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Biochemical and genetic risk factors for atherosclerosis in systemic lupus erythematosus

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Gene polymorphism;
High sensitive CRP;
Intima-media thickness;
Monocyte chemoattractant protein-1;
SLE

Abstract Introduction: Systemic lupus erythematosus (SLE) is associated with an increase in the risk of premature cardiovascular complications caused by accelerated atherosclerosis which significantly contributes to morbidity and mortality. Carotid ultrasonography is a very sensitive imaging tool to detect premature atherosclerosis and measurements of carotid intima–media thickness (IMT) assess the extent and the severity of systemic atherosclerosis. The pathogenesis of accelerated atherosclerosis in SLE is not clear; inflammation and endothelial dysfunction in addition to genetic risk factors represent important factors in the onset of atherosclerosis.

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Aim of the work: To evaluate the relation between asymmetric dimethylarginine (ADMA), high sensitive C-reactive protein (hs-CRP), monocyte chemoattractant protein-1 (MCP-1) (both serum levels and the genotypes of the MCP-1 A_2518G polymorphism) with the development of carotid atherosclerosis in patients with SLE and their relation to disease activity.

Patients and methods: In the present study, 30 non-menopausal SLE female patients and 20 healthy age-matched females were included. Both patients and controls were subjected to evaluation of body mass index (BMI), IMT, serum glucose, serum lipids, hs-CRP, ADMA, MCP-1 (both serum level and gene polymorphism). Serum ADMA, hs-CRP, and MCP-1, levels were measured by enzyme-linked immunosorbent assay. MCP-1 genomic variants were detected by polymerase chain reaction followed by restriction enzyme–fragment analysis.

Results: Values for IMT, hs-CRP, ADMA and MCP-1 were significantly higher in patients with SLE than in healthy controls with more significant increase in SLE patients with IMT > 1 mm than in those with IMT < 1 mm. Carotid IMT was significantly positively correlated with all the studied variables except for age, BMI and FBS, but significantly negatively correlated with HDL-C in all SLE patients. G/G genotype of MCP-1 A_2518G gene was more frequent in SLE patients than controls. IMT, hs-CRP, ADMA and MCP-1 from patients with G/G phenotypes were markedly higher than those from patients with the A/A genotype.

In multiple regression analysis, ADMA and MCP-1 were the strongest independent determinants of IMT in SLE patients.

Conclusions: Assessment of high levels of ADMA, hs-CRP, MCP-1, in addition to the MCP-1 A_2518G polymorphism may play a role in the pathogenesis of accelerated atherosclerosis in SLE patients and would be useful in identifying the risk of developing cardiovascular disease.

Kindly suggest: Development of a novel therapy targeting ADMA and MCP-1 may have a potential role in preventing the progression of increased IMT in SLE patients.

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1. Introduction

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors [1]. It is associated with an increased risk of atherosclerosis and related cardiovascular disease [2]. The mechanisms underlying the accelerated atherosclerosis in SLE are not clear because the traditional risk factors fail to account fully for the excess of cardiovascular events in lupus patients [3]. Therefore, it has been suggested that patients with SLE possess additional risks in addition to the traditional risk factors for the development of accelerated atherosclerosis [4].

Because atherosclerosis is a systemic disease, there is strong correlation between coronary atherosclerosis and that in the carotid arteries. Intima–media thickness (IMT) is a non-invasive predictor of early arterial wall alteration, which identifies and quantifies early structural vascular abnormalities and is currently considered as a marker of premature atherosclerosis [5].

Endothelial dysfunction may play pivotal roles in the initiation, progression and propagation of the atherosclerotic process [2]. Asymmetric dimethylarginine (ADMA) is the major endogenous inhibitor of nitric oxide synthase (NOS), the enzyme which synthesizes nitric oxide (NO) which has anti-atherosclerotic properties [6]. Increased plasma ADMA concentration causes impaired NO synthesis leading to endothelial dysfunction and atherosclerotic vascular disease [7].

Since atherosclerosis in part may be an inflammatory disease, circulating factors related to inflammation may be predictors of cardiovascular disease [8].

