# Study of early atherosclerosis in juvenile-onset systemic lupus erythematosus patients

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## Objective

The aim of this study was to investigate early atherosclerotic changes in juvenile-onset systemic lupus erythematosus (jSLE) patients and its relation with disease activity.

## Patients and methods

Thirty patients suffering from jSLE diagnosed according to the 2012 SLICC SLE criteria were included in the study. Clinical and laboratory parameters, disease activity, and traditional risk factors for atherosclerosis were assessed. B-mode ultrasound was performed to measure carotid intima–media thickness (CIMT) and the number and size of plaque deposits in both the left and the right common carotid arteries. A total of 20 healthy volunteers were taken as a control group.

## Results

The mean±SD age of the patients was 18.93±2.81. The mean±SD disease duration was 4.33±2.25. The mean±SD CIMT differed significantly between the patient and control ($n$=20) groups (0.74±0.21 vs. 0.38±0.05; $P<0.001$). The presence of lymphopenia, serum creatinine, total cholesterol, triglycerides, and low-density lipoprotein was positively associated with the progression of CIMT ($P$=0.049, $P$=0.02, $P\leq0.001$, $P\leq0.001$, and $P$=0.006, respectively).

## Conclusion

In patients with jSLE, some traditional and nontraditional risk factors such as increased low-density lipoprotein, triglycerides, total cholesterol, BMI, fasting blood sugar, and proteinuria for the development of subclinical atherosclerosis were identified. It is likely that good disease control is the optimum way to prevent premature atherosclerosis in jSLE.

## Introduction

Cardiovascular disease is a leading cause of mortality and morbidity in systemic lupus erythematosus (SLE) [1]. Although few studies have focused specifically on juvenile-onset systemic lupus erythematosus (jSLE), patients with jSLE have been found to have a higher risk for accelerated atherosclerosis compared with patients with adult-onset SLE [2].

Atherosclerosis is a multifactorial process involving inflammatory, immune-mediated mechanisms, oxidative stress, and endothelial dysfunction; early alterations of the arterial wall in jSLE document the development of accelerated atherosclerosis early [3].

Nowadays, it is widely accepted that several traditional risk factors, including dyslipidemia, sedentary lifestyle, high blood pressure, diabetes mellitus, cigarette smoking, and obesity are associated with atherosclerosis in jSLE [4].

Because cardiovascular and cerebrovascular events rarely occur before adulthood, surrogate vascular measures, such as carotid intima–media thickness (CIMT), flow-mediated dilation, and pulse wave velocity, are used to assess premature atherosclerosis in pediatric patients [5]. Increased CIMT has been shown to be predictive of coronary artery disease [6]. Flow-mediated dilation, which measures endothelial function, has been shown to predict cardiovascular events in adults [7]. Aortic pulse wave velocity is a measure of arterial stiffness and is also a strong predictor of cardiovascular events and all-cause mortality [8].

The CIMT is a valid marker of early atherosclerosis, and thus it has the potential to detect cardiovascular disease in its subclinical phase [9].

B-mode ultrasound has been proved to be a useful noninvasive means of quantitatively assessing the

amount of atherosclerosis in the carotid arterial system George *et al*. [10].

The aim of our study was to investigate the presence of both functional and morphological changes in the cardiovascular system in jSLE.

## Patients and method

This study was conducted on 28 female and two male jSLE patients diagnosed according to the 2012 SLICC SLE criteria [11]. The study was approved by the ethical committee of faculty of Medicine, Banha university on 16th November, 2014. Their ages ranged from 13 to 25 years. Patients were excluded from the study if they had clinically evident atherosclerosis, concurrent infection, collagen diseases other than SLE, renal failure, etc. Twenty healthy individuals (18 female and two male) of matched age (range from 13 to 23 years) were included as controls. The whole procedure was explained to every patient and written consent was obtained from every patient to ensure complete satisfaction.

