Multiple Sclerosis and Related Disorders 15 (2017) 11–14

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Investigation of the prognostic value of adipokines in multiple sclerosis

Arzu Çoban[a,*], Berna Düzel[b], Erdem Tüzün[c], Yusuf Tamam[b]

[a] Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
[b] Department of Neurology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey
[c] Department of Neuroscience, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey

ARTICLE INFO

Keywords:
Multiple sclerosis
Adipokine
Disability
Adiponectin
MCP-1
TNF-α

ABSTRACT

Background: Adipokines may be involved in multiple sclerosis (MS) as well as other inflammatory diseases. This study aimed to analyze the value of serum adipokine levels as biomarkers in determining the clinical progression of MS.

Methods: A total of 90 subjects including 40 healthy individuals and 50 MS patients [24 with classical clinical course of MS (C-MS), 26 with benign MS (B-MS)] were recruited for this study. The levels of serum adipokines and inflammatory mediators were measured using immunoassay methods.

Results: The levels of adiponectin, MCP-1, TNF-α and IL-6 were significantly higher in C-MS patients compared with B-MS patients and healthy controls. Only adiponectin and MCP-1 levels remained significantly high after Bonferroni correction. Adiponectin, MCP-1 and TNF-α levels showed a modest correlation with expanded disability status scale (EDSS) scores, which disappeared after Bonferroni correction.

Conclusions: Our findings suggest the potential role of adipokines in pathogenesis and clinical progression of MS. Adiponectin and MCP-1 might potentially serve as prognostic biomarkers in MS.

1. Introduction

Multiple sclerosis (MS) is the most common cause of neurological disability in young people and middle-aged adults. It has remarkable heterogeneity regarding clinical course, immunological features and pathogenesis (Lassmann et al., 2001). Although, the cause of MS is still fully unknown, there is consensus that immune system plays a critical role in the pathogenesis of MS (Bennett and Stüve, 2009; Graber and Dhib-Jalbut, 2011; Kasper and Shoemaker, 2010). As demonstrated in a few recent studies, adipose tissue as producer of adipocytokines including leptin, resistin and adiponectin may play an important role in MS pathogenesis (Emamgholipour et al., 2013; Frisullo et al., 2004; Guzik et al., 2006; Kraszula et al., 2012; Largo et al., 2007; Meier and Gressner, 2004).

Leptin is a cytokine secreted by adipose tissue and it is a potent modulator of immune responses (Otero et al., 2005). It affects both innate and acquired immunity. In innate immunity, leptin activates proliferation of monocytes, enhances phagocytosis activity of macrophages and production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL) − 6 and IL-12. In acquired immunity, leptin stimulates proliferation of naive T cells, and promotes memory T cell differentiation toward a T-helper 1 (Th1) profile, producing pro-inflammatory cytokines such as interferon gamma (IFNγ) and IL-2. Leptin also suppresses the production of the Th2cytokines IL-4 and IL-10 and thus modulates immune response toward a pro-inflammatory profile (Batocchi et al, 2003; Bernotiene et al., 2006; Guzik et al., 2006; Hasenkrug, 2007; Matarese et al., 2008; Meier and Gressner, 2004; Procaccini et al., 2015; Versini et al., 2014). Analysis of transcriptional profiling in MS lesions revealed that leptin expression is increased at the site of inflammation in brain (Robinson et al., 2003). Frisullo et al. noted an increased leptin level in the active phase of relapsing-remitting multiple sclerosis (RRMS) in untreated patients as compared to controls (Frisullo et al., 2004).

Resistin is a protein originating from monocytes and macrophages, and to a lesser extent, from adipocytes (Guzik et al., 2006). High concentrations of resistin were found in obese subjects, mostly during acute inflammatory reactions and after stimulation with some cytokines, i.e. TNF-α, IL-1, IL-6 (Bokarewa et al., 2005; Karbowska et al., 2009; Meier and Gressner, 2004; Tilg and Moschen, 2006).