High sensitive C-reactive protein (hs-CRP) is secreted by several cell types in inflammatory microenvironments, such as rheumatoid synovium and atherosclerotic lesions, induces the production of inflammatory cytokines from monocytes, and promotes monocyte chemotaxis [9]. It has been associated with increased IMT and suggested to cause initiation and progression of atherosclerosis [10].

Monocyte chemoattractant protein-1 (MCP-1) is a B-chemokine responsible for monocyte and lymphocytes T recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation; it also proposed that it is responsible for tissue inflammation in autoimmune diseases [11]. It was found to be highly expressed in human atherosclerotic plaques and postulated to have a crucial role in monocyte recruitment into the sub-endothelial lesions [12], so that mice deficient for MCP-1 develop less atherosclerosis. On the basis of this evidence, MCP-1 should be considered a potent atherosclerotic factor and a target for selective therapies [13].

A biallelic A/G polymorphism has been found in the MCP-1 distal gene regulatory region at position –2518 that affects the level of MCP-1 expression in response to an inflammatory stimulus. The effect of the G allele appears to be dose dependent as cells from individuals homozygous for G at –2518 produced more MCP-1 than cells from G/A heterozygotes [14].

The aim of this study was to evaluate the relation between ADMA, hs-CRP, MCP-1 (both serum levels and the genotypes of the MCP-1 A_2518G polymorphism) with the development of carotid atherosclerosis in SLE patients and their relation to disease activity.

2. Materials and methods

A total of 30 premenopausal SLE female patients and 20 age and sex matched healthy controls were enrolled in this study. All patients were fulfilling the latest version of the American College of Rheumatology (ACR) revised criteria for the classification of SLE [15]. The disease activity was assessed using the...
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SLE disease activity index score SLEDAI [16]. Patients were selected from out-patient clinic of Rheumatology and Rehabilitation Department of Tanta University Hospital. Written informed consent was obtained from all participants.

2.1. Exclusion criteria include

In order to avoid confusion with other known risk factors for atherosclerosis, the exclusion criteria were: clinically evident atherosclerosis, hypertension, or the use of antihypertensive medication; hyperlipidaemia, or the use of lipid-lowering medication; diabetes mellitus, and a history of ischaemic heart diseases or cerebrovascular events, infections in the previous 4 weeks, pregnancy or lactation in the previous 6 weeks, renal failure and age <18 or >65 years. Smoking habits both in SLE patients and healthy controls.

For all participants body mass index (BMI) and blood pressure were assessed.

2.2. IMT of the carotid arteries

All subjects underwent B-mode ultrasonography of extracranial carotid arteries using a 7.5 MHz probe which provides a direct and non-invasive assessment of subclinical atherosclerosis through IMT measurement and detection of atherosclerotic plaques. Common carotid artery (CCA), internal carotid, and external carotid arteries were evaluated carefully on both sides. CCA IMT measurements of the proximal and distal CCA posterior wall were done. Three measurements were made in a non-neighbouring fashion within an approximately 1 cm segment both from the left and right CCA proximal and distal portions. IMT values were then calculated by obtaining the arithmetic means of the measured values [5]. The mean IMT value for each subject was calculated using the formula [(Lt IMT + Rt IMT)/2] and this value was taken as the measure of current common carotid artery wall thickness [17].

Then the patients were classified into two groups with IMT < 1.0 mm (n = 17) and with IMT ≥ 1.0 mm (n = 13).

2.3. Biochemical assay

Venous blood sampling was obtained from all subjects by puncture of an ante-cubital vein at 8 a.m. after an overnight fasting. In order to obtain serum, blood was allowed to coagulate for at least 30 min–1 h at room temperature and then centrifuged at 3000 g for 10 min at 20 °C. The obtained serum was immediately frozen at −70 °C for future analysis of:

