3. Menguji Doktor

KODE K07

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KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS AIRLANGGA
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Nomor : 1105 /UN3.1.1/DL/2021 15 Pebruari 2021
Lamp : 1 Berkas
Hal : Mohon Kesediaan untuk menjadi Panitia Penguji Disertasi (Ujian Tertutup)

Yth.

1. Prof. Dr. Jusak Nugraha, dr., MS., Sp.PK(K) (Ketua)
2. Prof. Dr. H. Joewono Soeroso, dr., M.Sc.,Sp.PD.,K-R,FINASIM
3. Prof. Dr. Harianto Notopuro, dr., MS
4. Prof. Retnoflandajani, dr., MS., Ph.D
5. Prof. Dr. Nyoman Kertia, dr.,Sp.PD,KR
6. Dr. Gondo Mastutik, drh., M.Kes
7. Dr. Windhu Purnomo, dr., M.S

Dengan hormat,

Sehubungan dengan selesainya penulisan disertasi peserta Program Doktor angkatan tahun 2014/2015.

Nama : Awalia, dr.,Sp.PD ELPT 563
NIM : 011417017333
Judul : HUBUNGAN ANTARA *POLYMORPHISM* PROMOTER GEN C-REACTIVE PROTEIN (CRP) -821 A>G,-390 C>A/T, DAN KADAR INTERFERON-α DENGAN KADAR CRP SERUM PASIEN *SYSTEMIC LUPUS ERYTHEMATOSUS* (SLE) DI RSUD DR. SOETOMO SURABAYA

Promotor : Prof. Dr. H. Joewono Soeroso, dr., M.Sc., Sp.PD., K-R,FINASIM
Ko-Promotor : Prof. Dr. HariantoNotopuro, dr., MS

Ujian Disertasi rencananya diselenggarakan :

Hari, Tanggal : Jum'at, 26 Pebruari 2021
Pukul : 08.30 – 11.30 WIB
Tempat : Menguji secara online menggunakan aplikasi Zoom

Maka dengan ini mohon kesediaan Saudara untuk menjadi Ketua / Anggota panitia Penguji Disertasi tersebut, terlampir kami sampaikan pernyataan kesediaan untuk diisi dan dilampirkan pada kami dalam waktu yang tidak terlalu lama guna diproses lebih lanjut.

Demikian atas perhatian Saudara, kami ucapkan terima kasih.

An. Dekan
Wakil Dekan I,

Dr. Achmad Chusnu Romdhoni,dr.,Sp.THT-KL(K).,FICS
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Tindasan
- Kepala Sub. Bagian Sarana dan Prasarana
- Kepala Sub. Bagian Keuangan

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SURAT TUGAS

Nomor : 1106 /UN3.1.1/DL/2021

Wakil Dekan I Fakultas Kedokteran Universitas Airlangga dengan ini menugaskan :

1.	Prof. Dr. Jusak Nugraha, dr., MS , Sp.PK(K)	Ketua
2.	Prof. Dr. H. Joewono Soeroso, dr., M.Sc.,Sp.PD.,K-R,FINASIM	Anggota
3.	Prof. Dr. Hariarto Notopuro, dr., MS	Anggota
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5	Prof. Dr. Nyoman Kertia, dr.,Sp.PD.KR	Anggota
6.	Dr. Gondo Mastutik, drh , M.Kes	Anggota
7.	Dr. Windhu Purnomo, dr., M.S	Anggota

Sebagai Ketua / Anggota Panitia Ujian Tahap Pertama (Tertutup) Program Doktor Fakultas Kedokteran Universitas Airlangga atas nama Awalia, dr.,Sp.PD peserta Program Doktor Program studi Ilmu Kedokteran angkatan tahun 2014/2015 yang diselenggarakan pada tanggal 26 Pebruari 2021

Surat tugas ini diterbitkan sementara untuk menunggu keluarnya Surat Keputusan dari Dekan Fakultas Kedokteran Universitas Airlangga.

