**TNFSF13B rs9514828 C>T Polymorphism is Associated with Incidence of Atherosclerosis and Therapeutic Outcomes in Patients with Systemic Lupus Erythematosus**

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**Background:** Systemic lupus erythematosus (SLE) is a complex autoimmune disease with numerous clinical manifestations. Organ involvement can aggravate patients with SLE and cause comorbidities such as atherosclerosis. Recently, the TNFSF13B gene has been found to be linked with SLE events. This study aimed to analyze the association between single nucleotide polymorphisms of the TNFSF13B rs9514828 with incidence of atherosclerosis and therapeutic outcomes in patients with SLE.

**Patients and Methods:** This case-control study included 84 SLE patients, of whom 21 patients with SLE with atherosclerosis and 63 patients with SLE without atherosclerosis. Using enzyme-linked immunosorbent assay method, interleukin-6 and interferon gamma levels were quantified. The TNFSF13B gene polymorphism was evaluated using polymerase chain reaction followed by sequencing. The lupus low disease activity state (LLDAS) criteria were used to measure the therapeutic outcomes.

**Results:** The genetic variations of TNFSF13B rs9514828 were CC = 35, CT = 41, and TT = 8. There was an association between TNFSF13B rs9514828 C>T polymorphism in patients with SLE with and without atherosclerosis (p = 0.03; odds ratio (OR) 4.72, 95% confidence interval [CI] 1.22–18.37). Furthermore, the TNFSF13B rs9514828 C>T polymorphism had association with the therapeutic outcomes of patients with SLE who manifested with LLDAS (p = 0.00; OR 7.58, 95% CI 2.61–21.99).

**Conclusion:** The association of TNFSF13B rs9514828 C>T polymorphism and incidence of atherosclerosis as well as the therapeutic outcomes in patients with SLE indicate the potential utility of the gene variation as screening tool to employ personalized medicine to undertake preventive measures in order to prevent atherosclerosis and to predict a poor prognosis in SLE patient.

**Keywords:** systemic lupus erythematosus, atherosclerosis, genetic polymorphism, TNFSF13B, LLDAS

**Introduction**

Systemic lupus erythematosus (SLE) is an autoimmune disease that induces widespread tissue inflammation with complex clinical manifestations. This disease is caused by the interaction of three factors, including the environment, hormones, and genetics, that is characterized by organ and tissue injury.1–4 SLE prevalence varies depending on ethnic/racial groups and regions. In the United States, the prevalence of SLE ranges from 15 to 50 per 100,000 population, while in African–American women, the incidence of SLE is threefold higher than Whites. SLE affects more women than men with a ratio of 9:1 and is also common during the reproductive age group (15–40 years).5–7 In Hasan Sadikin Hospital in Bandung, Indonesia, 813 patients were diagnosed with SLE, revealed ratio of 22:1 for female: male.8
SLE pathogenesis involves innate and adaptive immune systems, including cell and tissue components. Autoantibodies are generated from B-cell hyperactivity through T-cell stimulation and antigen exposure to the apoptotic cell surface. Apoptosis (cell death) occurs in dying cells and dead cellular material that should be phagocytosed by phagocytic cells are recognized by cell surface antigens. In patients with SLE, the suboptimal disposal of apoptotic cells may trigger an immune response. Major histocompatibility complex in antigen-presenting cells (APC) binds to T-cell receptors. These receptors trigger B-cell hyperactivity and produce cytokines and antibodies, such as antinuclear antibodies, that are identified in patients with SLE. B lymphocyte hyperactivity, such as proliferation and maturation, is influenced by cytokine B-cell-activating factor of the TNF-family (BAFF) or commonly known as the B lymphocyte stimulator (Blys), TALL-1, or BAFF. This protein is encoded by the TNF superfamily member 13B gene (TNFSF13B) located in chromosome 13q.32–34. TNFSF13B rs9514828 polymorphisms (~871 C>T) are located at the promoter region. In patients with SLE, the TNFSF13B gene expression remains high. Thus, BAFF protein expression is increased. BAFF is expressed on the myeloid and epithelial cells stimulated by inflammatory cytokines such as interferon (IFN)-γ, IFN-α, interleukin (IL)-10, Toll-like receptor (TLR)-3, TLR-4, and TLR-7. BAFF is also known as a regulator of T-cell immunity on APCs including the production of inflammatory cytokines. In SLE, BAFF overproduction is associated with disease activity and increased autoantibody production.

