Decreased Serum Levels of Macrophage Derived Cytokine and Matrix Metalloproteinase-9 are associated with Disease Activity in the Patients with Systemic Lupus Erythematosus

Hamada S Ahmad1*, Gamal Othman2, Sherief E Farrag1

1 Rheumatology and Rehabilitation Department, Mansoura faculty of Medicine, Mansoura University, Egypt
2 Biochemistry Department, Mansoura Faculty of Medicine, Mansoura University, Egypt

Corresponding author: Hamada S Ahmad, Rheumatology and Rehabilitation Department, Mansoura faculty of Medicine, Mansoura University, Egypt, Tel: 00966506251110; E-mail: drhamada1970@yahoo.com

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Abstract

Aim: The aim of our study was to assess serum concentration of macrophage derived cytokine (MDC) and matrix metalloproteinase-9 (MMP-9) in patients with active and inactive SLE and in healthy volunteers.

Subjects and Methods: The study included 40 patients with SLE and 40 healthy control. The serum level of MDC and MMP-9 were evaluated and compared between the patients and the controls. The association of the MDC and MMP-9 with disease activity, renal involvement, drug intake were also evaluated.

Results: SLE patients had significantly lower MDC and lower MMP-9 than control (p=0.001). SLE patients who had nephritis had significantly lower MDC and lower MMP-9 than patients without nephritis (p=0.003, p=0.004 respectively). The lupus patients with +ve anti-dsDNA had significantly lower MMP-9 and MDC in their serum than patients with –ve anti-dsDNA (p=0.032, p=0.024 respectively). MDC and MMP-9 were inversely correlated with SLEDAI (p=0.008, p<0.003 respectively). No significant association was found between MDC and MMP-9 with disease duration or current drug intake.

Conclusion: These findings indicate that MDC and MMP-9 were involved in SLE pathogenesis, and MDC could be a marker for active SLE. Our findings indicated that MDC and MMP-9 seemed to be markers of renal involvement.

Introduction

Systemic lupus erythematosus (SLE) is a common systemic inflammatory autoimmune disease. The immune response in SLE can cause chronic inflammation leading to irreversible damage to organ systems [1]. In recent years, due to improved ability for earlier diagnosis and the introduction of new treatments, the survival of patients with SLE has increased significantly. However, because of prolonged life expectancy, SLE patients become exposed to an increased risk of morbidity related to the sequelae of disease activity, side effects of medications, and comorbid conditions, such as lupus nephritis (LN) [2,3]. The course of SLE is unpredictable, often with periods of flares and remissions. Therefore, identification of markers that accurately reflect activity status of the disease and organ involvement remains critical in the management of the disease.

SLE is characterized by many immunologic abnormalities, such as polyclonal activation of circulating B cells that generate a large amount of auto reactive antibodies. SLE is also characterized by T lymphocyte abnormalities and immune complex (IC) deposition. Chemokines and their receptors play a crucial role in the pathogenesis of SLE [4,5]. The differentiation of Th1 cell to Th2 cell is enhanced if monocyte/macrophage releases IL-10, while the differentiation to Th1 cell is enhanced if they release IL-12. Therefore, it seems that the cytokines that are derived from monocyte/macrophage play a key role in SLE pathogenesis. Moreover it was suggested that SLE is a Th2 dominant disease [4].

Macrophage derived cytokine, a CC chemokine, is a potent chemoattractant which activated Th2 lymphocytes via the chemokine receptor CCR4, and its receptor CC chemokine receptor 4 (CCR4) preferentially expressed on Th2 cells [6]. The production of MDC is down regulated by Th2 type cytokines IL-4 and IL-13. This may play a role in maintaining Th1/Th2 balance [7]. B-lymphocytes, macrophages and dendritic cells, all produce MDC constitutively, while NK cells, monocytes, and CD4+ T lymphocytes produce MDC upon stimulation. As many immune cells can produce MDC and MMP-9 can chemoattract many immune cells so, MDC may be essential in SLE pathogenesis.

Matrix metalloproteinase (MMP)-9 is involved in inflammation and immune system dysfunctions and has been implicated in angiogenesis [8,9]. The involvement of angiogenesis and angiogenic factors in pathogenesis of SLE has been previously suggested [10].