Both groups were subjected to the following: full medical history taking and examination were carried out to investigate traditional cardiovascular disease risk factors, Raynaud's phenomena, and secondary anti-phospholipid syndrome. Disease activity was evaluated at the beginning of the study using the SLE Disease Activity Index (SLEDAI) [12] and the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index (SLICC/ACRDI) [13]. Routine laboratory investigations [lipid profile, complete blood count, anti-nuclear antibodies, renal function tests, complete urine analysis, protein in 24 h urine collection, fasting blood glucose, Westergren erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein, C3, C4, lupus anticoagulant, immunoglobulin G (IgG) antibodies to dsDNA, and IgG/IgM anticardiolipin antibodies] and ECG were carried out.

### Measurement techniques

A carotid artery ultrasound was performed by an experienced radiologist to measure the combined thickness of intima and media layers of carotid artery - increase in thickness - due to hypertrophy of intima of medial layers or both. When the CIMT assessment to measure the combined thickness of intima and media layers of carotid artery -increase in thickness- due to hypertrophy of intima of medial layers or both focuses on a smaller number of sites. The patients and controls were scanned using GE P6 (LOGIQ e$^{TM}$, India) using 7.5 MHs probe. The

intima–media thickness measurements on each side were taken at the following points:

(1) Common carotid artery (10 mm before the bulb).
(2) Bulb 5–10 mm cranially to the start of the bulb.
(3) External carotid artery 10 mm after flow divider.

For each patient, the highest intima–media thickness among the six segments studied were recorded [14]. In addition, the number and size of carotid Atheromatas (AS) plaques were also assessed.

*Patient position*
We examined the carotid arteries with the patient in the supine position and with the examiner seated at the patient's head. In any case, exposure of the neck should be maximized by having the patient drop the ipsilateral shoulder as far as possible. Neck exposure is also optimized by tilting and rotating the head away from the side being examined [15].

*Transducer position*
Generally, the posterolateral and far-posterolateral positions are most useful for showing the carotid bifurcation and the Internal cartotid artery (ICA. The far-posterolateral approach often provides the best images of the distal reaches of the ICA. To use this view effectively, it is necessary to turn the patient's head far to the contralateral side and to place the transducer posterior to the sternomastoid muscle [15].

### Statistical analysis

The collected data were summarized in terms of mean ±SD and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the $\chi^2$-test and Fisher's exact test to compare differences between proportions as appropriate. Student's $t$-test was used to test mean differences between two groups. Pearson correlation coefficient ($r$) was used to examine the correlation of mean CIMT values and estimated parameters in SLE patients.

After the calculation of each of the test statistics, the corresponding distribution tables were referred to obtain the $P$-value (probability value). Statistical significance was accepted at $P$-value less than 0.05 (S). A $P$-value less than 0.001 was considered highly significant (HS) and a $P$-value greater than 0.05 was considered nonsignificant.

## Results

Thirty consecutive patients (28 female and two male) with jSLE were included, and 20 age-matched and sex-

matched apparently healthy volunteers were included as a control group (Fig. 1). The mean age at study entry was 18.93±2.81. The mean disease duration was 4.33±2.25 years. The controls had a mean age of 18.5±2.68. There were no statistically significant differences in age between patients and controls. A comparison as regards the means of clinical, laboratory, and radiological findings between the studied groups is shown in Table 1. In our study, 18 patients (60%) had malar rash, 18 patients (60%) had photosensitivity, four patients (13.33%) had oral ulcers, 10 patients (33.33%) had arthritis and/or arthralgia, five patients (16.67%) had pulmonary manifestation in the form of pleuritis and/or effusion, six patients (20%) had cardiac manifestations in the form of pericarditis, seven patients (23.33%) had lupus nephritis, and the mean of systolic blood pressure (SBP) and diastolic blood pressure (DBP) was 119.67±23.23 and 83.83±13.43 mmHg, respectively. However, none of our patients had any neurological affection. According to the SLEDAI, 10 patients (33.33%) had mild disease activity, 16 patients (53.33%) had moderate disease activity, and four patients (13.33%) had severe disease activity. SLICC damage index scores were as follows: score 0 in 16 patients (53%), score 1 in seven patients (23%), score 2 in four patients (13%), and score 3 in