1. Total cholesterol (TC), triglyceride (TG) concentrations and high density lipoprotein-cholesterol (HDLC) concentration were determined, low-density lipoprotein-cholesterol (LDLC) concentrations were calculated using Friedewald’s equation (LDLC = TC – HDLC – TG/5).
2. Fasting blood sugar (FBS) level.
3. Serum ADMA by ELISA Kit using Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim, according to Lu et al. [18].
4. Serum hs-CRP by ELISA Kit using Monobind Inc., Lake Forest, CA 92630, USA, according to Auer et al. [19].
5. Serum MCP-1 by ELISA Kit using RayBio® Human MCP-1 ELISA Kit Protocol according to Leonard and Yoshimura [20].
6. Genotyping of the MCP-1 –2518 polymorphism was performed as described by Rovin et al. [21]. Briefly, Genomic DNA was extracted from 200 μl of EDTA-anticoagulated peripheral blood leukocytes using the Qiagen mini blood DNA purification kits (Qiagen, Canada) according to the manufacturer’s instructions. MCP-1 genomic variants were detected by polymerase chain reaction (PCR) followed by restriction enzyme–fragment analysis using a PvuII site affected by the G/A polymorphism. Amplification with the primers 5'-CCGAGATGTCCAGCCACGCA-3' (forward) and 5'-CTGCTTTGCTTGTGCTCTFF-3' (reverse) (where A is adenine, C is cytosine, G is guanine and T is thymine) generated a 930 bp product. PCR is performed in 50 μl volumes containing 100 ng of DNA, 250 mmol/l of deoxynucleotides triphosphates, 1.5 mmol/l of MgCl2, 1 U of Hot Star Taq polymerase (Qiagen Inc., Valencia, CA), and 25 pmol/l of each primer. Cycle conditions were used were 95 °C for 15 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 1 min, and 72 °C for 1.5 min, with a final extension of 72 °C for 10 min. The PCR product was digested with 5 U of PvuII endonuclease (New England Biolabs, Beverly, MA) and analyzed by phototyping in 2% agarose gels containing ethidium bromide. Digestion with PvuII yields 708 bp and 222 bp fragments when G is at position –2518. The identified genotypes were named according to the presence or absence of the enzyme restriction site. So, samples showing only a 930 bp band were assigned as A/A, samples showing two bands of 708 and 222 bp were considered G/G and samples showing three bands at 930, 708 and 222 bp were typed A/G.
7. In SLE patients inflammatory parameters indices such as ESR mm/1st h were determined by Westergren and CRP was determined by semi-quantitative latex agglutination.

2.4. Statistical analysis

Statistical analysis in this study was performed using the MINITAB 1 and SPSS version 10. Most data are expressed as the mean (± SD) for quantitative variables and as numbers and percentages for categorical variables. Student’s t-test was applied to compare variables between 2 groups, whereas the frequencies of various alleles and genotypes were compared between the groups by Fisher’s exact. For each analysis, an odds ratio (OR) and 95% confidence interval (CI) were calculated. Pearson’s correlation analysis was used to examine the relationships between IMT and tested atherosclerotic risk factors and between ADMA, hs-CRP and MCP-1 and other variables.

Associations between IMT and other risk factors were evaluated by simple regression analysis followed by multiple linear regressions. All statistical tests were two-tailed and only a P value < 0.05 was considered statistically significant.

3. Results

Table 1 shows the clinical, laboratory and genetic characteristics of the studied groups; in the SLE group 9/30 patients (30%) presented with an IMT < 1.0 mm, while 21/30 (70%) with an IMT ≥ 1.0 mm. In the control group, IMT ≥ 1.0 was not detected. SLE patients with IMT ≥ 1.0 mm compared with
those with IMT < 1.0 had higher but non-significant differences in BMI, FBS, TG, LDL-C and lower HDL-C but higher with significant differences in the values of disease duration, IMT, hs-CRP, ADMA and MCP-1 serum level. In comparison with controls values for age, BMI, FBS and lipid profile were different genotypes with the highest values (the lowest value for HDL-C) present in those carrying the G allele (GG and AG). Genotyping of the MCP-1 gene polymorphism are shown in Table 5 in which there were significant differences in the IMT and serum levels of HDL-C, ADMA, hs-CRP and MCP-1 level, between the different genotypes with the highest values (the lowest value for HDL-C) present in those carrying the G allele (GG and GA). Genotyping of the MCP-1 −2518 polymorphism (GG, AG and AA) is represented in Fig. 1.

