

The Importance of HLA-DRB1 Alleles in Patients with Lupus Nephritis

Lupus Nefritli Hastalarda HLA-DRB1 Allellerinin Önemi

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Abstract

Introduction: Our aim was to re-evaluate the roles of HLA-DRB1 alleles on susceptibility, severity, activity and chronicity index of lupus erythematosus nephritis in Indonesia.

Materials and Methods: A total of 55 lupus nephritis patients and 52 healthy subjects as control were analyzed. HLA-DRB1 alleles examination was performed using polymerase chain reaction (PCR) followed by electrophoresis. Differences in HLA-DRB1 allele frequencies between lupus nephritis group and healthy group, between mild and severe lupus nephritis, and between high and low activity and chronicity index were analysed using Chi-square test, presented as odds ratio (OR). Results were considered significant at P value less than 0.05.

Results: HLA-DRB1*1501 was a susceptible allele for lupus nephritis group (OR=3.18; p=0.010). HLA-DRB1*1501 was also a susceptible allele for high activity index (OR=7.4; p=0.006). Although not statistically significant, HLA-DRB1*1501 was a susceptible allele for high chronicity index (OR=3.2; p=0.07). We found HLA-DRB1*0401 was a protective allele against severe class of lupus nephritis (OR=9.4; p=0.029).

Conclusion: HLA-DRB1*1501 was a susceptible allele for lupus erythematosus nephritis with high activity and chronicity index. HLA-DRB1*0401 was a protective allele against severe class of lupus nephritis.

Keywords: Lupus erythematosus, lupus nephritis, HLA-DRB1, allele, activity, chronicity

Öz

Giriş: Amacımız, Endonezyada yaşayan lupus eritematosus olup nefrit saptanan hastalardaki HLA-DRB1 allellerinin hastalığa olan eğilim, hastalığın ciddiyeti, etkinliği ve kronikliği üzerine etkisini incelemektir.

Gereçler ve Yöntemler: Toplam 55 lupus nefriti olan hasta ile 52 sağlıklı gönüllü HLA-DRB1 allellerinin araştırılması amacı ile irdelendi. Lupus nefriti olan hastalar ile sağlıklı kişilerin HLA-DRB1 allel frekansları, polimeraz zincir tepkimesi yöntemi ve ardından elektroforez kullanılarak saptandı. Lupus nefritli olgular ile sağlıklı bireyler arasındaki, ılımlı, ciddi nefritli hastalar ile düşük ve yüksek kroniklik düzeyi olan olgular arasındaki HLA-DRB1 allel frekansları arasındaki farklar Ki-kare testi kullanılarak incelendi. P değerinin 0.05'den düşük olması istatistiksel anlamlılık olarak kabul edildi.

Bulgular: HLA-DRB1*1501 alleli lupus nefriti olan hastalarda sorumlu allel olarak saptandı (Risk Oranı=3.18; p=0.01). Aynı allel, aynı zamanda yüksek aktivite birimi ile de ilişkili olarak saptandı (Risk Oranı=7.4; p=0.006). HLAB1*1501 alleli, her ne kadar istatistiksel olarak anlamlı düzeyde olmasa da yüksek kroniklik düzeyi ile ilişkili olarak saptandı (Risk Oranı=3.2; p=0.07). Ayrıca HLA-DRB1*1401 allelinin ise, ağır lupus nefritinden koruyucu olduğu saptandı (Risk Oranı=9.4; p=0.029).

Sonuç: HLA-DRB1*1501, lupus eritematosuslu hastalarda saptanan nefritin etkinliği ve kronikliği ile ilişkilidir. HLA-DRB1*0401 ise, ciddi lupus nefritine karşı koruyucudur.

Anahtar Kelimeler: Lupus eritematosus, lupus nefriti, HLA-DRB1, allel, aktivite, kronisite

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease. It may induce autoimmune nephritis (lupus nephritis).^[1] Several studies had shown that kidney involvement and severity of disease were genetically associated. HLA-DRB1 and HLA-DQ genes were known to predispose to SLE and lupus nephritis and were widely

investigated.^[1-3] Polymorphism of HLA-DRB1 were the most common. To date, there are 463 allelic variations of HLA-DRB1 gene. Research on the alleles of the HLA-DRB1 gene that affects the susceptibility and class of lupus nephritis resulted in different conclusions, depending on the population under study. In our study, we aimed to investigate the possible association of alleles of HLA-DRB1 gene with incidence, classes, activity and chronicity index of lupus nephritis.

