Serum interleukin-18 and its relationship with subclinical atherosclerosis in systemic lupus erythematosus

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Abstract

BACKGROUND: Interleukin-18 (IL-18) is a pro-inflammatory and pro-atherogenic factor, and its blood level has shown a direct correlation with atherosclerosis. We aimed to evaluate the serum IL-18 level in patients with systemic lupus erythematosus (SLE) and its relationship with the intima-media thickness (IMT) of the carotid artery in these patients, as an indicator of atherosclerosis.

METHODS: In this cross-sectional study, 60 patients as the patient group and 30 healthy volunteers as the control group [matched sex, age, and body mass index (BMI)] were selected, and their disease status and general data were gathered using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) form. A blood sample was also obtained from all participants to determine the serum level of IL-18 and other metrics, including high-sensitivity C-reactive protein (hs-CRP), cholesterol, triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), anti-double stranded deoxyribonucleic acid (anti-dsDNA), complement 3 (C3), and C4. The IMT of the carotid artery was calculated in both groups. We also evaluated the clinical cardiovascular manifestations.

RESULTS: The serum IL-18 levels in patients were significantly higher than in the control group (P < 0.005). It had no significant correlation with disease activity (P = 0.10). The patients with SLE with high IL-18 serum levels (> 280 pg/ml) had higher SLEDAI-2K (P = 0.02) than the patients with a low level (< 280), where 280 was the median of the IL-18 levels. The serum IL-18 level had no significant correlation with the carotid artery IMT.

CONCLUSION: A high level of IL-18 reflects the disease activity, but it was not significantly correlated with subclinical atherosclerosis, denoted by the carotid artery IMT.

Keywords: Systemic Lupus Erythematosus; Interleukin-18; Atherosclerosis

Date of submission: 21 Feb. 2020, Date of acceptance: 30 June 2021

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown etiology, with diverse manifestations and multi-organ involvement. The overproduction of proinflammatory cytokines has been recognized as the main feature of SLE. Interleukin-18 (IL-18) is an important inflammatory cytokine and a pro-inflammatory and pro-atherogenic factor. Its serum level has shown a direct correlation with atherosclerosis. We aimed to evaluate the serum IL-18 level in patients with systemic lupus erythematosus (SLE) and its relationship with the intima-media thickness (IMT) of the carotid artery in these patients, as an indicator of atherosclerosis.

level has a direct correlation with atherosclerosis in the coronary artery.\(^3\) We aimed to evaluate the relationship between the serum IL-18 level and the disease activity, with the carotid artery intima-media thickness (IMT) as a marker of atherosclerosis in patients with SLE.\(^6\)

**Materials and Methods**

This cross-sectional study was conducted in Ghaem Hospital of Mashhad, Iran, from February 2013 to September 2016, on 60 patients with SLE of 20 to 60 years old, with at least 5 months of disease history who fulfilled the Systemic Lupus Collaborating Clinics (SLICC) criteria 2012.\(^7\) The control group consisted of 30 healthy volunteers, with matched age, sex, and body mass index (BMI). Both groups completed Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) forms,\(^8\) after obtaining informed consent. Further, their medical history and different laboratory features through blood and morning urine samples were gathered in a single-blinded manner. Patients with other rheumatologic or non-rheumatologic inflammatory disorders, pregnant women, and very ill patients were excluded.

Blood samples, after separating the serum, were kept frozen at -70 °C. An IL-18 BMS267/2 enzyme-linked immunosorbent assay (ELISA) Research Kit (Bender MED) was used to determine the serum level of IL-18. All subjects underwent a B-mode duplex carotid Doppler ultrasonography (Medison 8000EX) with a linear 10 MHz probe to determine the IMT of the carotid artery. Atherosclerotic plaques were identified as a specific area with hyper-echogenicity or local elevations into the lumen of the artery, involving at least 50% of the artery circumference.

**Statistical analysis:** To present normally-distributed variables, mean ± standard deviation (SD) and for other quantitative variables, median and interquartile range (IQR) were used. We used the Kolmogorov-Smirnov test to identify if the samples followed a normal distribution pattern or not. In case that a sample did not follow a normal distribution pattern, we used non-parametrical tests including Mann-Whitney and and Kruskal-Wallis tests. We used independent samples t-test for data following a normal distribution pattern. A P-value of < 0.05 was considered statistically significant. Data were analyzed using SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA). The median of the IL-18 levels, equal to 280, was selected as the cut-off point.

**Results**

The female to male ratio was 55/5 in patients and 26/4 in controls (P = 0.474). The mean duration of SLE in patients was 57.80 ± 60.91 months (median: 36, IQR: 54, Q1 = 18.0, Q3 = 72.0), and the mean corticosteroid consumption duration was 49.80 ± 51.70 months. Descriptive statistics are provided in table 1.