Table 2 shows significant correlation between ADMA and all the studied parameters except for age and FBS, but both MCP-1 and hs-CRP are significantly correlated with carotid IMT and with each other.

In Table 3 simple regression analysis was carried out using IMT as the dependent variable, and other studied variables as independent variables. LDL-C, HDL-C, ADMA, hs-CRP, and MCP-1 were significantly associated with IMT.

In multiple regression analysis (Table 4), ADMA and MCP-1 were the strongest independent determinants of IMT in patients with SLE.

Atherosclerotic risk factors of all patients respective of MCP-1 gene polymorphism are shown in Table 5 in which there were significant differences in the IMT and serum levels of HDL-C, ADMA, hs-CRP and MCP-1 level, between the different genotypes with the highest values (the lowest value for HDL-C) present in those carrying the G allele (GG and GA). Genotyping of the MCP-1 −2518 polymorphism (GG, AG and AA) is represented in Fig. 1.

### 4. Discussion

A leading cause of morbidity and mortality in SLE patients is represented by an increased occurrence of cerebro-vascular and cardiovascular diseases attributable to the development of accelerated atherosclerosis [22]. These findings highlight the importance of recognizing and managing early stages of atherosclerosis for effective cardiovascular prevention [5,23].

In this study, the combination of classic risk factors (BMI, disease duration and lipid profile) and altered concentrations of ADMA, hs-CRP and MCP-1 (both serum level and gene polymorphism) appears to explain at least part of the relation between SLE and the change in carotid IMT.

In the current study there was no significant difference between SLE patients and controls in most of the classical risk factors for atherosclerosis, except for TG and HDL-C which
came in accordance with Sato et al. [24], but there was a significant difference as regards IMT, ADMA, hs-CRP and MCP-1. The present work together with several research groups has shown that IMT is significantly increased in SLE patients than in control subjects [5–24,25], and it was significantly correlated with all the studied parameters except for age, BMI and FBG as mentioned by Carotti et al. [26]. In contrast Del Sol et al. [27] have considered that IMT would not add substantially when used as a screening tool to discriminate subjects with high and low risk of coronary heart disease.

Although the precise mechanisms of atherosclerosis remain unclear, endothelial dysfunction and inflammation in addition to genetic risk factors represent important factors in the onset of atherosclerosis [28].

Endothelial dysfunction is a key event in atherogenesis appearing long before the formation of a structural atherosclerotic lesion in SLE. Various factors may give rise to alterations in vascular wall properties [29]. ADMA represents a new and well-characterized marker that has been associated with many traditional and novel risk factors in the setting of atherosclerosis. This biomarker represents nitric oxide bioavailability and also oxidative stress, thus identifying individuals at high cardiovascular risk even in an early stage, apart from traditional risk factors and inflammatory biomarkers [6].

In the present study, SLE patients have increased serum ADMA level, compared to that of control subjects with more significant increase in SLE patients with IMT \( > 1.0 \text{ mm} \). Moreover there was also a significant positive correlation between IMT, disease activity and serum ADMA levels. These came in agreement with studies of Bultink et al. [30]. High ADMA levels in SLE may be due to a number of mechanisms:

