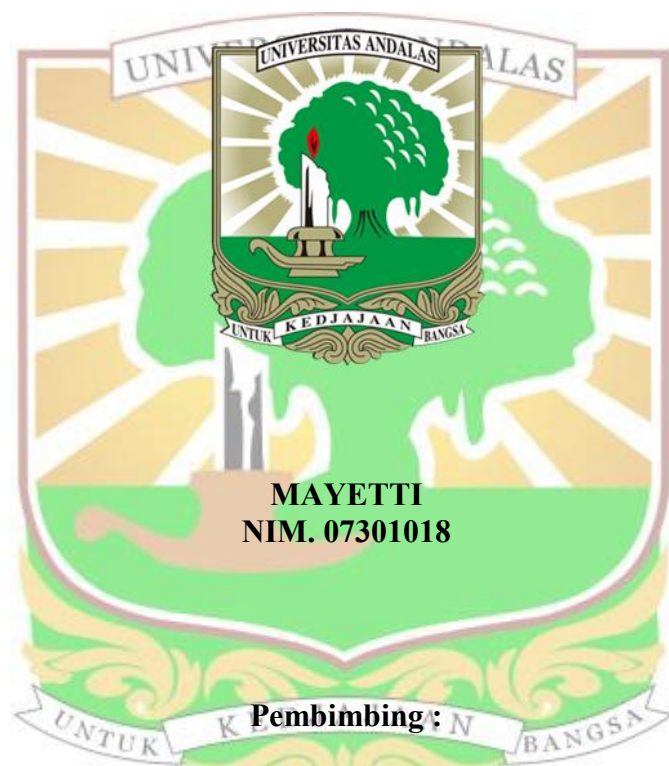


DISERTASI

HUBUNGAN POLIMORFISME GEN DAN KADAR ANGIOPOETIN 2 SERTA KADAR *VASCULAR ENDOTHELIAL GROWTH FACTOR* DENGAN TINGKAT KEPARAHAN PENYAKIT AKIBAT INFEKSI VIRUS DENGUE PADA ANAK



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PADANG
2017

ABSTRAK

Hubungan Polimorfisme Gen dan Kadar Angiopoetin-2 serta Kadar *Vascular Endothelial Growth Factor* dengan Tingkat Keparahan Penyakit Akibat Infeksi Virus Dengue pada Anak

Mayetti

Latar Belakang. Manifestasi klinis infeksi virus dengue sangat bervariasi, mulai dari bentuk yang asimtomatik sampai bentuk sangat berat, yaitu sindrom syok dengue (SSD) yang dapat menyebabkan kematian. Beberapa penelitian menunjukkan adanya pengaruh genetik terhadap keparahan penyakit akibat infeksi virus dengue. Beratnya penyakit disebabkan oleh integritas endotel vaskular. Angiopoetin-2 (ANGPT-2) dan *Vascular Endothelial Growth Factor* (VEGF) merupakan protein angiogenik yang berperan dalam terjadinya kebocoran plasma. Beberapa penelitian menunjukkan pengaruh polimorfisme gen ANGPT-2 pada beberapa penyakit, namun pada infeksi virus dengue belum pernah diteliti.

Tujuan penelitian. Mengetahui hubungan polimorfisme gen dan kadar ANGPT-2 serta kadar VEGF dengan tingkat keparahan penyakit akibat infeksi virus dengue pada anak.

Metode. Penelitian merupakan suatu penelitian *cross sectional* observasional pada anak dengan infeksi virus dengue di RSUP Dr M Djamil Padang. Sebanyak 108 anak dikelompokkan berdasarkan tingkat keparahan infeksi dengue menurut WHO 2011, yaitu demam dengue, demam berdarah dengue, dan sindrom syok dengue. Pemeriksaan kadar ANGPT-2 dan VEGF dengan metode *enzyme-linked immunosorbent assay* menggunakan Kit ANGPT-2 & Human Quantikine VEGF Immunoassay, Ray Biotech. Isolasi DNA menggunakan Mini Kit *genomic DNA*. Dilakukan *polymerase chain reaction* dan sekuensing DNA menggunakan 2 primer Exon 4-F, yaitu primer *forward* 5'-CACCCATATCCCACCTATCCT-3' dan primer *reverse* 5'-TGCCAGTCTCATCCTTCTA-3'. Primer disintesis oleh *Integrated DNA Technologies*, Singapura. Kadar ANGPT-2, VEGF dan jenis mutasi dihubungkan dengan tingkat keparahan penyakit. Analisis statistik menggunakan uji *chi-square* dan anova dengan signifikansi ($p < 0,05$). Jika distribusi data tidak normal, disajikan dalam median dan diuji dengan uji Kruskal Wallis atau Mann-Whitney.

