SINGLE NUCLEOTIDE POLIMORPHISMS PROMOTER GEN IL-10 SEBAGAI PREDIKTOR AKTIVITAS PENYAKIT *SYSTEMIC LUPUS ERYTHEMATOSUS*

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ABSTRACT

Interleukin -10 promoter Single Nucleotide Polymorphisms as predictor diseases activity of Systemic Lupus Erythematosus

Background : Systemic Lupus Erythematosus is a complex diseases, signifying that genetic and environmental factors are involved in diseases development. Although the role of genetic factor in many populations have been studied, the genetic factor associated with diseases activity is yet to be identified. Several study reported the association between serum level of IL-10 with SLE disease activity. This study was performed to determined polymorphisms in the IL-10 gene promoter with diseases activity SLE in Javanese population.
Methods : This study was performed in two procedures. The first procedure analyzed the genotype IL-10 gene proximal promoter in SLE patients using a pair of specific primer of the IL-10 gene proximal promoter and sequenced by Applied Biosystem method. The second procedure analyzed the influence of IL-10 in converting from anti-inflammatory into pro-inflammatory function using PBMC. This study enrolled 47 SLE patients (ACR criteria) in active diseases, dr Soetomo hospital Surabaya. Disease activity was evaluated using SLAM. The level of IL–10 was measured by Eliza kit.
Result : This study showed that there is no significant association between level of IL-10 with ANA and dsDNA, but the level of IL-10 was significant association with low level complement. There were three locus SNP at -294 A→G, -296 A→T and -301 A→G of IL-10 gene proximal promoter, with three variant combination: -294 A→G, -296 A→A, -301 A→G; -294 A→G, -296 A→T, -301 A→G and -294 A→A, -296 A→A, -301 A→A. The serum level of IL-10 among three group were significantly difference. The patients with variant -294 A→G, -296 A→A, -301 A→G had more significantly higher score of disease activity (severe diseases) compare with the other groups of patient. The combination -294 A→A, -296 A→A, -301 A→A is similiar to the sequence IL-10 gene proximal promoter from GenBank NCBI. The result of the study showed that interferon significantly induced pro-inflammatory function of IL-10.
Conclusion : This study result is Interleukin-10 proximal promoter SNP implicates diseases activity of Systemic Lupus Erythematosus by increasing the level of IL-10. Interferon converting the function of increasing level IL-10 as pro-inflammatory.

Keyword: SLE, SNP, IL-10, IFN, Javanese

RINGKASAN

Systemic Lupus Erythematosus (SLE) merupakan salah satu penyakit rematik kronik sistemik otoimun. SLE terkait dengan faktor genetik dan faktor lingkungan. Patogenesis SLE ditandai dengan proliferasi sel B yang membentuk bermacam-macam otoantibodi selain itu pada SLE juga didapatkan hilangnya toleransi sistem imun dan defek pembersihan komplek imun.

Belum ditemukan gen yang menentukan aktivitas penyakit. Di RSUD dr. Soetomo angka kematiannya masih tinggi dibandingkan pada populasi lain. Angka kematian penderita SLE di RSUD Dr. Soetomo sebagian besar disebabkan oleh karena penyakit yang aktif. Menurut Urowitz, *et al.* (1997) kematian penderita SLE pada fase awal penyakit akibat aktivitas penyakit.

Berdasarkan beberapa peneliti diduga SNP promoter gen IL-10 dapat sebagai prediktor aktivitas penyakit karena dengan adanya SNP di promoter terutama bagian regulator transkripsi akan meningkatkan kadar IL-10 terutama pada SLE yang aktif.

IL-10 berfungsi merangsang proliferasi, diferensiasi dan sintesa autoantibodi. IL-10 pada SLE yang aktif berubah fungsi menjadi pro-inflamasi. Berdasarkan penelitian-penelitian ini, maka dicoba mengeksplorasi genotip promoter IL-10 pada populasi jawa, apakah juga berperan dalam menimbulkan aktivitas penyakit.

Pada SLE yang aktif ditemukan kadar IL-10 yang tinggi dan terkait dengan aktivitas penyakit dilaporkan oleh beberapa peneliti. Peningkatan IL-10 ini disebabkan oleh adanya SNP di promoter gen IL-10. Kaitan antara peningkatan kadar IL-10 dengan aktivitas penyakit diduga karena perubahan sifat IL-10 dari anti-inflamasi menjadi pro-inflamasi karena IFN.

Perubahan sifat IL-10 dari anti-inflamasi menjadi pro-inflamasi melalui jalur signaling intraseluler. Pada kondisi normal IL-10 mengaktifkan faktor transkripsi STAT3 merupakan faktor transkripsi gen anti-inflamasi. Pengaruh IFN menimbulkan perubahan signaling IL-10 mengaktifkan STAT1 karena *cross signalling*. STAT1 merupakan faktor transkripsi gen pro-inflamasi.

