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ORIGINAL ARTICLE

Leptin hormone level in serum of opticospinal, neuromyelitisoptica and multiple sclerosis patients

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Abstract

Objectives A growing body of evidence shows that leptin acts as a pro-inflammatory cytokine in autoimmune disorders and is related to multiple sclerosis (MS) pathogenesis. The present study was an analysis of serum leptin levels among healthy volunteers and patients with different subtypes of MS, opticospinal MS (OSMS) and neuromyelitis optica (NMO).

Methods Leptin concentrations in the sera of 121 healthy volunteers and 201 patients with different subtypes of MS, as well as in 27 NMO and 27 OSMS, were measured.

Results Significant differences in leptin serum levels were observed between healthy volunteers, and MS, OSMS and NMO patients ($P < 0.001$). Furthermore, leptin serum concentration was in correlation with expanded disability status scale (EDSS) in primary progressive MS and secondary progressive MS groups. Interestingly, while the female-to-male ratio of leptin was approximately 2 in each group, the NMO female patients showed sevenfold higher levels of leptin than males.

Conclusion The present results show that leptin concentration is important in the pathogenesis of different neuroinflammatory diseases of the central nervous system, in particular NMO. (Clin. Exp. Neuroimmunol. doi: 10.1111/cen3.12092, January 2014)

Introduction

Multiple sclerosis (MS), characterized by autoinflammatory demyelination of the central nervous system (CNS), is one of the most common chronic disabling diseases of the CNS in young adults. The prevalence of MS differs from more than 100 cases per 100 000 population in North America to fewer than five cases per 100 000 in South-East Asia, where approximately 66% of all cases are female patients.[1] MS varies also in its onset, symptoms and progression. In the relapsing–remitting (RR) form of the disease, the course includes attacks and remission periods. Other forms of disease are described as secondary progressive (SP) and primary progressive (PP).

Compared with MS in the Caucasian population, involvement of the optic nerves and spinal cord is more common in the Asian form of MS called opticospinal MS (OSMS). The most important differences between OSMS and MS are older age at onset, marked female preponderance, fewer numbers of brain lesions on magnetic resonance imaging (MRI) and longitudinally extensive spinal cord lesions (LESCL) extending greater than three vertebrae compared with Caucasian MS (Fig. 1).[2]

Neuromyelitis optica (NMO; also known as Devic's disease) is an acquired inflammatory disease of the CNS predominantly involving the optic nerves and spinal cord. Although NMO closely mimics MS, it differs in that it is a relatively homogeneous disorder and shows distinct clinical and pathological