The major organ involvement in the patient group was the kidney (31.7%), followed by the heart (18.3%), with 1.7% cardiovascular intervention, 5.1% angina pectoris, and 11% pericarditis, and the central nervous system (CNS) (8.3%), including psychosis (3.3%), seizure (3.3%), and cerebrovascular accident (CVA) (1.7% of cases).

Regarding the qualitative variables in the patients’ group, they had a history of smoking (1.7%), diabetes (3.4%), angina pectoris (5.1%), cardiovascular intervention or CVA (1.7%), peripheral artery conditions (10.2%), coronary artery disease (CAD) in their 1st-degree relatives (5%), and CNS complications (8.3%), and no patient had a history of intermittent claudication. Further, in 78.3% of patients, the blood pressure was less than 140/90 mmHg, 26.7% of patients had a history of antihypertensive medication usage, only 15% had a history of premature ovarian failure (POF), and 18.3% reported a history of cardiovascular complications. Antinuclear antibody (ANA) was positive in 83.3% of the patients. All indicators were absent in the control group, except the CAD history in the 1st-degree relatives, which was 4.9%.

There was a significant difference in the history of taking antihypertensive medications (P = 0.04). There was also a significant difference in serum levels of IL-18 between patients and controls (P < 0.005). The mean of constraint-induced movement therapy (CIMT) was 0.396 ± 0.078 and 0.362 ± 0.080 for the patient and control groups, respectively (t = 1.92, P = 0.057).

Comparing SLEDAI between the two groups, only one significant difference was found, showing a higher disease activity in subjects with higher serum levels of IL-18 (P = 0.02). There was no significant correlation between the IL-18 serum level and the IMT of the carotid artery.

Regarding the patient group, there was no significant relationship (P = 0.133) between the serum level of IL-18 and the severity of the disease, dividing patients based on their SLEDAI score into three groups: < 5 (n = 30): 257.60 ± 54.01 pg/ml, 5-10 (n = 13): 427.46 ± 89.41, and > 10 (n = 17): 407.71 ± 71.99. No significant difference was found either between the IL-18 serum level and cardiovascular risk factors (Table 2).
Table 1. Comparison of quantitative variables in patients and controls (if present), and two groups of patients by the serum interleukin-18 (IL-18) level (if applicable)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients vs. controls (if applicable)</th>
<th>Comparison by serum IL-18 levels (patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient group (n = 60)</td>
<td>Control group (n = 30)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>28.80 ± 10.30</td>
<td>33.80 ± 9.10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.90 ± 4.70</td>
<td>24.40 ± 5.10</td>
</tr>
<tr>
<td>IMT (total) (mm)</td>
<td>0.39 ± 0.07</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>0.93 (0.30-3.60)</td>
<td>0.60 (0.31-2.50)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>182.00 ± 43.00</td>
<td>154.00 ± 50.00</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>135.00 ± 46.00</td>
<td>74.00 ± 38.00</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>121.00 ± 32.00</td>
<td>95.00 ± 43.00</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>45.00 ± 9.00</td>
<td>43.00 ± 5.00</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>336.93 ± 39.69</td>
<td>96.89 ± 16.15</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>4.50 (2.00-12.00)</td>
<td>-</td>
</tr>
<tr>
<td>Anti-dsDNA* (IU/ml)</td>
<td>107.00 (64.50-296.50)</td>
<td>-</td>
</tr>
<tr>
<td>C3 (mg/dl)*</td>
<td>48.00 (24.50-88.00)</td>
<td>-</td>
</tr>
<tr>
<td>C4 (mg/dl)*</td>
<td>22.00 (12.00-35.75)</td>
<td>-</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>84.40 ± 18.10</td>
<td>74.20 ± 18.30</td>
</tr>
<tr>
<td>Lymphocyte count*</td>
<td>5715.00 ± 2543.00</td>
<td>-</td>
</tr>
<tr>
<td>Platelet count*</td>
<td>231266.60 ± 85875.30</td>
<td>-</td>
</tr>
</tbody>
</table>

For non-normally-distributed variables, the Mann-Whitney test was applied and they were described by median and interquartile range (IQR) (Q1-Q3). For normally-distributed variables, the independent samples t-test was used for comparison and they were described by mean ± standard deviation (SD).

