SHORT PAPER

Correlation of Midkine Serum Level with Pro- and Anti-Inflamatory Cytokines in Multiple Sclerosis

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ABSTRACT

Background: Midkine (MK) is a heparin-binding growth factor with promoting effects in inflammatory responses through enhancing leukocytes migration. **Objective:** To study the correlation between MK serum levels and concentration of inflammatory cytokines in Multiple Sclerosis (MS) patients. **Methods:** We evaluated the MK level and its relationship with inflammatory cytokines (IL-17 and IL-23) and anti-inflammatory ones (IL-10 and TGF-β) in multiple sclerosis (MS) patients. The serum concentrations of MK and cytokines were assessed by ELISA in 32 MS patients in comparison with 32 healthy subjects. **Results:** Our data showed that the MK concentration in MS patients is lower than healthy controls (341.15 ± 40.71 Pg/ml vs. 620.15 ± 98.61 Pg/ml, respectively, p=0.015). We also observed a significant decrease in IL-10, IL-23, and TGF-β cytokine levels in MS patients. There was a significant correlation between MK and IL-23 concentrations in our study (r = +0.829, p≤ 0.001). **Conclusion:** These results confirm a role for MK in inflammatory reactions in MS.

***Shaygannejad** V, et al. Iran J Immunol. 2014; 11(2):134-138*

Keywords: IL-10, IL-17, IL-23, Midkine, Multiple Sclerosis, TGF-β

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune-mediated disease of the central nervous system (CNS) that occurs in genetically susceptible individuals (1). Numerous potential biomarkers have been considered for determining the trend of illness and also for response to the treatment of MS, but their clinical success has not yet been fully confirmed (2).

Midkine (MK) is a heparin-binding growth factor with various effects in different tissues of the body, including an important role in induction of oncogenesis, inflammation and restoration of tissues. MK induces inflammation via increasing leukocytes migration, induction of chemokine synthesis and preventing development of regulatory T cells (3). An important role for MK in inducing experimental autoimmune encephalitis (EAE) has been confirmed by disease attenuation in Mk deficient animals due to increasing regulatory T-cell (T_{reg}) population in peripheral lymphatic glands, also reducing activated T helper type 1 (Th1) and Th17 cell populations in these animal models (4).

Th17cells are a subset of CD4+ T cells with the ability of secreting inflammatory cytokines of IL-17 family and play a critical role in development of inflammatory responses (5). They have been shown to be the most important subset of T helper cells in pathogenesis of MS and EAE (4,6). *In vitro* studies suggest that IL-23 may provide a survival signal for already differentiated Th17 cells (1,5).

Another subset of CD4+ T cells namedregulatory T cells (T_{reg}), are considered to have a prominent role in controlling MS disease promotion, mainly through secreting anti-inflammatory cytokines such as IL10 and TGF-β (7). Onset of several autoimmune diseases is associated with lack of TGF-β1 expression or defect in signaling pathways in T cells related to this cytokine (7,8). Moreover, TGF-β has an important role in inducing regulatory phenotype and their differentiation to T_{reg} cells (4).

MK has been suggested to be used as a disease prognosis predictor of cardiac events in patients with chronic heart failure (9). Also a significant relationship between MK serum levels and EAE severity has been reported in animal studies (4,10,11), while such relation has not yet been studied in human subjects. The present study was designed to measure the serum MK levels and correlate it with the above mentioned Th17 and Treg related inflammatory and anti-inflammatory cytokines in MS patients. Present research aimed to address correlation between MK serum level and concentration of anti-inflammatory and inflammatory cytokines in MS patients.

MATERIALS AND METHODS

Human Subjects. Plasma samples were collected from 32 MS patients attending to neurology ward, Isfahan Kashani Hospital, Iran, between 2012-2013 which were kept at -80°C, and 32 age- and sex-matched healthy controls. All patients were definitely diagnosed with MS disease according to the McDonald criteria. The study was approved by the Ethics Committee of Isfahan University of Medical Sciences and all subjects signed an informed consent. 32 MS patients were included in this study of which 19 (59.4%) had relapsing remitting (RRMS), 11 (34.4%) had secondary progressive (SPMS) and 2 (6.2%) had primary progressive (PPMS) MS. The mean disease duration was 4.68 ± 3.81 years and the average EDSS (Expanded Disability

Status Scale) score at the time of sampling was 3.2 ± 2.09. The sampling among RRMS patients was performed in relapse phase.The mean age in MS and control groups was 32.4 ± 5.6 and 31.8 ± 4.3 years, respectively. All patients were under treatment with Interferon-β except primary progressive patients.

Cytokine levels were evaluated by enzyme linked immunosorbent assay (ELISA) method using MK and IL-23 assay kits (Glory Science, USA), and IL-10 , IL-17 and TGF-β assay kits (Boster, wuhan, china). Selected wave length to read the ELISA plates and measure the absorbances was 490 nm.

Statistical Analyses. The data were expressed as mean ± SEM (standard error of the mean). Student's *t-test*, ANOVA and Tukey's post hoc test were used where needed. Spearman correlation test was used to investigate the relationship between MK and other cytokine levels and disease severity and progression. $p<0.05$ was considered statistically significant. Data were analyzed by SPSS software 18.00.