Immune system dysregulation can trigger inflammation and injury to the vascular system, which are markers of atherosclerosis. In patients with SLE, risk factors that could trigger atherosclerosis in immunological mechanisms and cardiovascular risk factors, immune complexes, and inflammatory cytokine production such as type I and type 2 IFN, IL-6, and IL-17. SLE disease activity and glucocorticoid therapy use are specific factors. Traditional factors that contribute to the acceleration of atherosclerosis in SLE include hypertension, hypercholesterolemia, diabetes, and smoking history. Previous studies have revealed that an increased intima-media thickness (IMT) is a marker of atherosclerosis in SLE.

In autoimmune diseases such as SLE, the existence of genetic polymorphisms may influence the efficacy of therapy in terms of the choice of treatment and dosage management. In recent years, the study of polymorphisms has become a concern among researchers due to the development of personalized medicine. Each individual has a genetic profile and response to drug therapy that is different, which in turn remarkably affects the appropriate use of drugs. The main therapeutic options in the treatment of patients with SLE include immunosuppressants and glucocorticoids. Moreover, patients with SLE treated with high-dose prednisolone (>6 mg) accompanied by a long-term use have a significant risk of morbidity due to permanent tissue damage. The risk of cardiovascular events due to glucocorticoid use has also been reported.

This study aimed to analyze the association between single nucleotide polymorphisms of the TNFSF13B rs9514828 gene and therapeutic outcomes and atherosclerosis incidence in patients with SLE.

Materials and Methods

Study Populations

In this study, we were using biological archive (DNA) from patients diagnosed with SLE at the Rheumatology outpatient clinic at Dr. Hasan Sadikin General Hospital, Bandung, West Java, Indonesia. The Research Ethics Committee of Universitas Padjadjaran, Bandung, has approved all the procedures in this study. This study covers patient data confidentiality and compliance with the Declaration of Helsinki. This was a case-control study and all patients were receiving standard SLE therapy according to Recommendation form Indonesian Rheumatology Association and also Systemic Lupus International Collaborating Clinics (SLICC). A total of 84 patients with were enrolled with inclusion criteria namely diagnosis was established according to the criteria from Systemic Lupus International Collaborating Clinics (SLICC) in 2012, complete lupus low disease activity state (LLDAS) and medical records data including age, gender, IMT, duration of disease, glucocorticoid therapy, blood pressure, glucose, cholesterol and Body Mass Index (BMI). The total samples were divided into two groups: patients with SLE with atherosclerosis (21 patients) and without atherosclerosis (63 patients) measured by carotids Doppler ultrasound using GE Vivid S6 (Boston, USA). The SLE with atherosclerosis group are patients who manifested with an IMT ≥ 0.9 mm. The demographic characteristics and clinical data were obtained from the medical records. Patients who were receiving treatment for certain diseases were excluded in this study.
Serum IL-6 and IFN-γ Levels

The IL-6 and IFN-γ levels were quantified from the serum of patients with SLE using human IL-6 enzyme-linked immunosorbent assay (ELISA) KIT and human IFN-γ ELISA KIT (Sigma-Aldrich™) with product numbers RAB0306 and RAB0223. The samples were read at 450 nm via the Multiskan™ Go Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Genotyping

Genomic DNA was isolated from the whole blood using GeneJET Genomic DNA Purification Kit (Thermo Scientific) and isolated according to manufacturer’s instruction. TNFSF13B rs9514828C>T (bp 138) amplification was conducted using polymerase chain reaction (PCR) (BIO-RAD T100™ Thermal Cycler). The specific primer sequences were forward: 5’ TTGTACACCAGACCTGTTAGG 3’; reverse 5’ TGGAGTAAATCCACTGGGAAT 3’. DNA fragments were amplified under PCR conditions with an initial denaturation cycle at 94 °C for 3 min; 35 denaturation cycles for 30s at 94 °C; annealing for 20s at 60 °C; extension for 30s at 72 °C; and final extension for 1 min at 72 °C.