Surabaya, 15 Pebruari 2021

a.n. Dekan,
Wakil Dekan I,

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DISERTASI

HUBUNGAN ANTARA POLIMORFISME PROMOTER GEN C-REACTIVE PROTEIN (CRP) -821 A>G, -390 C>A/T, DAN KADAR INTERFERON-α DENGAN KADAR CRP SERUM PASIEN *SYSTEMIC LUPUS ERYTHEMATOSUS* (SLE) DI RSUD DR. SOETOMO SURABAYA

AWALIA

PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2021

HUBUNGAN ANTARA POLIMORFISME PROMOTER GEN C-REACTIVE PROTEIN (CRP) -821 A>G, -390 C>A/T, DAN KADAR INTERFERON-α DENGAN KADAR CRP SERUM PASIEN *SYSTEMIC LUPUS ERYTHEMATOSUS* (SLE) DI RSUD DR. SOETOMO SURABAYA

DISERTASI

Untuk Memperoleh Gelar Doktor
Dalam Program Studi Ilmu Kedokteran Jenjang Doktor
Pada Fakultas Kedokteran Universitas Airlangga dan
Dipertahankan di Hadapan Panitia Ujian Akhir
Tahap 1 (Tertutup)

Oleh :

AWALIA
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PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2021

LEMBAR PENGESAHAN

DISERTASI

HUBUNGAN ANTARA POLIMORFISME PROMOTER GEN C-REACTIVE PROTEIN (CRP) -821 A>G, -390 C>A/T, DAN KADAR INTERFERON-α DENGAN KADAR CRP SERUM PASIEN *SYSTEMIC LUPUS ERYTHEMATOSUS* (SLE)DI RSUD DR.SOETOMO SURABAYA

YANG TELAH DISETUJUI

PADA TANGGAL 15FEBRUARI 2021

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SUMMARY

Association of Polymorphisms in C-Reactive Protein (CRP) Promoter -821 A>G, -390 C>A/T, and Interferon-α (IFN-α) Level with Plasma CRP Level in Systemic Lupus Erythematosus (SLE) patients in Dr. Soetomo Academic General Hospital Surabaya

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease with unclear etiology and various clinical manifestation, disease progression, and prognosis. Inflammation in SLE can cause tissue damage due to deposit of immune complex. CRP as one of acute phase reactant will rapidly increase during inflammation with its function to clear apoptotic cells, incrase phagocytosis, and release inflammation cytokines. Unlike other inflammatory diseases, SLE patients generally have normal plasma CRP level unless there is bacterial infection. This can be caused by genetic variation on CRP gene and high level of IFN-α that may inhibit secretion of CRP by hepatocytes. There is no previous studies that explain which factor is more dominant.

There are many studies from various population showing some single nucleotide polimorphisms (SNPs) in CRP gene related to SLE or CRP level in SLE. One of them are SNPs in CRP -390 dan -821 in proximal promoter among Korean population. They related with low CRP basal and also during acute phase, and also related with SLE. These SNPs were also found among Afro-American SLE population. These SNPs location have never been studied in Indonesia. IFN-α is also said may cause low CRP level in SLE. Though there is high level of IL-6 and severe inflammation during SLE flare and viral infection, but CRP level is still normal. There are abundant of IFN-α during SLE flare and viral infection, so it is presumed that IFN-α may inhibit CRP production.

This study was an analytic observational study, with cross-sectional design held in Dr. Soetomo Academic General Hospital Surabaya. The aim of this study was to analyze the association of SNPs in CRP promoter -821, -390, other possible location, and plasma IFN-α level with CRP level of SLE patients. Those SNPs was also compared between SLE groups and control. Spondyloarthritis patients were used as control because generally there is increasing of CRP level in spondyloarthritis patients with high disease activity.