RESULTS

MK levels in MS patients was significantly lower than healthy controls (341.15 ± 40.71 pg/ml vs. 620.15 ± 98.61 pg/ml, respectively, p=0.015). Plasma levels of IL-10, TGF-β and IL-23 were also significantly decreased in MS patients (64.34 ± 15.56, 976.46 ± 132 and 144 ± 19.31 pg/ml, respectively) compared to healthy controls (615.93 ± 49.13, 1659.43 ± 258 and 212.65 ± 31.72 pg/ml, p=0.001, p=0.02 and p=0.04, respectively, Figure 1). However, elevation of IL-17 (18.65 ± 1.2 pg/ml) in plasma of MS patients in comparison with healthy controls (17.53 ± 3.71 pg/ml) was not significant (Figure 1). Among all cytokine/chemokine and clinical parameters analyzed in the relapse phase the only observed significant relationship was a positive correlation between plasma levels of IL-23 and MK ($r = +0.829$, $p \leq 0.001$). There was no correlation between plasma level of MK and MS severity or disease type.

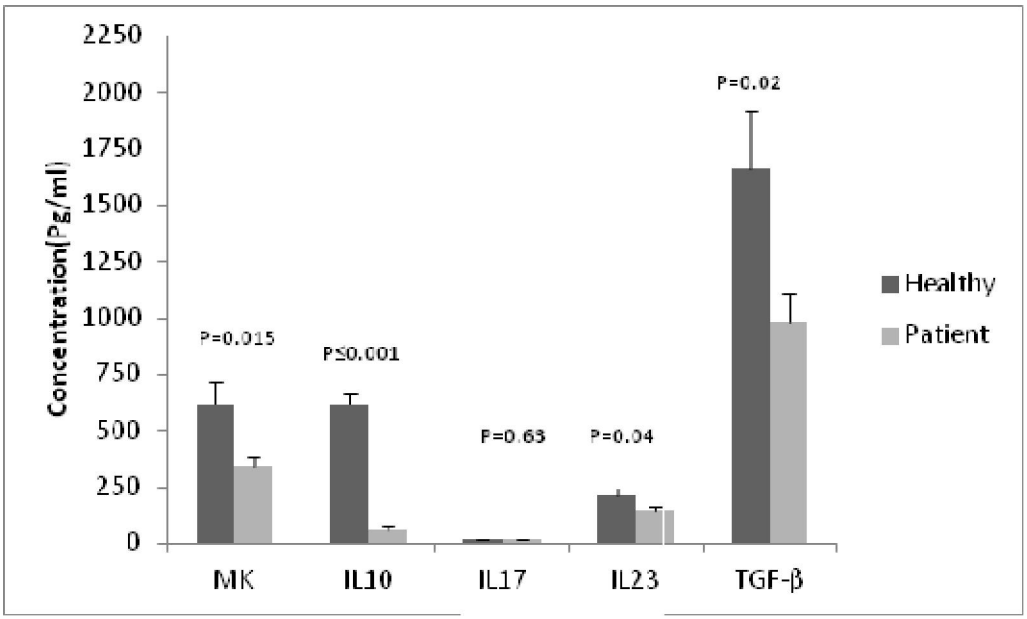

Figure1. Comparison of serum cytokine levels between healthy controls and MS patients.

DISCUSSION

Different types of immune cells and soluble mediators contribute to the complex mechanism underlying the onset and progression of MS, which is characterized by infiltration of auto-reactive T cells and activation of microglia, in the CNS (3,5,8). MK promotes inflammatory responses by enhancing the migration of inflammatory leukocytes (11-13), increasing chemokine synthesis (13), and suppressing regulatory T cells induction (4). However, the precise immunological function of MK remains to be elucidated. The critical role of Th17 cells in development of auto-immune disorders like MS has been revealed in recent years (14). It is supposed that IL-23 is an essential cytokine in expansion and survival of Th17 population (15).

Increased levels of MK in plasma of patients during inflammatory diseases such as Alzheimer's disease (16) and rheumatoid arthritis (17), also in animals with EAE (4) have been shown in previous reports. In the present study, we assessed plasma levels of this protein in MS patients in relapse phase in comparison with healthy subjects and explored its possible relation with the concentration of other mentioned cytokines and also the disease clinical parameters. Our results indicated a decreased MK level in MS patients compared with healthy controls. This is in controversy with the results of animal studies which have shown MK elevation in MS experimental models (4). The same discrepancy was also seen in comparison of IL-23 and IL-17 levels in MS patients with Healthy subjects. These inconsistencies may be due to the effects of IFN-β treatment in studied patients. Accordingly, several studies have demonstrated that IFN-β may exert its effects on MS by reduction of IL-17-associated immunity (18,19). A study has reported that administration of IFN-β decreases IL-23 production and its decrease could be responsible for indirect inhibition of Th17 (18).

The loss of Treg cell function which are the main producers of anti-inflammatory cytokines, TGF-β and IL-10, gives rise to various autoimmune diseases including MS (19-24). Our results showing a decrease in concentrations of both TGF-β and IL-10 cytokines in MS patients is in agreement with the previous reports.

A considerable result of the present study was the observed significant direct correlation between MK and IL-23 levels. This observation strongly confirms the inflammatory effects of MK and it suggest that the MK inflammatory functions may be exerted by mediating some influences on the production of IL-23 which indeed acts as the main cytokine in promoting the expansion and the survival of Th17 cells (25,26).

In conclusion our results suggest that midkine can play an indirect role in promotion of inflammatory reactions in MS disease. Also it should be considered that IFN-β therapy may exert its alleviating effect through decreasing MK production. More detailed studies in this regard are needed.

ACKNOWLEDGEMENTS

This article is extracted from thesis of medical students and the study wassupported by Isfahan University of Medical Sciences grant no 190126. We would like to thank all patients who participated in present study.

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