### Table 2: Correlation between ADMA, MCP-1 and hs-CRP and other variables in SLE patients (\( n = 30 \)).

<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE patients (( n = 30 ))</th>
<th>ADMA (( \mu \text{mol/l} ))</th>
<th>MCP-1 (ng/ml)</th>
<th>hs-CRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.16</td>
<td>&gt; 0.05</td>
<td>0.18</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.43</td>
<td>&lt; 0.01</td>
<td>0.23</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>0.37</td>
<td>&lt; 0.05</td>
<td>0.22</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.48</td>
<td>&lt; 0.001</td>
<td>0.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>0.20</td>
<td>&gt; 0.05</td>
<td>0.19</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0.38</td>
<td>&lt; 0.05</td>
<td>0.22</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>0.43</td>
<td>&lt; 0.01</td>
<td>0.20</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.39</td>
<td>&lt; 0.05</td>
<td>0.23</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>0.38</td>
<td>&lt; 0.05</td>
<td>0.21</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>0.47</td>
<td>&lt; 0.001</td>
<td>0.44</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ADMA (( \mu \text{mol/l} ))</td>
<td>–</td>
<td>–</td>
<td>0.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>0.48</td>
<td>&lt; 0.001</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>0.45</td>
<td>&lt; 0.001</td>
<td>0.37</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Complement (C3)</td>
<td>–0.034</td>
<td>&lt; 0.001</td>
<td>–0.38</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.544</td>
<td>&lt; 0.001</td>
<td>0.733</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 3: Lipid profile, hs-CRP, MCP-1 and ADMA as predictive factors for IMT among SLE patients (simple linear regression).

<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE patients (( n = 30 ))</th>
<th>Standardized ( B ) coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T )</td>
<td>( P )</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.249</td>
<td>2.029</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>–0.292</td>
<td>2.112</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0.100</td>
<td>0.953</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>0.010</td>
<td>0.088</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>0.612</td>
<td>5.359</td>
</tr>
<tr>
<td>ADMA (( \mu \text{mol/l} ))</td>
<td>0.480</td>
<td>4.079</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>0.361</td>
<td>2.724</td>
</tr>
</tbody>
</table>

\( B \) = regression coefficient. * Significance = \( P < 0.05 \).

### Table 4: Multiple regression analysis of ADMA, MCP-1, hs-CRP, LDL-C, HDL-C, TC and TG of the SLE patient regarding IMT.

<table>
<thead>
<tr>
<th>Variables</th>
<th>( B )</th>
<th>SE</th>
<th>( P )</th>
<th>( \text{Exp} (B) )</th>
<th>95% Confidence interval for ( \text{Exp} (B) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
<td>Upper limit</td>
</tr>
<tr>
<td>ADMA (( \mu \text{mol/l} ))</td>
<td>42.697</td>
<td>17.953</td>
<td>0.006*</td>
<td>1.05</td>
<td>1.012</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>0.044</td>
<td>0.016</td>
<td>0.026*</td>
<td>1.09</td>
<td>1.011</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>4.229</td>
<td>2.964</td>
<td>0.154</td>
<td>6.86</td>
<td>0.206</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.093</td>
<td>0.076</td>
<td>0.223</td>
<td>0.09</td>
<td>0.945</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>–0.731</td>
<td>0.440</td>
<td>0.097</td>
<td>0.48</td>
<td>0.203</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>–0.043</td>
<td>0.081</td>
<td>0.601</td>
<td>0.96</td>
<td>0.817</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>0.045</td>
<td>0.088</td>
<td>0.612</td>
<td>0.05</td>
<td>0.879</td>
</tr>
</tbody>
</table>

\( B \) = logistic regression coefficient. SE = standard error of \( B \). * Significance = \( P < 0.05 \).
hs-CRP has been considered as a good biomarker of systemic inflammation, and might also be the culprit in atherosclerosis. Therefore, increased hs-CRP concentration is associated with higher cardiovascular risk and enhances the progression of atherosclerosis and also reflects arterial wall inflammation [33].

In this study, there was a significant increase in hs-CRP level in SLE patients as compared with healthy controls with more significant increase in SLE patients with IMT $\geq$ 1.00 than, which came in accordance with Barnes et al. [34]. Also Corrado et al. [10] reported a significant correlation between IMT and hs-CRP which may potentially account for the complex role of hs-CRP and IMT in the pathogenesis of cardiovascular events. Roh et al. [35] concluded that measurement of the serum hs-CRP level is simpler and cheaper than ultrasonography. Thus, hs-CRP would be a useful screening marker for evaluating and estimating the degree of atherosclerosis. However, Olsen et al. [36] revealed that hs-CRP is only weakly related to cardiovascular damage after adjustment for traditional cardiovascular risk factors. Thus, although CRP likely plays a direct role in the pathogenesis of atherosclerosis, it is unclear if the CRP levels used to determine risk in the general population can also be applied in SLE patients [10].