Evaluation of Therapeutic Outcomes

Outcome or efficacy therapy of SLE was measured using the LLDAS tools which were assessed by clinician to monitor the progress of the patient’s condition according to the therapy administered. LLDAS was developed and validated by the Asia-Pacific Lupus Collaboration and has been widely used in daily practice as the basis for the treat-to-target. The LLDAS definition suggests a better predictor of patient outcomes including assessment of disease activity and treatment safety. LLDAS is attained if all of the following is exhibited by the patient: 1) SLE disease activity index 2000 (SLEDAI-2K) score of ≤4, with no activity in organ systems such as the renal, central nervous, and cardiopulmonary system and absence of vasculitis, fever, hemolytic anemia, or gastrointestinal derangements; 2) there are no new features of lupus disease activity compared to previous assessments; 3) physician assessment of global activity score (0–3) ≤1; 4) current prednisolone equivalent dose ≤ 7.5 mg/day; and 5) standard maintenance doses of approved immunosuppressive drugs and biologics are permitted.

Statistical Analysis

Descriptive and statistical analyses of data were performed with GraphPad Prism version 9 (GraphPad Software, San Diego, CA). The descriptive data indicate the percentage or as means ± standard deviation of cases. For comparisons of two groups one-tailed Chi-square tests were carried out. The Mann–Whitney U-tests was used to analyze the difference between the IL-6 and IFN-γ value in patients with SLE with and without atherosclerosis. The binary logistic regression test was used to analyze the relationship between the TNFSF13B rs9514828C>T genotypes and the incidence of atherosclerosis and therapeutic outcomes. The level of statistical significance was set ap < 0.05. The deviation of allele frequencies was analyzed using the Hardy–Weinberg equilibrium (HWE).

Results

Patient Characteristics

A total of 84 participants diagnosed with SLE in the study were female (100%). In terms of age, most of the participants (60 patients) were in the 15–40-year age group (71.4%), while 24 participants (28.6%) were in the 40–65 years age group. SLE affects more women of childbearing age (15–44 years), and the incidence of SLE was greater in women than men. According to chronicity, most patients belonged in the 6–10-year category (47.6%), followed by 0–5 years (23.8%), 11–15 years (17.9%), 16–20 years (7.1%), and 21–25 years (3.6%). The presence of age group and disease duration between SLE patients with atherosclerosis and without atherosclerosis was not associated (p = 0.37 and p = 0.98), see Table 1. Atherosclerosis status is indicated by the presence of intima thickening (>0.9 mm). In this study, the response variable was included together with the therapeutic outcomes as observed in the LLDAS assessment results. Moreover, all patients were undergoing glucocorticoid therapy (methyl-prednisolone as a standard therapy for SLE. There were 21 participants (25%) who had an IMT ≥ 0.9 mm, while
63 participants (75%) had an IMT < 0.9 mm. The participants who demonstrated improvement in LLDAS were 46 (54.8%), while those who did not were 38 (45.2%). The presence of LLDAS between SLE patient with atherosclerosis and without atherosclerosis was not associated (p = 0.80), see Table 1.

Table 1 Baseline Characteristics of the Patients with Systemic Lupus Erythematosus

