetic peptide production. The monoclonal antibodies WRD12, WRD11, WRD10, and WRD8 demonstrated reactivity toward the recombinant NS1 protein of DENV-3 at concentrations as low as 1:1000 in dot blotting assays, with WRD8 showing specificity for the synthetic β-roll peptide located in the N-terminal region of NS1 (HTWTEQYKFQ) at a concentration of 1:10. In contrast, WRD9 exhibited weak signals, indicating low affinity. ELISA results revealed that WRD12 and WRD11 had the highest reactivity toward NS1, with absorbance values of 0.37 and 0.32, respectively. WRD10 displayed moderate reactivity (0.25), while WRD8 and WRD9 showed lower reactivity (0.18 and 0.15, respectively). Validation via Western blotting confirmed that WRD12, WRD11, and WRD10 strongly detected NS1 in its monomeric (~55 kDa), dimeric (~80–109 kDa), and SUMO-conjugated forms (~68 kDa for the monomer and ~120 kDa for the dimer). WRD8 exhibited low affinity for both monomeric and dimeric NS1, whereas WRD9 demonstrated moderate but limited ability to detect SUMO-conjugated NS1.*
Conclusion. *In silico predictions identified eight conserved regions with two key antigenic epitopes, HTWTEQYKFQ and SQHNYRPGY. Immunisation of mice yielded five monoclonal antibodies, with WRD12, WRD11, and WRD10 demonstrating the highest diagnostic potential. In vitro assays revealed that WRD12, WRD11, and WRD10 recognised NS1 in all three tests (ELISA, dot blotting, and Western blot), WRD9 in two tests, and WRD8 exclusively in dot blotting, exhibiting high specificity towards the sequential epitope.*

Keywords: Dengue virus, NS1 protein, monoclonal antibody, rapid diagnostic test, sequential epitope, early detection