three patients (10%). There were highly statistically significant positive correlations ($P<0.001$) between mean CIMT values and SLEDAI score ($r=0.76$) and SLICC damage index score ($r=0.73$). There were statistically significant positive correlations ($P<0.05$) between mean CIMT values and BMI ($r=0.54$), disease duration ($r=0.55$), DBP ($r=0.37$), and age ($r=0.55$). There were correlations between mean CIMT values and SBP, which was nonsignificant ($r=0.09$) (Table 2). There were highly statistically significant positive correlations ($P<0.001$) between mean CIMT values and total cholesterol ($r=0.72$) and triglycerides (TGs) ($r=0.57$). There were statistically significant positive correlations ($P<0.05$) between mean CIMT values and ESR first hour ($r=0.37$), urine protein ($r=0.55$), low-density lipoprotein (LDL) ($r=0.49$), and serum creatinine ($r=0.42$). There was a statistically significant negative correlation ($P<0.05$) between mean CIMT values and C3 ($r=-0.42$) and white blood cells ($r=-0.36$). However, there were no statistically significant correlations ($P>0.05$) between mean CIMT values and fasting blood sugar ($r=0.07$), Serum glutamic oxaloacetic transaminase (SGOT) ($r=0.22$), and Serum glutamic pyruvate transaminase (SGPT) ($r=0.06$). There were statistically no

**Figure 1**
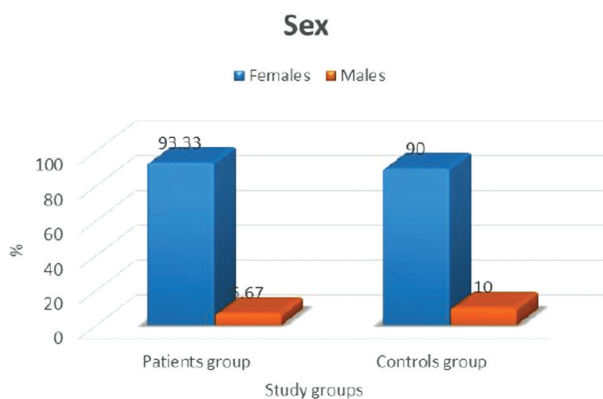


Sex distribution of the studied groups.

**Table 2 Correlations of the mean carotid intima–media thickness values and some variables in the systemic lupus erythematosus patient group**

| | CIMT | |
| --- | --- | --- |
| | r | P-value |
| Age (years) | 0.55 | 0.001 (S) |
| Disease duration (years) | 0.55 | 0.002 (S) |
| BMI (kg/m$^2$) | 0.54 | 0.002 (S) |
| SBP (mmHg) | 0.01 | 0.94 |
| DBP (mmHg) | 0.37 | 0.04 (S) |
| SLEDAI score | 0.76 | <0.001 (HS) |
| SLICC score | 0.73 | <0.001 (HS) |

CIMT, carotid intima–media thickness; DBP, diastolic blood pressure; SBP, systolic blood pressure. P>0.05=insignificant. P<0.05=significant. P<0.001=high significant.

**Table 1 Comparison of the means of clinical, laboratory, and radiological findings between the studied groups**

| Variables | Mean±SD | | t-Test | P-value |
| --- | --- | --- | --- | --- |
| | Patients (N=30) | Controls (N=20) | | |
| BMI (kg/m$^2$) | 32.23±11.14 | 23.3±2.9 | 3.50 | 0.001 (S) |
| Total cholesterol (mg/dl) | 218.77±38.32 | 165.7±20.88 | 5.65 | <0.001 (HS) |
| HDL (mg/dl) | 43.37±17.54 | 52±8.98 | 2.02 | 0.048 (S) |
| LDL (mg/dl) | 152.07±21.25 | 128.35±9.55 | 4.67 | <0.001 (HS) |
| TGs (mg/dl) | 168.52±88.56 | 69.75±15.14 | 4.87 | <0.001 (HS) |
| FBS (mg/dl) | 95.83±10.49 | 87.25±13.23 | 2.55 | 0.014 (S) |
| CIMT | 0.74±0.21 | 0.38±0.05 | 7.34 | <0.001 (HS) |