The aim of this study was to assess serum concentrations of MDC and MMP-9 in patients with active and inactive SLE and in healthy volunteers.
Methods

Subjects

This study was conducted on 40 consecutive patients with SLE (33 females and 7 males) who were attending the outpatient clinic of Rheumatology and Rehabilitation in different areas in Saudi Arabia, between February 2014 and August 2015. All patients met the SLICC SLE 2012 criteria for diagnosis of SLE [11]. The age of the patients ranged from 18 to 47 years and their disease duration ranged from 3 to 12 years. The study included also 40 apparently healthy control volunteers who are age and sex-matched with the patient group.

The clinical assessment of the patients included interview for history taking to report demographic and clinical data regarding age, sex, and duration of disease. The medical history and drugs used for the treatment were obtained during the interview and from the medical files of the patients. Lupus disease activity was calculated using the SLE Disease Activity Index (SLEDAI) [12].

Three milliliters of venous peripheral blood were collected from each subject, centrifuged to get serum, and then stored at -80°C. ELISA measured the concentrations of anti-dsDNA, MDC and MMP-9. ELISA kits for HGF, MDC and MMP-9 were purchased from R&D Systems Inc. (Catalog No: DMD00, DMP00). Assays were performed according to the manufacturer's instructions.

Statistical analysis

All statistical analysis was performed using SPSS for windows version 20.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ± standard deviation (SD), while categorical data were expressed in number and percentage. Continuous data were checked for normality and equality of distribution, prior to any analysis being performed. Comparison between the continuous was tested using the Student's t test while comparison between categorical data was tested using chi square test. Correlation co-efficient test was used to evaluate relationship between continuous variables. All analysis was 2-tailed. Statistical significance was set at p<0.05.

Results

This study included 40 SLE patients (33 females and 7 males) and 40 control volunteers. No significant difference in the age and gender between SLE patients and controls was found. On the other hand, SLE patients had significantly lower MDC than controls (447.1 ± 93.7 vs 618.9 ± 20.2 pg/ml respectively, p<0.001). Additionally, the serum level of the MMP-9 was significantly lower in the SLE patients as compared to the controls (108 ± 36.2 vs 336.9 ± 59 pg/ml respectively, p<0.001) (Table 1).

Of the patients with SLE, 20 had LN. SLE patients who had nephritis had significantly lower MDC than patients without nephritis (407.6 ± 95.8 vs 492.5 ± 71.4 pg/ml respectively, p=0.003) and lower MMP-9 (93 ± 38 vs 125.4 ± 26.3 ng/ml respectively, p=0.004) (Table 2).

Anti-dsDNA was positive in 26 (65%) of the SLE patients participated in this study while 14 (35%) were anti-dsDNA negative. Patients with +ve anti-dsDNA had significantly lower serum level of MDC than those with –ve anti-dsDNA (p=0.032). Similarly, the serum level of the MMP-9 was significantly lower in the SLE patients with +ve anti-dsDNA than those with –ve anti-dsDNA (p=0.024) (Table 3).

MDC and MMP-9 were inversely correlated with SLEDAI (p=0.008 and p=0.003 respectively) (Figures 1 and 2). On the other hand, MDC and MMP-9 did not correlate significantly with disease duration (r=−0.016; p=0.922 and r=−0.010; p=0.909 respectively) (data not shown).

As shown in Table 4, no significant association was found between the serum levels of MDC and MMP-9 and the current drug intake.
Discussion

The main finding of the current study is that serum level of MDC in the SLE patients was significantly lower than the controls and the MDC serum level was inversely correlated with SLEDAI score in the SLE patients and was significantly lower in the patients with +ve anti-ds DNA than patients with –ve anti-dsDNA. Moreover, MDC was significantly lower in the patients who had LN than in the patients without LN. Our results were in agreement with that of Liu et al. [13] who reported that serum MDC is significantly lower in patients with SLE than in controls and significantly lower in active lupus patients than patients with inactive lupus. In agreement with our results, Robak et al. [14] reported that the concentrations of MMP-9 were unexpectedly lower in lupus patients when compared with control groups and detected lower concentration of MMP-9 in lupus patients with active disease when compared with lupus patients with inactive disease. In agreement with the current results, Liu et al. [15] found lower levels of MMP-9 in SLE patients in comparison with healthy subjects. In addition, they observed similar findings of lower concentration of MMP-9 in serum of patients with active SLE as compared with inactive disease. On the other hand, Faber-Elmann et al. [16] found the increased activity of MMP-9 in serum of patients with SLE than in controls. They also did not demonstrate any correlation between serum MMP-9 level and the Systemic Lupus Erythematosus Disease Activity Index.