Several lines of evidence suggested that chemokines may play an important role in the inflammatory process and in the development of atherosclerosis [28]. Over expression of MCP-1 increased the progression of atherosclerosis. In mouse models [37], deficiency of MCP-1 reduced atherosclerosis [37], and plasma levels of MCP-1 correlated with monocyte infiltration in atherosclerotic lesions [38]. Consistent with the previous data, in the present study, the SLE patients have increased serum MCP-1 level, compared to that of control subjects with more significant increase in SLE patients with IMT $\geq$ 1.00 with a significant positive association between plasma MCP-1 and carotid IMT, which came in agreement with Brown et al. [39]; also, McMahon and Hahn [28] confirm the importance of MCP-1 in the development of atherosclerosis plaque which is emphasized by the finding that elevated circulating levels of MCP-1 are positively related to increased carotid artery IMT in human. Deo et al. [40] found higher levels of MCP-1 to be associated with an increased probability of significant levels of coronary calcium, which is a surrogate measure for the burden of coronary atherosclerosis. Also MCP-1 may promote lipid oxidation which is the main contributor to

### Table 5  Atherosclerotic risk factors of all SLE patients regarding MCP-1 gene polymorphism.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AA ($n = 9$)</th>
<th>AG ($n = 10$)</th>
<th>GG ($n = 11$)</th>
<th>$F$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42 ± 3</td>
<td>42 ± 2</td>
<td>43 ± 4</td>
<td>0.4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>10.4 ± 3.3</td>
<td>10.6 ± 2.8</td>
<td>11 ± 2.8</td>
<td>0.12</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.9 ± 2.7</td>
<td>26.5 ± 3.3</td>
<td>27.2 ± 3.3</td>
<td>1.4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>90.7 ± 3.4</td>
<td>90.7 ± 3.4</td>
<td>103.6 ± 16.3</td>
<td>4.4</td>
<td>0.02*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>93.3 ± 12.2</td>
<td>99 ± 9.9</td>
<td>105.6 ± 16.3</td>
<td>1.5</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>167.9 ± 15.3</td>
<td>168.35 ± 15.5</td>
<td>168.35 ± 15.5</td>
<td>0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>102.4 ± 17</td>
<td>109.9 ± 15.4</td>
<td>109.9 ± 15.4</td>
<td>1.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>32.4 ± 3.9</td>
<td>32.4 ± 3.9</td>
<td>32.4 ± 3.9</td>
<td>1.7</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ADMA (umol/l)</td>
<td>3.8 ± 0.76</td>
<td>3.8 ± 0.76</td>
<td>3.8 ± 0.76</td>
<td>7.9</td>
<td>0.002*</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>274.5 ± 30.9</td>
<td>274.5 ± 30.9</td>
<td>307.7 ± 51.7</td>
<td>3.5</td>
<td>0.04*</td>
</tr>
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</table>

* Significance $= P < 0.05$. 

Figure 1  Agarose electrophoresis of the PCR products after cutting with PvuII restriction endonuclease. The bands were visualized using ethidium bromide and 2% agarose (lanes 2 and 6 represent normal homozygote (MCP-1/C0 2518 AA), lanes 1, 3, 4 and 7 represent homozygote (MCP-1/C0 2518 GG) and lane 5 represents the heterozygote (MCP-1/C0 2518 AG).
atherogenesis [41]. So, it can be hypothesized that the increased serum levels of MCP-1 may play a role in the progression of chronic atherosclerotic lesions in SLE patients. On the basis of this evidence, MCP-1 should be considered an atherosclerotic factor and a target for selective therapies, such as using antibodies anti-MCP-1 and anti-MCP-1 gene therapies [42].

