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Title: The Serum Level of Midkine in Patients with Multiple Sclerosis and Neuromyelitis Optica

Running title: Midkine Level in MS and NMO

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Highlights

- The mean of MK level in MS patients is higher than NMO patients and healthy control group.
- The level of MK is higher in NMO group compared with the healthy one.
- The healthy subjects have the lowest mean of MK level compared with the other groups.
- Sex and age have no significant association with the MK level.

Plain Language Summary

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are two distinct diseases with the similar Clinical symptoms, so it's necessary to distinguish these disorders from each other. Although there are many studies to find biomarkers for this goal, only a few of them validated for routine clinical practice. Due to the inflammatory nature of MS and NMO, our research group claims to evaluate the utility of midkine (MK) as a heparin-binding growth factor that implicated in inflammation. Our results demonstrated that MS patients have higher MK level means in comparison to NMO patients and the healthy participants. Also, the sex and age have no significant association with the MK level. Therefore, MK may be a reliable biological marker to considerate in differentiating of MS and NMO.
ABSTRACT

Introduction: Midkine (MK), a heparin-binding growth factor, is involved in neurological diseases by mediating the inflammatory responses through enhancing the leukocyte migration. The present study assesses the serum concentration of this growth factor among newly multiple sclerosis (MS) and neuromyelitis optica (NMO).

Methods: The present research, as a cross-sectional study, which performed at Isfahan University of Medical Sciences. All samples were selected from patients who visited Kashani and Alzahra hospital during two years 2014-2016. The MK level was assessed in 80 new cases of MS, 80 NMO patients and 80 healthy subjects. After collecting blood sera samples, MK serum level was measured using the ELISA. Data were analyzed by SPSS statistical software.

Results: The mean of MK level was 1038.58 ± 44.73 pg/ml in MS group, which was significantly higher compared with the mean of MK level in NMO (872.62 ± 55.42 pg/ml) and control groups (605.02 ± 9.42 pg/ml).

Conclusion: Overall, these results demonstrated MK plays a prominent role in inflammatory reactions and also in neuroautoimmune diseases, especially in MS. So, the MK level may be applied to earlier diagnosis and also prevention of disease progression by using a special inhibitor.

Keywords: Midkine, Multiple Sclerosis, Neuromyelitis Optica
Introduction

Multiple Sclerosis (MS) is a chronic autoimmune neurological disease of the central nervous system (CNS) (1) that leads to inflammatory demyelination of axons (2). Neuromyelitis Optica (NMO, Devic’s syndrome) is another immune-mediated chronic inflammatory disorder of the CNS that predominantly targets the optic nerve and spinal cord (3). Distinguishing of the two diseases has become increasingly important because the treatments differ and interferon-beta (IFN-β) can actually make NMO worse (4). NMO usually can be differentiated from MS by the presence of a serum immunoglobulin (Ig)G autoantibody (NMO-IgG) against the astrocytic water channel aquaporin-4 (AQP4) (5). Although it constitutes a sensitive and highly specific biomarker, it is challenging to diagnose NMO patients that are negative for this marker from MS patients (6). However, despite many studies which claim to have discovered markers for potential utility only a few clinically useful biomarkers have been successfully validated for routine clinical practice (7).

Midkine (MK) also known as heparin-binding growth factor is a Cytokine implicated in a variety of pathological conditions ranging from neurodegenerative diseases to inflammation and cancer (8). MK expression has been shown to be enhanced during induction and progression of experimental autoimmune encephalitis (EAE), the animal model of MS (9). According to the need for inflammatory marker available in MS and NMO diseases, the current study aimed at investigating the serum levels of MK in patients with MS and NMO and also healthy controls.

Methods

Subjects and Samples

In this cross-sectional study, subjects were selected among newly diagnosed MS cases on the basis of McDonald criteria (2010) and the patients with newly diagnosed NMO according to the Wingerchuk criteria (2006) who were referred to the MS clinics of Alzahra and Kashani hospitals in Isfahan. They were the patients who had no concomitant inflammatory diseases and no history of steroid or immunosuppressive medication. Individuals with infectious disease, blood abnormalities and autoimmune disease were excluded. The control group was chosen from non-first-degree relatives of the patients as well as hospital staff who did not have any neurological problem. Each group consisted of 80 patients from both sexes, who were between 16-55 years old.

Blood samples were taken from all subjects in the case and control groups. After centrifugation, serum specimens isolated and stored frozen at -20°C until further analyses. The serum MK level was measured by enzyme linked immunosorbent assay (ELISA) using a MK kit (Glory Science, USA). Then the concentration of MK in the samples was determined by comparing the optical density (OD) of the samples in 450 nm to the standard curve.