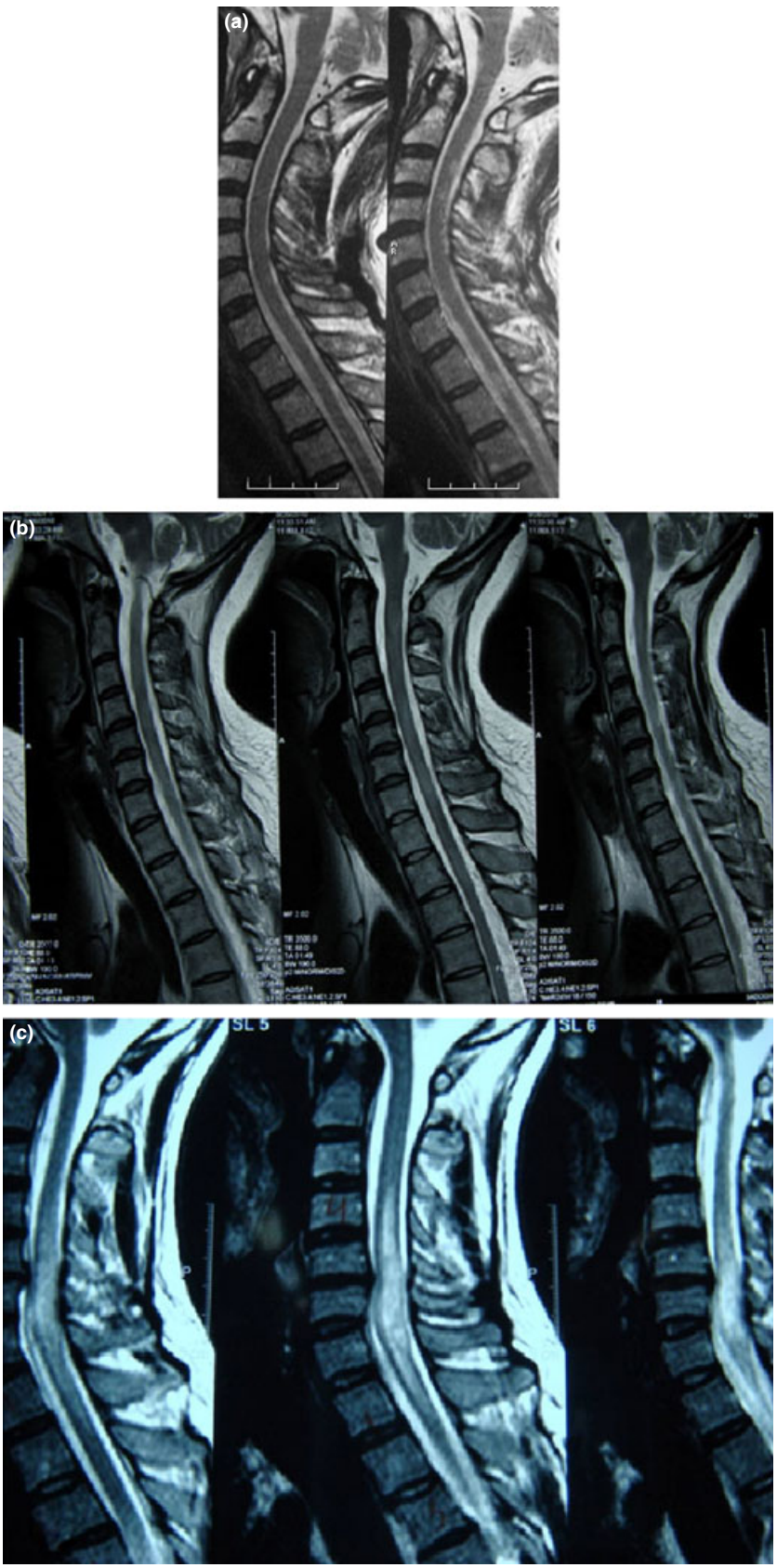

Figure 1 T2-weighted magnetic resonance imaging (MRI) comparison of spinal cord lesions in patients with opticospinal multiple sclerosis (OSMS), neuromyelitis optica (NMO) and multiple sclerosis (MS). (a) Sagittal T2-weighted image of the spinal cord of an OSMS patient showing multiple scattered and patchy hyperintense signals mainly in the cervical and upper thoracic cord areas. (b) T2 imaging MRI of a NMO patient with longitudinally extensive lesions in the cervical region. (c) T2 imaging MRI of a MS patient with scattered lesions in the cervical region.

features.[3] In addition to demyelination, the clinical picture of NMO is often dominated by optic neuritis and myelitis, which is associated with significant malady and disability with more severe attacks.[4,5] Furthermore, the presence of NMO-immunoglobulin G (IgG; anti-aquaporin-4 IgG) in most of NMO patients has facilitated the differentiation between MS and NMO. Clinical hallmarks of NMO are the presence of LESCL extending more than 3 vertebrae, NMO-IgG and the absence of brain MRI lesions (Fig. 1).[6]