While leptin and resistin have pro-inflammatory activity, adiponectin has been consistently shown to be an important anti-inflammatory factor (Koerner et al., 2005; Meier and Gressner, 2004; Natarajan et al., 2015). It inhibits the activation and proliferation of T and B lymphocytes and phagocytic activity of macrophages, as well as synthesis of pro-inflammatory cytokines (IL-6, TNF-α, INF-γ), and induces production of anti-inflammatory cytokines, such as IL-10 and IL-1 (Guzik et al.,

* Corresponding author.
E-mail address: arzucoban2002@yahoo.com (A. Çoban).

http://dx.doi.org/10.1016/j.msard.2017.04.006
Received 29 December 2016; Received in revised form 17 March 2017; Accepted 19 April 2017

2006; Meier and Gressner, 2004; Tilg and Moschen, 2006).

To investigate whether, adipokines are involved in clinical progression and disability in MS, we compared serum levels of several adipokines in MS patients with a benign or classical clinical course. Furthermore, potential correlations between adipokine levels and disability scores were investigated.

2. Material and Methods

2.1. Subjects

The study included 50 patients with MS [24 MS patients with a classical clinical course (C-MS), 26 MS patients with a benign clinical course (B-MS)] and 40 age-and gender-matched healthy individuals. Patients were diagnosed with RRMS according to the McDonald criteria (Polman et al., 2011). Patients with more than ten years of disease duration and expanded disability status scale (EDSS) score of ≥3.0 were considered C-MS. Patients with more than ten years of disease duration and EDSS score of < 3.0 were considered B-MS. The clinical and demographic information, EDSS scores and body mass indexes (BMIs) of all participants were recorded. All patients were under remission during blood sample collection and none of the patients had received immunosuppressive or immunomodulatory therapy for at least one month prior to sample collection. Primary progressive and secondary progressive MS patients were not included. All sera were collected between 8:00–10:00 A.M. and stored at −80 °C. The study was approved by the local ethics committee and all individuals gave written informed consent.

2.2. ELISA

Serum levels of leptin, resistin, adiponectin, MCP-1, IL-1β, IL-6, IL-8 and TNF-α were determined by immunoassay (ELISA) using a R & D System kit (R & D System, St Charles, MO, ABD) in the direction of manufacturer's instructions. The results were obtained as OD values and were converted to mg/L, ng/mL, ng/L or pg/mL with the help of the curves generated from the OD values of the standards of each parameter.

2.3. Statistical analysis

Adipokine levels were compared among C-MS, B-MS and healthy control groups using Kruskal-Wallis test and Dunn's post-hoc analysis, whereas age and BMI values were compared with ANOVA. Numerical variables were reported as the mean ± standard deviation. Appropriate parametric (Student *t*-test) and nonparametric analysis (Mann-Whitney U and Chi-square test) methods were used for the comparison of the clinical characteristics of the MS patient subgroups. Spearman correlation was used to investigate the correlation between adipokine levels with demographic and clinical characteristics of the MS patient groups. Differences were considered statistically significant at level of $p < 0.05$. Bonferroni's correction for multiple testing was used for cytokine statistics.

3. Results

Demographic and clinical characteristics of MS patient subgroups (C-MS and B-MS) are presented in Table 1. There were no significant differences between the two patient groups regarding age, sex, body mass index (BMI), EDSS score, MS duration, MS age of onset and the dominant clinical finding in the first episode (Table 1).