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
<th>Patient With SLE With Atherosclerosis n (%)</th>
<th>Patient With SLE Without Atherosclerosis n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>84 (100)</td>
<td>21 (25)</td>
<td>63 (75)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>84 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–40 years</td>
<td>60 (71.4)</td>
<td>31 (36.9)</td>
<td>29 (34.5)</td>
<td>0.37</td>
</tr>
<tr>
<td>40–65 years</td>
<td>24 (28.6)</td>
<td>15 (17.9)</td>
<td>9 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5 years</td>
<td>20 (23.8)</td>
<td>11 (13.1)</td>
<td>9 (10.7)</td>
<td>0.98</td>
</tr>
<tr>
<td>6–10 years</td>
<td>40 (47.6)</td>
<td>21 (25)</td>
<td>19 (22.6)</td>
<td></td>
</tr>
<tr>
<td>11–15 years</td>
<td>15 (17.9)</td>
<td>9 (10.7)</td>
<td>6 (7.1)</td>
<td></td>
</tr>
<tr>
<td>16–20 years</td>
<td>6 (7.1)</td>
<td>3 (3.6)</td>
<td>3 (3.6)</td>
<td></td>
</tr>
<tr>
<td>21–25 years</td>
<td>3 (3.6)</td>
<td>2 (2.4)</td>
<td>1 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Glucocorticoid therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>84 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLDAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46 (54.8)</td>
<td>12 (14.3)</td>
<td>34 (40.5)</td>
<td>0.80</td>
</tr>
<tr>
<td>No</td>
<td>38 (45.2)</td>
<td>9 (10.7)</td>
<td>29 (34.5)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (32.5)</td>
<td>10 (13)</td>
<td>15 (19.5)</td>
<td>0.05*</td>
</tr>
<tr>
<td>No</td>
<td>52 (67.5)</td>
<td>10 (13)</td>
<td>42 (54.6)</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (23.5)</td>
<td>12 (14.8)</td>
<td>7 (8.6)</td>
<td>0.00*</td>
</tr>
<tr>
<td>No</td>
<td>62 (76.5)</td>
<td>9 (11.1)</td>
<td>53 (65.4)</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (36.3)</td>
<td>9 (11.3)</td>
<td>20 (25)</td>
<td>0.46</td>
</tr>
<tr>
<td>No</td>
<td>51 (63.8)</td>
<td>12 (15)</td>
<td>39 (48.8)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23 (29.5)</td>
<td>6 (7.7)</td>
<td>17 (21.8)</td>
<td>0.81</td>
</tr>
<tr>
<td>No</td>
<td>55 (70.51)</td>
<td>13 (16.7)</td>
<td>42 (53.9)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: *Significance value P < 0.05 using Chi-square test.
Abbreviation: LLDAS, lupus low disease activity state.
In addition to specific factors for atherosclerosis progression in patients with SLE, traditional risk factors also influence its occurrence such as hypertension, hypercholesterolemia, diabetes mellitus, smoking history, and obesity. Figure 1 shows data that several participants suffered from SLE accompanied by comorbidities such as hypertension, hyperglycemia, hypercholesterolemia, and obesity. Twenty-five (32.5%) of 77 of total patients had a history of hypertension. Nineteen (23.5%) of 81 of total patients had a history of hyperglycemia. Among 80 of total patients, hypercholesterolemia was documented in 29 patients (36.3%), while in the 78 of total patients, 23 had a history of obesity (29.5%). The presence of hypertension and hyperglycemia between SLE patient with atherosclerosis and without atherosclerosis was associated (p = 0.05 and p = 0.00). The presence of hypercholesterolemia and obesity between SLE patient with atherosclerosis and without atherosclerosis was not associated (p = 0.46 and p = 0.81), see Table 1. Several study patients did not have complete clinical data, which was a limitation in this study.

The ELISA results for the IFN-γ and IL-6 levels are presented in Table 2. The mean of the IFN-γ level in patients with SLE with atherosclerosis was 647.9 ± 440.4 pg/mL and patients with SLE without atherosclerosis was 582.2 ± 308.7 pg/mL. The results revealed absence of a significant relationship (p = 0.73). The IL-6 level in patients with SLE with atherosclerosis was 148 ± 109.3 pg/mL, and patients with SLE without atherosclerosis was 197.19 ± 145.5 pg/mL (p = 0.12) using the Mann–Whitney U-test (Figure 2).