The product of the *ob* gene, a 167-amino acid hormone called leptin, is produced predominantly by adipocytes, but also in lesser amounts by brown adipose tissue, the placenta (syncytiotrophoblast), ovaries, skeletal muscle, stomach (lower part of fundic glands), mammary epithelial cells, bone marrow, pituitary, liver and immune cells.[7] The main physiological roles of leptin are related to the control of metabolism and energy homeostasis, but also a growing body of evidence shows that leptin acts as a pro-inflammatory cytokine in immune responses and autoimmunities, such as autoimmune encephalomyelitis, intestinal bowel inflammation and type 1 diabetes.[8–11] Leptin regulates the CD4+ Th1 response and shifts the Th1/Th2 responses balance towards Th1, resulting in increased production of pro-inflammatory cytokines, such as tumor necrosis factor-α, interferon-γ, interleukin (IL)-6 and IL-12.[12–14] It has been proposed by some authors that there is an intricate relationship between leptin and MS.[15,16]

In the present study, we analyzed the possible relationship between serum leptin levels, as a pro-inflammatory cytokine that can affect immune responses, and different neuroinflammatory diseases of the CNS, including MS, OSMS and NMO. Our data show that despite some similarity in the symptoms and pathology of NMO, OSMS and MS, leptin levels vary between the three entities, possibly reflecting distinct pathophysiology.

Methods

MS patients

Consecutive patients, who were referred to Isfahan Multiple Sclerosis Clinic and Isfahan Devic's Disease Clinic at Al-Zahra Hospital, Isfahan University of Medical Sciences in Isfahan, Iran, were screened for the present study. According to the revised McDonald's criteria, as well as neurological evaluation, spinal cord and MRI, 147 cases were diagnosed with MS (Table 1 and 2).[17]

NMO and OSMS patients

Patients were evaluated by a neurologist, and based on the initial abnormal spinal MRI scan, longitudinally extensive spinal cord lesions (LESCL) and NMO-IgG seropositivity, were divided into two groups:[18] (i) 27 patients who presented with optic neuritis and myelitis with LESCL and positive anti-AQP4 antibody included as Devic's disease (NMO; Fig. 1); and (ii) 27 patients showing NMO seronegativity with typical involvement of optic nerves and myelitis without or minimal involvement of other areas of the CNS and classified as opticospinal MS (OSMS) in the present study.[2,19,20]

Demographic and clinical history using specially designed questionnaires were obtained from all the patients in the present study, and appropriate tests for exclusion were carried out. The study was evaluated and approved by the local ethics committee, and all patients signed an informed consent form before the study.

Healthy controls

Additionally, 121 age-, sex- and body mass index (BMI)-matched healthy individuals were selected from the Isfahan Blood transfusion organization (IBTO), in Isfahan, Iran. Clinical and demographic characteristics of normal donors were recorded in

Table 1 Age, sex, body mass index and leptin serum levels in normal donor control and patient groups

			Sex						*P*-value			
	n	Age (years)	BMI (kg/m^2)		Leptin (ng/mL)		Male	Female	MS	NMO	OSMS	ND CTRL
Patients												
MS	147	30.3	34	113	22.7	23.63	29.8	36.8		0.000	0.000	0.000
NMO	27	32.2	7	20	24.3		67.5		0.000		0.012	0.000
OSMS	27	31.6	7	20	23.9		47.2		0.000	0.012		0.000
ND CTRL	121	30	29	92	24.9		15.1		0.000	0.000	0.000	

BMI, body mass index; MS, multiple sclerosis; ND CTRL, normal donor control; NMO, neuromyelitis optica; OSMS, opticospinal multiple sclerosis.

Table 2 Clinical profile and mean leptin serum level in different multiple sclerosis subtypes

	n	Mean age (years)	Sex		BMI (kg/m^2)	Leptin (ng/mL)
			Male	Female		
MS subtypes						
RRMS	119	28.4	23	96	23.5	42.1
PPMS	16	35.6	7	9	22.3	70.6
SPMS	12	41.3	4	8	21.9	25.8

BMI, body mass index; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

questionnaires. Five milliliters of peripheral blood were collected, and separated serum was stored at –80°C until analysis.