Serum levels of adipokines were compared between MS patient subgroups and healthy control group (Fig. 1). Adiponectin level was significantly higher in C-MS patients compared with B-MS patients and healthy controls ($p < 0.001$). The level of leptin was increased in C-MS and B-MS patients compared with healthy controls ($p = 0.017$). There was not statistically significant difference between C-MS and B-MS patients. The levels of MCP-1 ($p = 0.004$), TNF-α ($p = 0.013$) and IL-6 ($p = 0.023$) in the C-MS group were significantly higher than those of B-MS and healthy control groups. Notably, in TNF-α and IL-6 comparisons, the statistical significance did not cease when two C-MS patients with outliner values (> 50 pg/mL) were excluded ($p = 0.027$ and $p = 0.043$ for TNF-α and IL-6, respectively). There was no statistically significant difference between C-MS, B-MS and healthy controls, regarding the levels of resistin ($p = 0.152$), IL-1β ($p = 0.442$) and IL-8 ($p = 0.074$) (Fig. 1). Since leptin levels are influenced by the amount of the adipose tissue, BMI adjusted leptin levels (leptin level/BMI value) were also compared. Similar to leptin levels, BMI adjusted leptin levels were comparable among C-MS and B-MS patients and significantly lower in healthy controls than MS groups (mean ± standard deviation values for C-MS, B-MS and healthy controls are 0.08 ± 0.09, 0.06 ± 0.10 and 0.02 ± 0.01, respectively; $p = 0.016$ by Kruskal-Wallis test). After Bonferroni correction [p value (0.05) / number of parameters (8) = adjusted p value (0.006)], adiponectin and MCP-1 values were still significantly higher in C-MS patients than B-MS patients and healthy controls. Table 2 shows correlations of studied adipokines with demographic and clinical characteristics in participants. There were positive correlations between EDSS scores and adiponectin ($p = 0.037$, $R = 0.294$), MCP-1 ($p = 0.043$, $R = 0.287$) and TNF-α ($p = 0.019$, $R = 0.329$) levels in MS patients (Table 2, Fig. 2). When BMI adjusted values were used in correlation tests, adiponectin level/BMI value ($p = 0.030$, $R = 0.267$), MCP-1 level/BMI value ($p = 0.031$, $R = 0.266$) and TNF-α level/BMI value ($p = 0.011$, $R = 0.323$) were also found to be correlated with EDSS scores indicating that obesity is not a confounding factor in correlation statistics. However, these significant correlations had relatively modest correlation coefficient (R) values (< 0.4) and none of the p values remained significant after Bonferroni correction [p value (0.05) / number of correlations (32) = adjusted p value (0.0016)]. None of the other demographic-clinical parameters showed a significant correlation with adipokine levels.

Table 1
Demographic and clinical characteristics of patient population.

	C-MS (n = 24)	B-MS (n = 26)	HC (n = 40)	P value
Age (years)	39.3 ± 7.2	37.8 ± 7.6	38.9 ± 7.2	0.757*
Gender (female/male)	15/9	22/4	26/14	0.152**
BMI (kg/m^2)	23.1 ± 2.7	23.4 ± 2.9	23.5 ± 2.8	0.911*
EDSS	4.4 ± 1.4	1.8 ± 0.6	NA	< 0.001***
MS duration (years)	12.0 ± 1.3	11.8 ± 1.3	NA	0.229†
MS age of onset (years)	22.0 ± 5.1	21.5 ± 5.9	NA	0.372†
First episode clinical finding	9 ON, 15 others	10 ON, 16 others	NA	> 0.999**

C-MS: classical multiple sclerosis; B-MS: benign multiple sclerosis; HC: healthy control; NA: not applicable; BMI: body mass index; EDSS: expanded disability status scale; ON: optic neuritis
Values are expressed as mean ± SD. Comparisons between two groups were performed by *ANOVA, **chi-square test, ***Mann-Whitney U or †Student's *t*-test.

4. Discussion

The contribution of adipokines in regulation of immune system has been recognized, but little is known about their impact on MS. In this study, we analyzed the levels of adipokines (leptin, resistin, adiponectin, MCP-1, IL-1β, IL-6, IL-8 and TNF-α) in sera of MS subgroups. Serum levels of adipokines were compared between MS patient subgroups and healthy control group. The effect of adipokines on the clinical progression of MS was investigated.