Makowski and Ramsby [17] examined a correlation between MMP-9 concentration and anti-dsDNA levels showing reverse correlation with anti-dsDNA, which is a specific marker of SLE. Similarly, Liu et al. [15] observed lower concentration of MMP-9 in patients with LN as compared with patients with SLE without renal impairment. These observations are consistent in patients with active SLE as compared with patients with inactive disease. These findings were in agreement with our results.

However, the lower serum levels of MDC and MMP-9 in lupus patients, especially with active disease detected either in the current work or in studies performed by other authors together with the finding that the peripheral blood mononuclear cells (PBMC) in patients with SLE form and secrete more MMP-9 than their healthy volunteers seem conflicting [9]. Moreover, the most increased pro-MMP-9 activity inside the PBMCs was identified for relapsed SLE subgroup. It can be assumed that in lupus patients, more MDC and MMP-9 are transported from blood to the lipoid tissues, especially blood vessels in the more active lupus patients. Mawrin et al. [18] identified that MMP-9 could be detected in vessel walls of nerves in patients with SLE and peripheral neuropathy while in healthy subjects they were not found. The authors suggest that up-regulation of MMP-3 and MMP-9 within the vessels may be responsible for vascular damage seen in SLE.

Table 4: Association of MDC and MMP-9 between with current drugs used in the SLE patients.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No current use of the drug</th>
<th>Current use of the drug</th>
<th>P</th>
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<tbody>
<tr>
<td>Corticosteroids</td>
<td></td>
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</tr>
<tr>
<td>MDC</td>
<td>492.4 ± 65.8</td>
<td>435.8 ± 96.9</td>
<td>0.128</td>
</tr>
<tr>
<td>MMP-9</td>
<td>123.2 ± 24</td>
<td>104.3 ± 38</td>
<td>0.19</td>
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<tr>
<td>Azathioprine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>465 ± 78.2</td>
<td>437.5 ± 101.1</td>
<td>0.382</td>
</tr>
<tr>
<td>MMP-9</td>
<td>115.8 ± 29.2</td>
<td>103.9 ± 39.3</td>
<td>0.33</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>443.4 ± 93.7</td>
<td>468.1 ± 99</td>
<td>0.558</td>
</tr>
<tr>
<td>MMP-9</td>
<td>106.8 ± 36.4</td>
<td>115.4 ± 37.5</td>
<td>0.597</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td></td>
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</tr>
<tr>
<td>MDC</td>
<td>484.6 ± 73.3</td>
<td>436.2 ± 97.1</td>
<td>0.176</td>
</tr>
<tr>
<td>MMP-9</td>
<td>122 ± 28.4</td>
<td>104 ± 37.5</td>
<td>0.191</td>
</tr>
<tr>
<td>Biologics</td>
<td></td>
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</tr>
<tr>
<td>MDC</td>
<td>446.3 ± 92.4</td>
<td>454.5 ± 120</td>
<td>0.87</td>
</tr>
<tr>
<td>MMP-9</td>
<td>107.5 ± 35.6</td>
<td>113.3 ± 47</td>
<td>0.763</td>
</tr>
</tbody>
</table>
SLE tends to damage multiple organs, including the kidney. Our study showed that the serum levels of MDC and MMP-9 were significantly lower in lupus nephritis patients than those without renal involvement. These findings indicated that MDC and MMP-9 might be involved in the pathogenesis of LN and seemed to be markers of renal involvement. Garcia and his colleagues [19] reported, they found that MDC was critically involved in the development at anti-GBM GN from acute glomerular injury to irreversible tissue damage. It was previously reported that MDC induced strong migratory response in inflammatory cells prepared from the nephritic glomeruli [20].

Conclusion

These findings indicate that MDC and MMP-9 were involved in SLE pathogenesis, and MDC could be a marker for active SLE. Our findings indicated that MDC and MMP-9 seemed to be markers of renal involvement.

References