Hasil. Terdapat peningkatan yang bermakna secara statistik ($p < 0,05$) kadar ANGPT-2 sesuai tingkat keparahan penyakit. Kadar VEGF cenderung meningkat sesuai tingkat keparahan penyakit namun tidak bermakna. Polimorfisme gen ANGPT-2 ditemukan pada 8 dari 10 SNP yang diperiksa, mutasi terbanyak pada Exon 4 rs3020221 G/A (35,18%). Ditemukan SNP baru (4c.46981), berlokasi pada urutan basa ke-232, mengubah asam amino *lysin* menjadi *glutamate*. Kadar ANGPT-2 kelompok mutan rs7834131 lebih tinggi dibandingkan non mutan. Polimorfisme gen ANGPT-2 tidak memengaruhi tingkat keparahan penyakit.

Kesimpulan. Terdapat peningkatan bermakna kadar ANGPT-2 sesuai tingkat keparahan penyakit, kadar VEGF juga meningkat tapi tidak bermakna. Polimorfisme pada rs7834131 diduga mempunyai efek protektif terhadap keparahan penyakit infeksi virus dengue pada anak.

Kata Kunci : Infeksi virus dengue , anak, ANGPT-2, polimorfisme gen, VEGF

ABSTRACT

Association of Gene Polymorphism, Angiopoietin-2 Level and Vascular Endothelial Growth Factor Level with Dengue Virus Infection Severity in Children

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Background: Clinical manifestations of dengue virus infection vary widely, ranging from asymptomatic to heavy forms, dengue shock syndrome (DSS) which can cause death. Recent several studies have demonstrated that there is genetically influence such as mutation and polymorphism toward dengue infection severity. Angiopoietin-2 (ANGPT-2) and Vascular Endothelial Growth Factor (VEGF) are protein molecule that playing role on integrity and damage of endothelial. Several studies have shown that polymorphism influence in ANGPT-2 gene toward some diseases. On contrary ANGPT-2 gene polymorphism in infected children has been yet observed.

Objective: This study aimed to determine association of gene polymorphism, ANGPT-2 level and VEGF level with dengue infection severity in children.

Methods: This study was observational method with cross sectional study in 108 infected dengue children in Dr. M. Djamil Hospital Padang. All patient was classified according to World Health Organization (WHO) 2011 into 3 DF (34), DHF (39), and DSS (35). Level of ANGPT-2 and VEGF were examined by Enzyme-Linked Immunosorbent Assay (ELISA) using ANGPT-2 & Human Quantikine VEGF Immunoassay Kit, Ray Biotech. Gene DNA was isolated by using genomic DNA mini kit, PCR using direct DNA sequencing method by using two primary Exon 4; primary forward 5'-CACCCATATCCCACCTATCCT-3', and primary reverse 5'-TGCCAGTCTCATCCTTCTA-3'. Primary was synthesized by Integrated DNA Technologies (IDT), Singapore then conducted BLAST analysis and sequencing all samples. Angiopoietin-2 level, VEGF and type of mutation are associated with dengue infection severity. Analytical statistic was chi-square test and anova with significant level ($p < 0,05$) if distribution data was not normal so it was presented in median data and tested by Kruskali-Wails, Mann-Whitney

Result: The results showed an increase in ANGPT-2 levels according to the severity and that increasing was statistically significant ($p < 0.05$). While VEGF levels increased along with severity of the disease though it was not significantly. Polymorphism was found in 8 of the 10 SNPs examined, and the most mutation occurred at Exon 4 rs3020221 G/A (35,18%). This study also found a new SNP 4c.46981 located on the sequence of bases to 232 changing lysine to glutamine. The level of ANGPT-2 in the mutation group rs7834131 was higher than the normal. ANGPT-2 gene polymorphism didn't affect the severity of the disease.

Conclusion: there is an increase of ANGPT-2 levels according to the severity, VEGF levels is also increasing but it's not statistically significant. ANGPT-2 gene polymorphism at rs7834131 has been assumed that has protective effect toward the severity of the disease due to dengue virus infection in children.

Keyword: dengue virus infection, children, ANGPT-2, gene polymorphism, VEGF