Inclusion criteria of this study were SLE patients aged 18-60 years old willing to join by signing informed consent. SLE diagnosis was based on ACR 1997 classification criteria. Sample size was determined using sample size formula for correlation test with coefficient correlation based on previous study. Minimal sample size was 40. Inclusion criteria for control was spondyloarthritis patients aged 18-60 years-old willing to join this study by signing informed consent. Spondyloarthritis diagnosis was based on ASAS 2010 criteria. We excluded patients with history of cancer, diabetes mellitus, cardiovascular diseases, mixed connective tissue disease, patients with infection, CRP > 50 mg/dl, and obese patients. SLE disease activity score was measured using *systemic lupus activity measure* (SLAM).

History taking, physical examination, and blood sample were obtained for every patients in both groups. Laboratory test taken for SLE groups were complete blood count (CBC), ANA, anti ds-DNA, plasma complement C3, C4, IFN-α, and PBMC to isolate DNA, perform convensional PCR, and DNA squencing. Laboratory test for control group were only CBC, CRP, and DNA isolation till DNA sequencing. Serum CRP level test was quantitative with immunoturbidimetry technique. IFN-α level was measured using ELISA technique with Human IFN alpha ELISA Kit Invitrogen BMS216/BMS216TEN. Plasma samples for IFN-α test were stored at -80^{0}C. Upon completion of a sample assay using the kit protocol, absorbance was determined at 450 nm on Microplate reader: iMark (BioRad).

There were 40 SLE patients, all were women with median age was 31.5 years old. Median age for control group was 39 years old (range 18-59 years old). Median SLAM score was 8.5 (range 0-26). Mean CRP level was 5.19 ± 2.69 mg/L, and median IFN-α level was 46,02 pg/ml. There was no significant difference of SNPs CRP -821 and -390 in both groups. A new SNP was detected in CRP -456 in 5 SLE subjects but none in control group. There was significant difference of SNP CRP -456 in SLE group compared to control. SNP in CRP -456 might increase the risk of SLE by 2.143 times. There was a moderate negative correlation between plasma IFN-α and CRP level. There was also positive moderate correlation between plasma IFN-α and SLE disease activity (SLAM score). Multivariate analysis had shown only plasma IFN-α correlated with CRP level.

We concluded that there was moderate negative correlation between plasma IFN-α and CRP level either bivariately or by using linear regression. There was no association between SNPs in CRP -821, -390, nor -456 with plasma CRP level. There was significant difference of SNP CRP -456 in SLE patients compared to control, with increasing risk of SLE on subject having SNP CRP -456 A>G compared to those who did not.

ABSTRAK

Hubungan Antara Polimorfisme Promoter Gen *C-Reactive Protein* (CRP) -821 A>G, -390 C>A/T, dan Kadar Interferon-α dengan Kadar CRP Serum Pasien *Systemic Lupus Erythematosus* (SLE) di RSUD Dr. Soetomo Surabaya

Awalia

Latar Belakang: Pasien SLE aktif meskipun berat umumnya memiliki kadar CRP plasma yang normal, padahal CRP sebagai protein fase akut dibutuhkan untuk *clearance* sel apoptosis dan deposit kompleks imun pada SLE. CRP tidak meningkat pada SLE aktif diduga karena adanya variasi genetik gen CRP dan tingginya interferon-α (IFN-α) yang dapat menghambat sekresi CRP oleh hepatosit. Penelitian ini bertujuan menganalisis hubungan antara *single nucleotide polymorphisms* (SNPs) di promoter gen CRP dan kadar IFN-α dengan kadar CRP serum pasien SLE. Dianalisis juga hubungan antara SNPs di promoter gen CRP dengan kejadian SLE.

Metode: Empat puluh pasien SLE dan 40 pasien spondiloartritis sebagai kontrol dilibatkan dalam studi ini. Pada subjek SLE dilakukan tes darah rutin, kadar CRP (dengan metode *immunoturbidimetry*), kadar IFN-α serum (diukur dengan ELISA), dan dilakukan sekuensing DNA untuk melihat SNPs di promoter gen CRP melalui isolasi DNA dari *peripheral blood mononuclear cells* (PBMC). Pada kontrol hanya dilakukan pengambilan darah untuk sekuensing DNA.