Materials and Methods

Subjects

We analyzed the Javanese descendant patients who visited outpatient clinic and inpatient wards of Internal Medicine Department in Dr. Saiful Anwar General Hospital in Malang. Patients were diagnosed with nephritis caused by lupus erythematosus based on the American College of Rheumatology (ACR) 1997 criteria.^[4] This study was approved by the Ethics Committee of Medical of the University of Universitas Brawijaya and Dr. Saiful Anwar General Hospital. All participants signed informed consent.

HLA-DRB1 examination

HLA-DRB1 alleles examination was performed using polymerase chain reaction (PCR) techniques with sequence specific primers (PCR-SSP method). Specific primers were designed to selectively amplify target sequences which were specific to a single or group of alleles. The presence of amplified DNA fragments indicated existence of specific allele sequences within DNA. Morgan HLA SSP DRB Typing kitTM with catalog number 33280 was used for determining this.

Identification of HLA-DRB1 gene polymorphism was done using 3 ml blood samples with EDTA through the steps of DNA isolation, PCR and electrophoresis. DNA isolation was performed using Qiagen kit and DNA concentration was expected between 10–40 ng/ μ l. Thermal cycler in PCR ran for approximately 1 hour 25 minutes. PCR products (12 μ L) were continued to 2% agarose gel electrophoresis which separated DNA fragments by sizes. Amplified fragments were stained with ethidium bromide followed by ultraviolet light exposure. A worksheet was provided by manufacture to assist the determination of HLA types by analysing PCR results.

Statistic analysis

Normality and homogeneity was analysed with Kolmogorov-Smirnov and Levene test, respectively. Differences in HLA-DRB1 allele frequencies between lupus nephritis patients and healthy controls, between mild and severe classes and between high and low activity and chronicity index were analysed using Chi-square test and the relative risk was stated by odds ratio (OR). p value less than 0.05 was considered significant. The statistical analysis software used was SPSS 22(IBM Inc.; USA).

Results

In two years, 55 patients (94% of them were women) were diagnosed as lupus nephritis. All patients were on treatment. Based on ISN/RPS 2004 classification, there were 10 patients (18%) with class II lupus nephritis,^[5] 24 patients (44%) with class III, 8 patients (14%) with class IV and 13 patients (23%) with mixed class V mixed. Severe classes consisted of class III, IV and mixed V; so there were 45 patients (82%) with severe classes and the other patients were the patients with class I and II disease. Classification of activity and chronicity index were based on National Institutes of Health (NIH) criteria.^[6] Thirteen patients (24%) had high activity index. There were 29 patients (52.7%) patients with high chronicity index. Patients with severe classes experienced significantly more severe proteinuria than patients with Class I or II disease. (805.17 ± 706.54 vs 99.52 ± 92.6 ; $p=0.001$).

Table 1 shows that frequency of HLA-DRB1*1501 was significantly higher in lupus nephritis than that of control group (OR=3.18; $p=0.010$); whereas other HLA-DRB1 allele frequencies were not found to be different between two groups. Table 2 shows the differences in frequencies of HLA-DRB1 alleles among patients with mild and severe classes of lupus nephritis. HLA-DRB1*0401 were more common in mild classes (OR=9.4; $p=0.029$). Table 3 shows differences in frequencies of HLA-DRB1 alleles between the patients with low and high activity index lupus nephritis. HLA DRB1*1501 was more common in patients with high activity index group (OR=7.4; $p=0.006$). Table 4 shows that frequency of the HLA-DRB1*15 was higher in patients with high than that of in low chronicity index group, although the difference was not statistically significant (OR=3.2; $p=0.07$).

Table 1. Distribution of HLA-DRB1 alleles in patients with lupus nephritis and control group. LN: Lupus nephritis, OR: Odds ratio. CI: Confidence interval.