Number of patients, N=30. CIMT, carotid intima–media thickness; FBS, fasting blood sugar; HDL, high-density lipoprotein; LDH, low-density level lipoprotein; TGs, triglycerides. P>0.05=nonsignificant. P<0.05=significant. P<0.001=highly significant.

**Table 3 Correlations of the mean carotid intima–media thickness values and laboratory data in the systemic lupus erythematosus patient group**

|  | CIMT | |
| --- | --- | --- |
|  | r | P-value |
| Hb% (g/dl) | −0.31 | 0.09 |
| RBCs ($\times 10^6$)/$cm^2$ | −0.15 | 0.44 |
| WBCs ($\times 10^3$)/$cm^2$ | −0.36 | 0.049 (S) |
| PLTs ($\times 10^3$)/$cm^2$ | −0.35 | 0.06 |
| Serum creatinine (mg/dl) | 0.42 | 0.02 (S) |
| Urine protein | 0.55 | 0.002 (S) |
| SGOT (U/l) | 0.22 | 0.24 |
| SGPT (U/l) | 0.06 | 0.76 |
| Total cholesterol (mg/dl) | 0.72 | <0.001 (HS) |
| HDL (mg/dl) | −0.35 | 0.06 |
| LDL (mg/dl) | 0.49 | 0.006 (S) |
| TGs (mg/dl) | 0.57 | <0.001 (HS) |
| FBS (mg/dl) | 0.07 | 0.72 |
| ESR 1st hour (mm/h) | 0.37 | 0.045 (S) |
| Complement (C3) (mg/dl) | −0.42 | 0.02 (S) |

Number of patients, N=30. CIMT, carotid intima–media thickness; ESR, erythrocyte sedimentation rate; FBS, fasting blood sugar; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density level lipoprotein; PLT, platelet count; RBCs, red blood cells; TGs, triglycerides; WBCs, white blood cells. P>0.05=nonsignificant. P<0.05=significant. P<0.001=highly significant.

significant correlation ($P>0.05$) between mean CIMT values and Hb ($r=-0.31$) and red blood cells ($r=-0.15$). There was a negative correlation between thrombocytes and the mean CIMT values, which was nonsignificant (Table 3).

## Discussion

The present study was essentially planned aiming to investigate early atherosclerotic changes in jSLE patients. Twenty healthy individuals of matched age participated in this study. There were statistical differences between the study group and the control group as regards BMI, total cholesterol, LDL, TGs, fasting blood sugar, high-density lipoprotein, and CIMT.

In our study, there were statistically significant positive correlations ($P<0.05$) between mean CIMT values and age ($r=0.55$). In our current study, we found that there were statistically significant positive correlations ($P<0.05$) between mean CIMT values and DBP ($r=0.37$). This increased blood pressure in children and adolescents is associated with endothelial dysfunction in systemic arteries, increased thickness of intima media, impaired arterial compliance, and distensibility [16].

In our current study, we found that there were statistically significant positive correlations ($P<0.05$) between mean CIMT values and LDL, TGs, and total cholesterol.

Our results are in agreement with those of Barsalou and colleagues (2013), who described that pediatric SLE patients with active disease before corticosteroid therapy had elevated TGs, very low-density lipoprotein-cholesterol, and had depressed high lipoprotein-cholesterol often referred to the active lupus lipid profile.

In contrast to our result, Sozeri et al. (2013) demonstrated that LDL and total cholesterol and other traditional risk factors had no correlation with functional and morphological changes in the cardiovascular system. They found that these changes correlated with disease activity scores in early disease stages.