Hasil: Didapatkan 40 wanita SLE dengan median umur 31,5 tahun dan median skor SLAM 8,5. Median umur kontrol adalah 39 tahun. Rerata kadar CRP subjek SLE adalah 5,19 ± 2,69 mg/L, dan median kadar IFN-α adalah 46,02 pg/ml. Tidak terdapat perbedaan bermakna kejadian SNPs di CRP -821 maupun di -390 antara kelompok subjek SLE dan kontrol. Ditemukan lokasi SNP baru di CRP -456 A>G pada 5 subjek kelompok SLE, dan tidak pada kelompok kontrol. SNP di CRP -456 A>G ini akan meningkatkan risiko SLE 2,143 kali dibanding normal. Terdapat hubungan bermakna (korelasi negatif sedang) antara kadar IFN-α dengan kadar CRP serum. Secara multivariat juga hanya kadar IFN-α yang berhubungan bermakna dengan kadar CRP serum.

Kesimpulan: IFN-α berkorelasi negatif dengan kadar CRP serum. Tidak didapatkan hubungan bermakna antara SNPs di CRP -821, -390, dan -456 dengan kadar CRP serum. Terdapat perbedaan bermakna kejadian SNP di CRP -456 dari kelompok subjek SLE dibanding kontrol. Subjek yang mengalami SNP CRP -456 A>G akan berisiko terkena SLE 2,143 kali lebih besar dibanding yang normal.

Kata kunci: promoter gen CRP, interferon-α, kadar CRP, *systemic lupus erythematosus*

ABSTRACT

Association of Polymorphisms in C-Reactive Protein (CRP) Promoter -821 A>G, -390 C>A/T, and Interferon-α (IFN-α) Level with Plasma CRP Level in Systemic Lupus Erythematosus (SLE) patients in Dr. Soetomo Academic General Hospital Surabaya

Awalia

Background: Active SLE patients generally have normal plasma CRP level. CRP as an acute phase reactant is needed to clear apoptotic cells and immune complex in SLE. Unresponsive CRP in SLE is may be caused by genetic variation and high level of IFN-α that can inhibit CRP secretion by hepatocytes. The aim of this study was to analyze association of *single nucleotide polymorphisms* (SNPs) in CRP promoter and IFN-α level with plasma CRP level in SLE patients. We also analyzed association of this SNPs with SLE.
Methodes: Fourty SLE and 40 spondyloarthritis (as control) patients were included in this study. SLE subjects underwent routine blood test, test for CRP level (*immunoturbidimetry*), serum IFN-α (ELISA methods), and DNA squencing to detect SNPs in CRP promoter through DNA isolation from *peripheral blood mononuclear cells* (PBMC). Control group only underwent DNA squencing.
Results: Fourty SLE women were included with median age 31.5 years old and median SLAM score was 8,5. Median age of control group was 39 years old. Average CRP level was 5.19 ± 2.69 mg/L, and median IFN-α level was 46.02 pg/ml. There was no significant difference of SNPs in CRP -821 or -390 between SLE subjects and control. New SNP was found in CRP -456 A>G in 5 SLE subjects, but none in control group. This SNP would increase SLE risk by 2.143 times more compared to normal. There was moderate negative correlation between IFN-α level and plasma CRP. Linear regression only showed IFN-α level correlated with serum CRP.
Conclusion: Plasma IFN-α correlated with CRP level. There was no association of SNPs in CRP -821, -390, and -456 with CRP level. There was significant difference of SNP in CRP -456 between SLE group and control. SNP CRP -456 A>G would increase the risk of SLE 2.143 times more compared to normal

Keywords: CRP promoter, interferon-α, CRP level, *systemic lupus erythematosus*