Frequency Distribution of TNFSF13B rs9514828 C>T Polymorphisms on Incidence of Atherosclerosis and Therapeutic Outcomes in Patients with SLE

The genotype distribution and deviation of TNFSF13B rs9514828 in patients with SLE is analyzed using the HWE with p = 0.29 (Table 3). The distribution of TNFSF13B rs9514828 C>T polymorphisms on incidence of atherosclerosis and therapeutic outcomes in patients with SLE is presented in Table 4. Patients with T alleles (CT and TT) showed a higher incidence of atherosclerosis (85.7%) than those without (49.2%). This finding was verified by statistical analysis showing that the TNFSF13B rs9514828 has a significant correlation with the incidence of comorbid atherosclerosis in patients with SLE (p = 0.01) (Table 4).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patient with SLE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Atherosclerosis (pg/mL)</td>
<td>Without Atherosclerosis (pg/mL)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>647.9 ± 440.4</td>
<td>582.2 ± 308.7</td>
</tr>
<tr>
<td>IL-6</td>
<td>148 ± 109.3</td>
<td>197.19 ± 145.5</td>
</tr>
</tbody>
</table>

**Notes:** Result in mean ± S.D.

**Abbreviations:** IFN-γ, interferon gamma; IL-6, interleukin-6.
The incidence of the \textit{TNFSF13B} rs9514828 has a significant correlation with the therapeutic outcomes according to the LLDAS criteria in patients with SLE (p = 0.00) (Table 4). The CT and TT genotype showed a greater incidence of LLDAS (80.4%) than in the not-LLDAS (31.6%) group. Moreover, the wildtype (CC) variant was higher (68.4%) in the not-LLDAS group.

The Association Between TNFSF13B rs9514828 C>T Polymorphisms with Incidence of Atherosclerosis and Therapeutic Outcomes in Patients with SLE

This study revealed that the \textit{TNFSF13B} rs9514828 gene polymorphism was associated with the occurrence of SLE with atherosclerosis using a case-control analysis (Table 5). The \textit{TNFSF13B} rs9514828 C>T has a significant effect on

**Table 3** The Genotype Distribution of TNFSF13B rs9514828 in SLE Patient Was Analyzed Using the Hardy-Weinberg Equilibrium Test

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype (n)</th>
<th>Allele C</th>
<th>Allele T</th>
<th>Observed Frequency n (%)</th>
<th>Expected Frequency n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFSF13B</td>
<td>CC (35)</td>
<td>70</td>
<td>0</td>
<td>41 (48.8)</td>
<td>42.3 (50.4)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>CT (41)</td>
<td>41</td>
<td>41</td>
<td>37 (44.1)</td>
<td>35.3 (42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT (8)</td>
<td>0</td>
<td>8</td>
<td>6 (7.1)</td>
<td>7.1 (8.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (84)</td>
<td>111</td>
<td>49</td>
<td>84 (100)</td>
<td>84 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Analyzed using the Hardy-Weinberg equilibrium test.

**Table 4** Comparison of TNFSF13B rs9514828 with Incidence of Atherosclerosis and Therapeutic Outcomes in Patients with SLE

<table>
<thead>
<tr>
<th>Genotype, n (%)</th>
<th>Patients with SLE</th>
<th>P-value</th>
<th>Therapeutic Outcome</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Atherosclerosis n (%)</td>
<td>Without Atherosclerosis n (%)</td>
<td></td>
<td>LLDAS n (%)</td>
</tr>
<tr>
<td>CC, 35 (41.7)</td>
<td>3 (14.3)</td>
<td>32 (50.8)</td>
<td>0.01*</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>CT, 41 (48.8)</td>
<td>14 (66.7)</td>
<td>27 (42.9)</td>
<td></td>
<td>31 (67.4)</td>
</tr>
<tr>
<td>TT, 8 (9.5)</td>
<td>4 (19)</td>
<td>4 (6.3)</td>
<td></td>
<td>6 (13)</td>
</tr>
<tr>
<td>84 (100)</td>
<td>21 (100)</td>
<td>63 (100)</td>
<td></td>
<td>46 (100)</td>
</tr>
</tbody>
</table>

Notes: *Significance value P<0.05 using Chi-square test. LLDAS definition suggests a better predictor of patient outcomes including assessment of disease activity and treatment safety.
atherosclerotic comorbidities in patients with SLE (p = 0.03). The TNFSF13B rs9514828 gene polymorphism in patients with SLE was indicated by the presence of heterozygous CT and homozygote TT variants compared to wild-type CC as a reference variable. Using the OR, the tendency of the TNFSF13B rs9514828 gene polymorphism to influence the incidence of atherosclerosis in patients with SLE was determined. The results showed that the risk of atherosclerotic events in patients with SLE with the TNFSF13B rs9514828 gene polymorphism (CT and TT genotype) was 4.72-fold higher than the wild-type group (CC genotype) (Table 5).