Sozeri et al. (2013) had demonstrated that chronic low-grade inflammation and traditional risk factors will eventually accelerate the progression of the disease; the side effects of medications such as steroids may also play a role in disease progression.

There were statistically significant positive correlations ($P<0.05$) between mean CIMT values and ESR first hour ($r=0.37$).

Tyrrell et al. [17] demonstrated that elevated inflammatory markers, including fibrinogen, albumin, C-reactive protein (CRP), and ESR levels, were predictors of cardiovascular disease.

High CRP level is a marker of risk for cardiovascular disease [18]. In our current study we found that the mean CIMT in positive CRP patients was 0.86±0.27, whereas it was 0.67±0.12 in negative CRP patients.

Our results are in agreement with those of Sozeri et al. (2013), who described a higher CRP level in SLE patients compared with controls.

Our results are in agreement with those of Huang et al. [19], who reported that a higher level of CRP at baseline was associated positively with progression of CIMT.

In our study, there were highly statistically significant positive correlations ($P<0.001$) between mean CIMT values and SLEDAI score ($r=0.76$) and SLICC damage index score ($r=0.73$).

In contrast to our result, Falaschi et al. [20] demonstrated that the results of CIMT were not correlated to SLEDAI score and SLICC/ACR damage index score and laboratory indicators of SLE activity.

In our current study, we found that there was a statistically significant negative correlation ($P<0.05$) between mean CIMT values and C3 ($r=-0.42$).

In our current study, we found that there was a statistically significant negative correlation ($P<0.05$) between mean CIMT values and white blood cells ($r=-0.36$).

Our results are in agreement with those of Huang *et al.* [19], who described that only lymphopenia at diagnosis was significantly related to CIMT progression. Lymphopenia is associated with lupus disease activity. It is associated positively with renal involvement, leukopenia, anti-dsDNA antibodies, anti-RO antibodies, the use of corticosteroid, azathioprine, methotrexate, aggregation of factors simultaneously related to acceleration of atherosclerosis and lymphopenia. Lymphopenia is the only factor showing a significant effect on accelerated atherosclerosis. Patients with active SLE have lymphopenia and associated high titers of anti-T cell and anti-CD4 antibodies as well as markedly reduced level of CD4 T cells [21]. Decreased level of peripheral CD4 T cells are associated with decreased accelerated atherosclerosis; it is possible to build up a linkage between lymphopenia and accelerated atherosclerosis based on CD4 T cells defects [19].

In our current study, we found that there were statistically significant positive correlations ($P<0.05$) between mean CIMT values and serum creatinine ($r=0.42$).

Our results are in agreement with those of Laura *et al.* (2009), who described that creatinine clearance ($P=0.031$) was associated only with increased mean common CIMT; both proteinuria and creatinine clearance were associated with CIMT, but only creatinine clearance remained significant.

Our results are in agreement with those of Huang *et al.* [19], who described that a higher level of serum creatinine at baseline was associated positively with progression of CIMT. SLE is characterized by systemic inflammation and vasculitis; the kidneys are particularly susceptible to vascular damage [22]. Thus, monitoring kidney function provides useful information on the vascular effects of SLE; high serum creatinine levels reflect active systemic inflammation and vasculitis and consequently accelerate atherosclerosis.

## Conclusion
There are significant functional and morphological changes that occur in jSLE patients; increase risk of

atherosclerosis if there's renal involvement, leukopenia, anti-double strand DNA "with disease activity". Disease activity is brought under control, steroid therapy is being weaned and proteinuria improves, the lipid values normalize. These results suggest that disease control rather than long-term lipid-lowering therapy may be the most important factor to control dyslipidemia in jSLE. No disease activity, so stopping or decreasing steroid dose lead to normalization of lipid value preventing premature atherosclerosis in jSLE.

### Conflicts of interest
There are no conflicts of interest.

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