Single nucleotide polymorphisms in the MCP-1 regulatory region (i.e., –2518 G/A substitutions) have been identified and shown to affect transcription of the gene. In the present study significant increased frequencies of MCP-1 –2518 GG genotype and G allele existed in SLE patients as compared with healthy controls, and the percentage of GG genotypes in SLE with IMT 1 mm (71.5%) is significantly higher than in those with IMT <1 mm (44.4%), and both groups were significantly higher than controls (5%). Many studies concerning the relationship between MCP-1 gene polymorphism and inflammatory diseases have been performed in the last several years; Szalai et al. [43] reported that the GG genotype of the –2518 MCP-1 gene is associated with susceptibility to CVD. Nyquist et al. [14], also reported that this polymorphism was associated with myocardial infarction and with serum MCP-1 level in participants of the Framingham Heart Study. Aguilar et al. [11] demonstrated an association between the presence of G at position –2518 in the MCP-1 promoter region and the presence of cutaneous vasculitis among SLE patients. Also, Tucci et al. [44] show that the A/G and G/G MCP-1 polymorphisms are associated with a greater likelihood of renal disease in SLE patients. Their explanation is based on the fact that the –2518 A/G polymorphism in the promoter region of the MCP-1 gene is functionally important. It could potentially affect MCP-1 transcription and expression. In contrast Sánchez et al. [45] found no significant differences in the allele and genotype frequencies of the MCP-1 –2518 A/G polymorphism between SLE patients and controls. Also, Tabara et al. [46] reported that although the plasma level of MCP-1 showed a significant association with carotid IMT, the –2518 A/G polymorphism did not directly correlate with carotid IMT. These contradictory results may be due to gene variations between various countries, as well as among different areas in the same country. So the mechanism underlying this association needs to be elucidated.

However, in the concurrent study significant differences in the IMT and serum levels of HDL-C, ADMA, hs-CRP and MCP-1 level were found among the different genotypes with the highest values present in those carrying the G allele (GG and GA). These results came in accordance Buraczynska et al. [47], who found that the G allele was associated with higher levels of serum MCP-1, in both patient groups and controls.

As mentioned previously, this polymorphism seems to influence the transcriptional activity because cells obtained from individuals with G/G or A/G genotype produce more MCP-1 protein than cells isolated from individuals with A/A genotype [48]. Consequently, it would be reasonable to speculate that under the same stimulatory conditions, individuals bearing GG at position –2158 produce more MCP-1 protein than individuals with the genotype A/A and, in consequence, could make a stronger inflammatory response with higher tissue damage [23]. Also, the concurrent study demonstrated a significant correlation between –2518 A/G polymorphism and carotid IMT; thus, genetic polymorphisms in the regulatory regions of the MCP-1 gene could be implicated in the susceptibility or progression of atherosclerosis in SLE.

In the present study, HDL-C, LDL-C, ADMA, hs-CRP, and MCP-1 (both serum level and MCP-1 –2518 AG + GG polymorphism) were shown to be candidate risk factors for IMT in simple regression analysis but in multiple regression analysis, only ADMA and MCP-1 were the strongest independent determinants of IMT in SLE patients.

These results came in accordance with Furuki et al. [49] who revealed that baseline ADMA was the only predictor of IMT progression after adjustments for age, sex, baseline IMT, and the four major risk factors plus hyperuricaemia.

In conclusion the present data confirm that ADMA, hs-CRP, and MCP-1 are identified as important predictors of atherosclerosis progression and suggest that these factors have potent damaging effects on the vasculature in SLE patients. The synergistic effects of higher levels of ADMA, hs-CRP and MCP-1 in addition to MCP-1 gene polymorphism most likely contribute to increased IMT as a marker of sub-clinical atherosclerosis. According to the present findings individuals homozygous for G at –2518 of the MCP-1 gene regulatory region are at increased risk. So screening for MCP-1 polymorphisms may be applied to determine the relative risk of developing atherosclerosis in a patient with an established diagnosis of SLE.

Kindly suggest: Further studies using larger series might lead to more significant results. There is no specific treatment for ADMA and MCP-1. Reversing the effects of increased ADMA or the reduction of ADMA levels (by increasing the DDAH) may be meaningful goals for the treatment of endothelial dysfunction in SLE patients.

Therefore, development of a novel therapy targeting ADMA and MCP-1 may have a potential role in preventing the progression of increased IMT.

References