Furthermore, we examined the association between the TNFSF13B rs9514828 C>T has a significant effect on therapeutic outcomes (LLDAS) in patients with SLE (p = 0.00). Disease improvement, demonstrated by the LLDAS score, was smaller in patients with the TNFSF13B rs9514828 gene polymorphism (CT and TT genotypes). Additionally, patients with SLE with polymorphisms have a 7.58-fold risk no improvement after therapy than those without the TNFSF13B rs9514828 gene polymorphism (reference group) (CC genotype) (Table 5). The TNFSF13B rs9514828 gene polymorphism in patients with SLE was demonstrated by the presence of a heterozygous CT genotype variant and a homozygous TT genotype variant compared to the liar type CC as a reference variable.

Table 5 The Association of TNFSF13B rs9514828 with Incidence of Atherosclerosis and Therapeutic Outcomes in Patients with SLE

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SLE Patients with Atherosclerosis</th>
<th>Therapeutic Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odd Ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>CC</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>4.72 (1.22–18.37)</td>
<td>0.03*</td>
</tr>
<tr>
<td>TT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *Significance value P<0.05 using binary logistic regression test.

Discussion

This study comprised entirely of female patients. As previously established, females have a higher prevalence of SLE than males. In line with previous studies indicating a significantly higher incidence of SLE in females compared to males, with prevalence ratios ranging from 7 to 9 to 1 for SLE. The increased incidence of SLE in women is attributed to hormones that are important in disease manifestations. Also, the disparities in the clinical manifestations of SLE between males and females are not significantly fundamental. This study predominantly involved individuals within the reproductive or childbearing age (15–40 years old; 60% of the sample); linear studies elucidated that SLE occurred predominantly between the ages 25 and 39 years. This is attributed to the recognized influence of sex hormones on the immune system function. Sex hormones have been acknowledged for their potential role as triggers or protectors of SLE development. The acceleration of atherosclerosis in patients with SLE is caused by specific risk factors such as activity and illness duration and use of glucocorticoids. The utilization of immunosuppressive and systemic steroid agents may potentially influence the comorbidities in patients with SLE. Incidence of cardiovascular was significantly higher in patient with SLE. This study identified atherosclerosis as the primary comorbidity, followed by hypertension, hypercholesterolemia, obesity, and hyperglycemia. Hypertension and hyperglycemia show significant differences in plaque development in SLE patients. The incidence of obesity is associated with an increase IMT. Thus, when SLE is not effectively managed, it may lead to comorbid conditions such as cardiovascular disease, a sequela of atherosclerosis. This is related to traditional cardiovascular risks. SLE patients who receive steroid therapy have the potential to be 4 times more likely to develop diabetes mellitus and hypertension. Use of a 7.5 mg dose of prednisone was associated with a 2.5-fold increase in cardiovascular risk. The effects of cardiovascular drugs depend on disease activity and traditional risk factors in SLE patients.