Metode penelitian ini adalah menganalisis DNA dari pasien SLE aktif yang dikelompokkan menjadi berat dan ringan berdasarkan skor SLAM. Proses untuk mengetahui pola SNP maka DNA sampel dilakukan PCR dan sekuensing. Pada penelitian juga dilakukan pembuktian IL-10 berperan sebagai pro-inflamasi karena paparan IFN dilakukan percobaan in vitro dengan PBMC.

Hasil penelitian dilakukan uji statistik. Karakteristik pasien, frekuensi kriteria ACR, frekuensi SNP dilakukan uji statistik deskriptif. Hubungan SNP dan kombinasi SNP dengan kadar IL-10 dan skor SLAM dilakukan uji anova dengan software SPSS versi 17.

Untuk mencari hubungan antara kadar IL-10 dan peningkatan antibodi serta penurunan komplemen dilakukan dengan analisis jalur. Pada kultur PBMC untuk melihat perbedaan sekresi TNF-α sebelu dilakukan paparan dan setelah dilakukan paparan dilakukan uji *paired T-test.*

Hubungan SNP dengan kadar TNF-α pada kultur PBMC dilakukan uji independent dua sampel bebas, sedangkan hubungan kombinasi SNP dengan kadar TNF-α pada kultur PBMC dilakukan uji *one way anova* dengan metode Brown-Forsythe.

Hasil dari penelitian ini didapatkan tiga SNP yaitu pada posisi -294 A→G, -296 A→T dan -301 A→G. Ketiga SNP ini membentuk kombinasi 1)-294 A→G, -296 A→A, 2)-301 A→G; -294 A→G, -296 A→T, -301 A→G dan 3)-294 A→A, -296 A→A, -301 A→A. Kombinasi SNP -294 A→G, -296 A→A, -301 A→G dan -294 A→G, -296 A→T, -301 A→G signifikan meningkatkan kadar IL-10 (p=0,000). Kombinasi SNP -294 A→G, -296 A→A, -301 A→G lebih tinggi meningkatkan kadar IL-10 dibandingkan kombinasi -294 A→G, -296 A→T, -301 A→G.

Kombinasi SNP -294 A→G, -296 A→A, -301 A→G didapatkan peningkatan kadar IL-10 (338,16 ± 8,58 pg/ml) 20x lipat dari normal sedangkan pada kelompok penyakit yang ringan yaitu kombinasi SNP -294 A→G, -296 A→T, -301 A→G peningkatan kadar IL-10 mencapai (83,22 ± 5,41 pg/ml) meningkat 8x lipat. Kombinasi SNP -294 A→A, -296 A→A, -301 A→A merupakan kombinasi yang sesuai dengan sekuen promoter gen IL-10 (14,62 ± 3,42 pg/ml).

Hubungan antara SNP pada posisi -294 A→G, -296 A→T dan -301 A→G dengan aktivitas penyakit berdasarkan skor SLAM hasilnya signifikan (p=0,000). Hubungan antara kombinasi SNP signifikan (p=0,000) dengan skor SLAM. Pada kombinasi SNP -294 A→G, -296 A→A, -301 A→G menunjukkan aktivitas penyakit yang berat menurut skor SLAM (rerata 29,81±7,31), sedangkan pada kombinasi SNP -294 A→G, -296 A→T, -301 A→G menunjukkan aktivitas penyakit yang ringan (rerata 15,70 ± 2,95).

Dalam menganalisis pengaruh IL-10, antibodi ANA dan dsDNA dan penurunan komplemen, dilakukan analisis jalur. Hasil uji yang diperoleh kadar IL-10 signifikan mempengaruhi aktivitas penyakit berdasarkan skor SLAM dan penurunan komplemen, sedangkan dengan antibodi ANA dan dsDNA tidak signifikan dengan peningkatan kadar IL-10.

Mencari hubungan kadar TNF-α dengan berbagai paparan (IL-10, IFN,ConA, IL-10 dan IFN) pada kultur PBMC. Pada penelitian ini kadar TNF-α sebelum menunjukkan kadar yang tinggi dari harga normal. Setelah dilakukan paparan IFN, IL-10, ConA dan kombinasi IL-10 dan IFN didapatkan hasil kadar TNF-α signifikan dengan semua paparan (p=0,000) kecuali dengan paparan IL-10 (p=0,351). Pada penelitian ini disimpulkan bahwa IL-10 karena pengaruh IFN berubah fungsi menjadi pro-inflamasi.