HLA-DRB1 alleles	LN (n=55)	Control (n=52)	p	OR	95% CI
DRB1*0401	3 (5.5%)	0 (0%)	0.090	5.526	0.27–112.93
DRB1*0701	6 (10.9%)	2 (3.8%)	0.180	2.778	0.54–14.4
DRB1*0801	0 (0%)	1 (1.9%)	0.242	2.373	0.01–12.82
DRB1*0820	0 (0%)	1 (1.9%)	0.242	2.373	0.01–12.82
DRB1*0832	0 (0%)	2 (3.8%)	0.110	4.840	0.01–4.69
DRB1*0901	2 (3.6%)	0 (0%)	0.161	3.621	0.16–82.11
DRB1*1101	0 (0%)	2 (3.8%)	0.110	4.840	0.01–4.69
DRB1*1105	0 (0%)	1 (1.9%)	0.242	2.373	0.01–12.82
DRB1*1201	21 (38.2%)	27 (51.9%)	0.063	2.006	0.23–1.07
DRB1*1401	1 (1.8%)	0 (0%)	0.298	1.780	0.06–54.14
DRB1*1404	0 (0%)	1 (1.9%)	0.242	2.373	0.01–12.82
DRB1*1423	0 (0%)	1 (1.9%)	0.242	2.373	0.01–12.82
DRB1*1446	2 (3.6%)	0 (0%)	0.161	3.621	0.16–82.11
DRB1*1501	22 (40%)	8 (15.4%)	0.010	3.184	1.27–7.98
DRB1*1517	1 (1.8%)	2 (3.8%)	0.397	2.360	0.04–4.81
DRB1*1601	2 (3.6%)	4 (7.7%)	0.263	2.417	0.07–2.36

Table 2. Distribution of HLA-DRB1 alleles in patients with mild and severe classes of lupus nephritis. OR: Odds ratio. CI: Confidence interval.

HLA-DRB1 alleles	Class n (%)		p	OR	95% CI
	Mild (n=10)	Severe (n=45)			
DRB1*0401	2 (20%)	1 (2.2%)	0.029	9.400	1.29–114.01
DRB1*0701	1 (10%)	5 (11.1%)	0.669	1.279	0.08–7.39
DRB1*0901	1 (10%)	1 (2.2%)	0.239	4.273	0.25–73.75
DRB1*1201	3 (30%)	18 (40%)	0.359	1.800	0.13–2.32
DRB1*1401	0 (0%)	1 (2.2%)	0.524	1.880	0.06–59.31
DRB1*1446	0 (0%)	2 (4.4%)	0.406	1.087	0.04–21.72
DRB1*1501	5 (50%)	17 (37.8%)	0.580	1.303	0.36–4.74
DRB1*1517	0 (0%)	1 (2.2%)	0.524	1.880	0.06–59.31
DRB1*1601	0 (0%)	2 (4.4%)	0.406	1.087	0.04–21.72

Table 3. Distribution of HLA-DRB1 alleles in patients with low and high activity index lupus nephritis. OR: Odds ratio. CI: Confidence interval.

HLA-DRB1 alleles	Activity index n (%)		p	OR	95% CI
	Low (n=34)	High (n=11)			
DRB1*0401	1 (2.9%)	0 (0%)	0.565	1.435	0.02–22.19
DRB1*0701	4 (11.8%)	1 (9.1%)	0.806	1.333	0.13–13.37
DRB1*0901	0 (0%)	1 (9.1%)	0.075	6.900	0–4.64
DRB1*1201	16 (47.1%)	2 (18.2%)	0.089	4.000	0.75–21.33
DRB1*1401	0 (0%)	1 (9.1%)	0.075	6.900	0–4.64
DRB1*1446	1 (2.9%)	1 (9.1%)	0.390	3.300	0.02–5.29
DRB1*1501	9 (26.5%)	8 (72.7%)	0.006	7.407	1.6–34.21
DRB1*1517	1 (2.9%)	0 (0%)	0.565	1.435	0.02–22.19
DRB1*1601	2 (5.9%)	0 (0%)	0.411	1.438	0.06–34.27

Table 4. Distribution of HLA-DRB1 alleles in patients with low and high chronicity index lupus nephritis. OR: Odds ratio. CI: Confidence interval.

HLA-DRB1 alleles	Chronicity index n (%)		p	OR	95% CI
	Low (n=21)	High (n=24)			
DRB1*0401	1 (4.8%)	0 (0%)	0.280	2.450	1.08–76.85
DRB1*0701	1 (4.8%)	4 (16.7%)	0.205	4.000	1.03–5.44
DRB1*0901	0 (0%)	1 (4.2%)	0.344	1.870	1.02–16.77
DRB1*1201	8 (38.1%)	10 (41.7%)	0.807	1.161	0.26–2.85
DRB1*1401	0 (0%)	1 (4.2%)	0.344	1.870	1.02–16.77
DRB1*1446	1 (4.8%)	1 (4.2%)	0.923	1.150	0.07–19.6
DRB1*1501	5 (23.8%)	12 (50%)	0.071	3.200	1.09–8.13
DRB1*1517	0 (0%)	1 (4.2%)	0.344	1.870	1.02–16.77
DRB1*1601	0 (0%)	2 (8.3%)	0.176	3.909	1.01–6