Statistical Analyses
All analyses were performed using IBM SPSS Statistics Version 25. The significance level was 0.05 and the data were expressed as mean ± SD. One-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test was used to compare the mean of MK level among the three groups.

**Results**

The characteristics of the study cases are summarized in Table 1. All groups were matched in terms of age and gender therefore there were no significant difference between the three groups (P > 0.05)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MS (n =80)</th>
<th>NMO (n = 80)</th>
<th>Control (n = 80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.60 ± 9.91</td>
<td>34.77 ± 10.26</td>
<td>34.83± 10.07</td>
<td>0.687</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>26 (32.5%)</td>
<td>28 (35%)</td>
<td>30 (37.5%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>54 (67.5%)</td>
<td>52 (65%)</td>
<td>50 (62.5%)</td>
</tr>
</tbody>
</table>

Main results are summarized in Table 2. There was a statistically significant difference between the mean of MK level among the three groups so that the MS group had the highest mean compared to the NMO and control groups (P < 0.0001). The data have been shown in figure 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>1081.04 ± 405.56</td>
<td>0.316</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1018.14 ± 399.59</td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>Age (years)</td>
<td>&lt; 25</td>
<td>1066.99 ± 237.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25-35</td>
<td>1008.74 ± 458.86</td>
</tr>
<tr>
<td>Age (years)</td>
<td>MK</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>863.12 ± 333.92</td>
<td>0.474</td>
<td></td>
</tr>
<tr>
<td>25-35</td>
<td>970.28 ± 596.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 35</td>
<td>808.33 ± 481.45</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>MK</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>768.96 ± 304.37</td>
<td>0.431</td>
</tr>
<tr>
<td>Female</td>
<td>928.44 ± 567.95</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>MK</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>614.87 ± 86.39</td>
<td>0.392</td>
</tr>
<tr>
<td>25-35</td>
<td>585.95 ± 77.13</td>
<td></td>
</tr>
<tr>
<td>&gt; 35</td>
<td>613.93 ± 87.99</td>
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</tr>
</tbody>
</table>

Figure 1 The mean of MK level in groups.
By pairwise comparisons of three groups, Tukey's test revealed that the mean of MK level was 163.523 ± 66.59 in MS group, which was significantly higher compared with NMO group ($P < 0.0001$). Also, it was observed that the mean of MK level was significantly higher in MS group than control group (433.565 ± 66.59) ($P < 0.039$) and NMO than control group (270.042 ± 66.59) ($P < 0.0001$).

No significant difference was reported among the three studied groups in the mean of MK level according to age and sex ($P > 0.05$), as presented in Table 2.

Brain and spinal cord MRI were displayed in Figures 2.
Figure 2 (A) and (B) The MRI brain of 26 years female with MS. (C) The MRI spinal cord of 14 years female with NMO.

**Discussion**

Based on our results, despite the fact that MS and NMO are both autoimmune disorders and have the same clinical manifestations, it seems that inflammatory responses are greater in patients with MS. Moreover, age and sex have not any considerable association with MK levels. Increased levels of MK in autoimmune and inflammatory diseases have been shown in prior reports.

Our result was consistent with Salama et al. which reported that MK levels in patients with Alzheimer’s disease, as another neurological disease, is more than healthy persons (10). Plasma MK was elevated in patients with systemic lupus erythematosus which was correlated with IL-17 (11). In rheumatoid arthritis (RA) patients, high MK levels were found to implicated in the pathogenesis of RA and was associated with IL-6 and IL-8 (12, 13). In acute and chronic kidney injuries like ischemia/reperfusion injury and diabetic nephropathy, the expression of MK was upregulated and promotes migration of neutrophils and macrophages, hence MK deficiency may show less renal damage (14, 15). In inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn's disease serum MK level was positive associated with disease activity index (16, 17).
Our previous study indicated that MS patients received IFN-β treatment had the lower level of MK and IL-23 than healthy controls which has a controversy with our recent research, performed on newly diagnosed MS patients. It reveals that the serum level of this marker increases in neurodegenerative diseases in the early onset of them. So that MK might be helpful for the prognosis of the disease stage (18).

It can be concluded that the MK level in newly diagnosed MS and NMO patients was more than healthy individuals. The higher MK level might lead to inflammatory cytokine induction and the development of neuronal cells and therefore disease progression. Although we cannot confirm it only with a cross-sectional survey, this research can be a beginning for cohort assessment and further investigation.

**Ethical Considerations**

**Compliance with ethical guidelines**

Written informed consent was obtained from all participants and the research protocol was approved by the medical ethics committee of Isfahan University of Medical Sciences.

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**Authors contributions**

All authors contributed in preparing this article.

**Conflict of interest**

The authors declared no conflict of interest.

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