Menganalisis kombinasi SNP yaitu -294 A→G, -296 A→A, -301 A→G dan -294 A→G, -296 A→T, -301 A→G dengan peningkatan kadar TNF-α, yang menunjukkan angka signifikan meningkatkan kadar TNF-α, adalah -294 A→G, 296 A→A, 301 A→G dan -294 A→G, -296 A→T, -301 A→G, tetapi -294 A→G, -296 A→A, -301 A→G lebih tinggi dibanding -294 A→G, -296 A→T, -301 A→G dalam meningkatkan kadar TNF-α.

Pada penelitian ini dapat disimpulkan bahwa SNP dapat sebagai prediktor aktivitas penyakit SLE, khususnya pada suku Jawa di Rumah Sakit Dr. Soetomo.

SUMMARY

Systemic Lupus Erythematosus is a complex diseases, signifying that genetic and environmental factors are involved in diseases development. Although the role of genetic factor in many populations have been studied, the genetic factor associated with diseases activity is yet to be identified. Several study reported the association between serum level of IL-10 with SLE disease activity. This study was performed to determined polymorphisms in the IL-10 gene promoter with diseases activity SLE in Javanese population.

Single Nucleotide Polymorphisms (SNP) assembled to evaluation disease linkage gene disorder, because SNP are less variable and widely used to characterize human molecular variation and evolution. SNPs are more common in population and less mutationally complex than microsatellite polymorphism. Previous finding is Single Nucleotide Polymorphisms (SNP) in the Interleukin-10 (IL-10) gene promoter genetically determined inter individual differences in production. As known, the promoter is regulator gene transcriptions.

Aberrant biological activities by numerous cytokines have been described in patients with SLE. Patients with active SLE have been reported to have significantly higher levels of serum pro-inflammatory cytokines, TNF. Correlations between in vitro IL-10 production and the promoter genotypes have been reported, it particularly happen to the active disease of SLE patients. Elevated levels of IL-10 have been demonstrated in SLE patients with active disease, and IL-10 levels have been shown to correlate with parameters of disease activity. Several lines of evidence indicate that interleukin-10 gene is a strong candidate gene in disease activity of SLE. IL-10 is considered a regulatory/tolerogenic cytokine, because it inhibits monocyte and dendritic cell pro-inflammatory function and promotes the differentiation of T cells. In B cells it induces activation, differentiation and antibody production.

IL-10 exerts its biologic effects on cells by interacting with a specific cell surface receptor. Engagement of the IL-10 receptor has been shown to activate the JAK-STAT signaling pathway. Specifically, IL-10 effects the activation of JAK1 and Tyk2 and induces the activation of Stat1, Stat3, and in some cells Stat5. Stat1 is a transcription factor of cytokines gene pro-inflammatory. IFN brief confers a pro-inflammatory gain of function on IL-10 through cross talk signaling JAK-STAT pathway which more activate Stat1 than Stat3.

This study was performed in two procedures. The first procedure analyzed the genotype IL-10 gene proximal promoter in SLE patients using a pair of specific primer of the IL-10 gene proximal promoter and sequenced by Applied Biosystem method. The second procedure analyzed the influence of IL-10 in converting from anti-inflammatory into pro-inflammatory function using PBMC. This study enrolled 47 SLE patients (ACR criteria) in active diseases, dr Soetomo hospital Surabaya. Disease activity was evaluated using SLAM. The level of IL–10 was measured by Eliza kit.

This study showed that there is no significant association between level of IL-10 with ANA and dsDNA, but the level of IL-10 was significant association with low level complement. There were three locus SNP at -294 A→G, -296 A→T and -301 A→G of IL-10 gene proximal promoter, with three variant SNPs combination: 1)-294 A→G, -296 A→A, -301 A→G; 2)-294 A→G, -296 A→T, -301 A→G and 3)-294 A→A, -296

A→A, -301 A→A. The serum level of IL-10 among three group were significantly difference. The patients with variant SNPs combination -294 A→G, -296 A→A, -301 A→G had more significantly higher score of disease activity (severe diseases) compare with the other groups of patient. The SNPs combination -294 A→A, -296 A→A, -301 A→A is similiar to the sequence IL-10 gene proximal promoter from GenBank NCBI. The result of the study showed that interferon significantly induced pro-inflammatory function of IL-10.

According to the previous report, IFN alter the function IL-10 through cross talk signaling JAK-STAT pathway. On the PBMC study, the result is interferon significantly associated convert IL-10 as pro-inflammatory. This result is founded on PBMC which is induced by IFN (250pg/ml) and high dose IL-10 (50 ng/ml) and the correlation genotypes of SNP -294 A→G, -296 A→A, -301 A→G had most significantly resulting of TNF-α production. IL-10 in the vicious cycle is maintains B cell hyperactivity. This condition correlate associated with disease activity.

This study result is Interleukin-10 proximal promoter SNP implicates diseases activity of Systemic Lupus Erythematosus by increasing the level of IL-10. Interferon converting the function of increasing level IL-10 as pro-inflammatory.