*These factors only were checked in patient group

BMI: Body mass index; IMT: Intima-media thickness; TG: Triglyceride; hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; FBS: Fasting blood sugar; IL-18: Interleukin-18; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: Double stranded deoxyribonucleic acid
Table 2. Comparison of classified cardiac risk factors and interleukin-18 (IL-18) serum level in patients with systemic lupus erythematosus (SLE)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable level</th>
<th>Percentage</th>
<th>Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>Less than 25</td>
<td>65</td>
<td>357 ± 56</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>More than 25</td>
<td>35</td>
<td>301 ± 47</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>Less than 200</td>
<td>30</td>
<td>340 ± 55</td>
<td>0.960</td>
</tr>
<tr>
<td></td>
<td>More than 200</td>
<td>70</td>
<td>336 ± 52</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>Less than 140</td>
<td>75</td>
<td>348 ± 51</td>
<td>0.797</td>
</tr>
<tr>
<td></td>
<td>More than 140</td>
<td>25</td>
<td>324 ± 55</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>Less than 50</td>
<td>23</td>
<td>358 ± 47</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>More than 50</td>
<td>77</td>
<td>268 ± 71</td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>Less than 110</td>
<td>5</td>
<td>432 ± 30</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>More than 110</td>
<td>95</td>
<td>332 ± 42</td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>Less than 3</td>
<td>33</td>
<td>268 ± 55</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>More than 3</td>
<td>67</td>
<td>373 ± 54</td>
<td></td>
</tr>
</tbody>
</table>

For non-normally-distributed variables, the Mann-Whitney test was applied and for normally-distributed variables, the independent samples t-test was used.

BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; FBS: Fasting blood sugar; hs-CRP: High-sensitivity C-reactive protein; SD: Standard deviation

Discussion

The present study showed significantly higher serum levels of IL-18 in patients with SLE compared to the healthy population, indicating the role of IL-18 as an inflammatory marker in patients with SLE.

Recent studies have demonstrated that the plasma level of IL-18 in patients with SLE is significantly higher than that in the healthy population and is associated with the disease activity and its clinical manifestations. In our study, the higher levels of serum IL-18 in patients with SLE could be considered as an inflammatory factor. However, even though the disease activity was also higher in higher IL-18 levels, no significant relationship between the level of IL-18 and subclinical atherosclerosis was found.

A systematic review by Jefferis et al. and Teixeira and Tam studied significantly increased levels of triglyceride (TG) and C-reactive protein (CRP) and decreased levels of high-density lipoprotein (HDL) in patients with higher serum levels of IL-18. Yamagami et al. also reported a significant positive relationship between the IL-18 level and age, BMI, TG, and high-sensitivity CRP (hs-CRP), and a negative correlation with fasting blood sugar (FBS), total cholesterol, and HDL. Tso et al. reported a difference in serum levels of TG between patients with a high and low serum level of IL-18. However, we found no such significant relationships between patients with low and high serum IL-18 levels. It could be because our subjects were in different stages of the disease, therefore, with different types and doses of anti-inflammatory medications. The lack of correlation between IL-18 and low-density lipoprotein (LDL) in this study can also be due to multiple factors such as differential disease duration, the severity of the disease, and the presence of immunosuppressive treatments.

As reported by Yamagami et al. and Correale et al., IL-18 has demonstrated a positive correlation with the carotid IMT, as an indicator of systemic atherosclerosis. Other authors have also reported a significant difference between the patients with SLE and the control group in terms of IMT. We also found such a significant difference for the both right lower and left lower carotid artery, where patients had a higher IMT. These findings suggested that IMT could be attributed as a subclinical atherosclerosis indicator in patients with SLE. However, no significant difference in IMT was found between the two groups of patients based on IL-18 level.

Other cytokines may play a role in the increased risk of premature atherosclerosis in patients with SLE. Race and genetics may also influence the inflammatory response leading to plaque formation.

One limitation of this study is to enroll patients after five months of disease. In future works, patients should be evaluated over a longer period.

Conclusion

The serum level of IL-18 was found to be significantly higher in patients with SLE. The level of IL-18 ≥ 280 was significantly correlated with the disease activity, but no significant relationship was found between the serum IL-18 and IMT and
subclinical atherosclerosis. Studies with a larger sample size and longer follow-up are recommended.

Acknowledgments
This study was part of a thesis, approved by the Ethics Committee of Mashhad University of Medical Sciences (#87601), and was supported by a grant from the Vice-Chancellor of Research of Mashhad University of Medical Sciences (#2260).

Authors’ Contribution
ZR: Designing the study, referring the patients, interpretation of the data, revising the intellectual content, final revision of the manuscript
MA: Writing the manuscript, gathering data, interpretation of data, revising the comments
NS: Revising the intellectual content, final revision of the manuscript
MS: Author of the primary draft of the manuscript and following up the procedure, analysis of data, final revision of the manuscript
RO: Gathering data, interpretation of data
SS: Gathering data, interpretation of data
HE: Analysis of data, final revision of the manuscript
MRA: Doing Doppler ultrasound and analysis of IMT, interpretation of data
AF: Revising the manuscript, interpretation of data, analysis of data
EA: Analysis of data

Conflict of Interests
Authors have no conflict of interests.

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http://arya.mui.ac.ir  15 Nov.
Serum interleukin-18 role in lupus patients