Cytokine markers, such as IFN-γ and IL-6, are known to play a crucial role in accelerating atherosclerosis in patients with SLE. IFN-γ is a pro-inflammatory cytokine that may stimulate foam cell formation, a specific immune response from T helper 1 (Th1) cells, and atherosclerotic plaque development. IFN-γ secreted by macrophages may induce gene expression. In atherosclerotic lesions, Th1 cells, macrophages, and APC express IFN-γ. In normal cells, during
sterol homeostasis, balance in cholesterol absorption exists; however, if an imbalance occurs due to pathology, thereby increasing IFN-γ, it may affect the formation of foam cells and cause a decline in cholesterol absorption and an increase in oxidized LDL (OxLDL) absorption. IL-6 involvement in the production of inflammatory cytokines and lipid homeostasis is associated with cardiovascular disease mortality. Serum IFN-γ levels in patients with SLE have not been reported; however, IFN-γ overexpression may play a role in the immunopathogenesis of SLE via the induction of sBLyS/BAFF by monocytes and macrophages causing B-cell activation and maturation. In this study, a statistically significant association was not found between IFN-γ and IL-6 levels and atherosclerosis incidence in patients with SLE. However, in this study, it can be observed from the data obtained that IFN-γ levels were slightly higher in those with comorbid atherosclerosis compared to those without (647.9 ± 440.4 pg/mL vs 582.2 ± 308.7 pg/mL and 148 ± 109.3 pg/mL vs 197.19 ± 145.5 pg/mL, respectively). The values obtained are in concordance with those found in previous study showed that IL-6 levels approximately ranged from 4.272 ± 0.4222 to 123.71 ± 81.783 pg/mL in patients with SLE and 0.93 ± 0.95 to 10.46 ± 4.33 pg/mL in healthy controls. The IL-6 levels were found to be high in patients in this study. As previously elucidated, both IL-6 and IFN-γ are associated with atherosclerosis in SLE. However, atherosclerosis itself is a complex and multifactorial condition with numerous contributory pathways and factors. While the immune response and inflammation play a critical role, other important aspects of atherosclerosis pathogenesis include endothelial dysfunction caused by factors such as high blood pressure, smoking, and high LDL cholesterol levels. The dysregulation of lipid metabolism, hemodynamic factors, and elevated levels of homocysteine (an amino acid) are also associated with an increased atherosclerosis risk. Numerous markers are directly associated with atherosclerosis pathogenesis, such as hs-CRP, lipid levels, and homocysteine levels, whereas IL-6 and IFN-γ are indirectly associated with atherosclerosis. Additionally, the genetic marker could also predispose an individual to the risk of atherosclerosis especially genes that are related to the immune response such as TNFSF13B. The TNFSF13B gene encodes the BAFF protein, which plays a critical role in B-cell modulation. The increase in serum BAFF levels is higher in individuals at risk of atherosclerosis compared to those who are not at risk. This increase is associated with BAFF protein upregulation encoded by the TNFSF13B gene on SLE with atherosclerosis as a comorbidity.

This study showed that variations in the TNFSF13B rs9514828 genotype were found in the SLE patient population at Hasan Sadikin Hospital Bandung, Indonesia. The genotype distribution of patients with SLE was analyzed using the HWE test which states that the genetic variation in a population will remain constant from one generation to the next (p = 0.29). However, genetic variation changes will occur when there are disturbing factors such as mutations, environmental changes, random mating, and migration. This study found that TNFSF13B rs9514828 gene polymorphisms were present in both patients with SLE with and without comorbid atherosclerosis. The frequency of the TNFSF13B rs9514828C>T polymorphism was noted to be higher in patients with SLE with atherosclerosis (66.67%) compared to those without atherosclerosis (42.86%). Although the number of patients who experienced atherosclerotic events was smaller, a significant correlation (p = 0.01) was identified, indicating that the TNFSF13B rs9514828C>T polymorphism may potentially contribute to the development of atherosclerosis in patients with SLE (OR = 4.72). However, it is noteworthy that the number of participants was higher in the group of patients with SLE without comorbid atherosclerosis. This difference in group sizes may be related to the therapies administered to patients, particularly the use of corticosteroid-class drugs, which are commonly used as the primary treatment in patients with SLE. The prevalence of atherosclerotic plaques is reported to be approximately twofold higher in patients with SLE compared to the general population. Owing to their pro-atherogenic properties, corticosteroids are known to contribute to early atherosclerosis, which have adverse effects on metabolic factors such as body fat distribution, blood pressure, and glucose metabolism. This clinical condition has been shown to result in an increase in LDL cholesterol and triglyceride levels while decreasing HDL cholesterol.