Discussion

HLA-DRB1 gene which is a risk factor for lupus nephritis has different alleles.^[1] Bastian's study that was conducted in multi-ethnic population in The United States of America showed that HLA-DRB1*1503 was a risk factor for lupus nephritis.^[2] In contrast, HLA-DR3 and HLA-DQB1*0201 were found to be protective against lupus nephritis.^[2] A study that was performed in 155 children and adolescent SLE in Brazil also reported that HLA-DRB1*15 was a risk allele for lupus nephritis.^[7] It was also documented that HLA-DRB1*15 was associated with elevated levels of anti-dsDNA, anti-Sm, anti-U1-RNP and anti-SSA/Ro antibodies.^[7] In Italy, it was found that HLA-DQA1*0101 and HLA-DRB1*1501 were risk alleles for lupus nephritis.^[1] The article published by Walid (2014) in Saudi Arabia also indicated that HLA-DRB1*15 was associated with lupus nephritis.^[8]

A Taiwanese study stated that no alleles of HLA-DRB1 gene were risk factors for lupus nephritis.^[9] On the other hand a study from Malaysia emphasized that alleles as risk factor for lupus nephritis were HLA-DR, HLA-DQB1*0501.^[7] Whereas, in a Korean study, risk allele for lupus nephritis was reported to be HLA-DRB107.^[8] Another study from Hungary stated that HLA-DR3 and HLA-DR7 were risk alleles for lupus nephritis.^[9]

Our study showed that HLA-DRB1*1501 was a risk allele for lupus nephritis (OR=3.18; p=0.010). But unlike other studies, there was no protective allele described against lupus nephritis in our study. HLA-DRB1*1202 was reported to be protective against lupus nephritis in Taiwanese population.^[9] In a study performed in The

United States of America indicated that, protective alleles against lupus nephritis were HLA-DR3 and HLA-DQB1*0201.

Our results showed that HLA-DRB1*1501 allele was not only a risk allele for lupus nephritis, but also a risk factor for development of lupus nephritis with high activity and high chronicity index. These results were similar to those published. An Italian study stated that 80% of patients with HLA-DRB1*15 fell into severe classes of diffuse proliferative (class IV), the remaining 20% into focal proliferative (class III).^[1] On the other hand in Brazilian patients, HLA-DRB1*15 and HLA-DRB1*07 were significantly associated with class IIB and V disease.^[4] HLA-DR3 was found to be a risk factor for development of class III, IV and V disease in Egyptian population. Pan et al. did not find a specific allele of HLA-DRB1 that affected the severity of lupus nephritis.^[9]

Yung et al. (2006) showed that cultured human mesangial and tubular epithelium cells, induced with anti-DNA antibodies, produced pro-inflammatory cytokines, IL-1 β , IL-6, TNF- α , TGF- β 1 and matrix protein accumulation, fibronectin, in mesangial cells.^[10]

Another possible risk factor associated with HLA-DRB1*1501 is related to the amino acid sequence.^[11] HLA-DRB1 has a specific amino acid sequence which is able to present the antigen.^[11] Kim et al. (2014) conducted the first mapping study in Korea to find relationship between SLE with HLA-DRB1 at the amino acid level.^[11] Arg13 and Tyr13 were found to be associated with SLE, while His13 and Ser13 were found to be negatively correlated with the disease. There were two HLA-DRB1 alleles that were risk

for SLE: HLA-DRB1*1501 (containing Arg13) and HLA-DRB1*0301 (containing Tyr26).^[11] These amino acids were risk factors because they were hydrophobic and were located in the epitope-binding groove area.^[10] Hydrophobic residues made the protein easy to fold, formed a unique three-dimensional structure and they are especially necessary for biological activity, the ability to present antigens.^[11]

We were also able to find that HLA-DRB1*0401 was protective against severe nephritis. Another study by Jacob et al. also indicated that DR4 (HLA-DRB1*0401) was protective against lupus nephritis. Protective properties of HLA-DRB1*0401 might be attributable to TNF- α secreting effect. TNF- α inhibits viruses and parasites and has anti-tumour activity. Presumably, high levels of TNF- α was associated with protective effect on lupus nephritis.^[12]

We conclude that HLA-DRB1*1501 was associated with higher probability of lupus nephritis with high activity and high chronicity index. HLA-DRB1*0401 seems to be protective against development of severe lupus nephritis.

Ethics Committee Approval: This study was approved by the Ethics Committee of Medical Faculty Universitas Brawijaya and Dr. Saiful Anwar General Hospital Malang.

Informed Consent: All participants signed informed consent.

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Conflict of Interest: No conflict of interest was declared by the authors.

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