The standard therapy is administered in severe cases or during relapses. The prescribed dosage is ≤7.5 mg/day of prednisone. Pulse glucocorticoid therapy involves an intravenous administration of 0.5–1 gram of methylprednisolone for three days. If the patient’s condition flares, the prednisolone dose is increased according to guideline therapy. Additionally, the use of sparing agents aids in facilitating the adjustment of corticosteroid doses and mitigates their
side effects. In this study, patients utilized sparing agents (immunosuppressive) such as azathioprine, mycophenolate mofetil (MMF), cyclophosphamide, and methotrexate. It is noteworthy that participants in this research had not been exposed to biologic agents as part of their therapy. We did not analyze the effect of other immunosuppressive agents as the confounding factors of outcome therapy (LLDAS). Some studies showed that steroids, MMF, azathioprine, cyclophosphamide, and hydroxychloroquine have no effect on atherosclerosis progression in animal model or SLE patients, although in a small study showed that MMF effect on plaque progression still need to be explored. This also one of limitation in our study, although previous studies showed that immunosuppressive agents do not affect the progression of atherosclerosis in SLE patients.

In this study, the therapeutic outcomes in patients with SLE are categorized into two groups: the LLDAS group and the non-LLDAS group. LLDAS is used as a target goal in the treatment of patients with SLE. The therapeutic response of each individual is different depending on disease severity with organ involvement. Several studies have demonstrated that the achievement of LLDAS is associated with reduced flares and organ damage so it can be a target in treatment strategies. In this study, the frequency and distribution analysis revealed a significant correlation (p = 0.00) between the genotype and the LLDAS. The CT and TT genotype demonstrated a greater incidence of LLDAS (80.43%) than in the non-LLDAS (31.58%) group. Moreover, the wildtype (CC) variant was higher (68.42%) in the non-LLDAS group. The OR (7.581), calculated based on the genotype for LLDAS, indicates that disease improvement, as indicated by a tendency toward achieving LLDAS, was less noticeable in patients with the rs9514828 gene polymorphism (CT and TT genotypes). As previously noted in a Mexican study, the rs9514828 C>T polymorphism appears to elevate TNFSF13B gene expression. An elevated BAFF expression is linked to active disease, particularly renal and hematological involvement in patients with SLE. Another study showcased consistent results, showing that this specific SNP is associated with increased gene expression in autoimmune patients. Findings from both studies suggested that TNFSF13B is indirectly linked to disease activity through BAFF expression. In a separate pharmacogenetics study, a combined analysis of various SNPs, including IFN regulatory factor 5, and TNFSF13B, displayed a strong, direct association with favorable response to rituximab in SLE therapy.

This study suggests the potential necessity for considering multiple genetic markers in predicting clinical outcomes. However, the conflicting results found in this study and previous research might be explained by the factors that influence the LLDAS score. LLDAS is a combination of both low SLE disease activity and the use of a low prednisone dose (<7.5 mg daily). In the multivariable model, factors such as an African–American ethnicity, the presence of anti-RNP and anti-dsDNA antibodies, low complement levels, history of serositis, history of vasculitis, decreased renal activity, arthritis, malar rash, discoid rash, thrombocytopenia, and duration of immunosuppressant drug use remained as negative LLDAS. Other studies elucidated that certain marker related to nephritis (nephritis-associated markers, including urinary protein and serum creatinine) and lower C3 levels (complement component 3) at the beginning of the study had a negative impact on achieving LLDAS. This implies that higher levels of urinary protein and serum creatinine and lower C3 levels were associated with a reduced likelihood of achieving LLDAS. Thus, despite the LLDAS score being influenced by various factors other than BAFF levels, this study shows that the TNFSF13B rs9514828 C>T polymorphism is correlated with atherosclerosis risk. Therefore, we suggest that when combined with effective therapy, it will be significantly associated with improved disease activity in SLE.

Conclusion

TNFSF13B rs9514828 C>T polymorphism has a significant association with incidence atherosclerosis and therapeutic outcomes in patients with SLE (p < 0.05). Health workers need to underscore the TNFSF13B rs9514828 gene to undertake preventive measures in order to prevent atherosclerosis and poor prognosis in patients with SLE.

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**Disclosure**

The authors report no competing interest exists in this work.

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