Leptin measurement

Levels of serum leptin hormone were measured using commercially available enzyme-linked immunosorbent assay kits (DY398; R&D Systems, Minneaplois, MN, USA) following the manufacturer's instructions.

Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Science version 19 (SPSS 19) software package (SPSS, Chicago, IL, USA). Student's unpaired *t*-test was used to evaluate differences between groups, and the Mann–Whitney *U*-test, as well as Spearman's rank correlation coefficient, was used to determine the relationship between variables. In all cases, *P*-values of 0.05 were considered statistically significant.

Results

Study group

The characteristics of the 322 participants studied have been summarized in Table 1. The average age and BMI was matched between groups, and the male-to-female ratio was on average 0.31. The average age of onset in patients was 30 years, in accordance with the WHO global report.[1]

Statistically significant differences were observed between leptin of patients and the matched age, sex and BMI control group ($P < 0.001$; Table 1, Fig. 2). Further analyses of patient groups showed significantly elevated levels of leptin in patients with the NMO manifestation in comparison with other patients, including those with MS and OSMS (Fig. 2). The maximum variation was observed between the NMO and MS groups ($P < 0.001$).

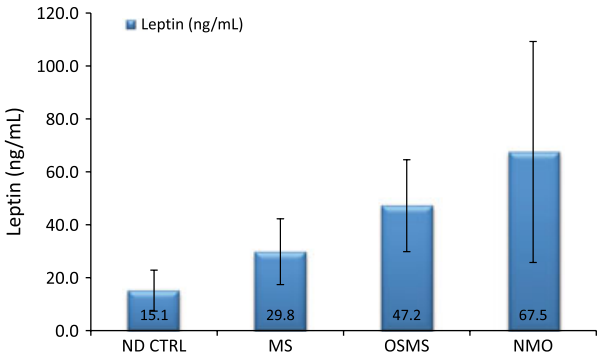

Figure 2 Leptin serum concentration in different groups. leptin serum level was statistical significant ($P < 0.001$) higher in MS, OSMS and NMO groups than age, sex and BMI matched CTRL group. The minimum difference of leptin serum concentration was observed between OSMS and NMO groups ($P < 0.02$). ND CTRL, Normal Donor Control; MS, Multiple Sclerosis, OSMS, Opticospinal Multiple Sclerosis, NMO, Neuromyelitis Optica.

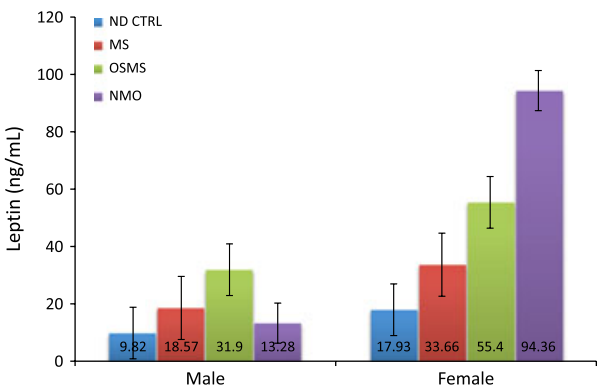

Figure 3 Gender differences in serum leptin levels in control and patient groups. Females in CTRL, OSMS and MS groups had more than twice higher levels of leptin, NMO females patients possessed more than seven times higher leptin than males.

Mean serum leptin concentrations were more than twice as high in females than in males in each group. Strikingly, the NMO group females showed levels that were more than sevenfold higher than males within this group (Fig. 3).

Furthermore, in the MS group, a statistically significant difference was observed between serum leptin levels of SPMS and PPMS patients (25.8 *vs* 70.6 ng/mL, $P < 0.01$), as well as between RRMS and PPMS (42 *vs* 70.6 ng/mL, $P < 0.05$) (Table 2). This difference in leptin serum concentration was in correlation with the expanded disability status scale (EDSS) in

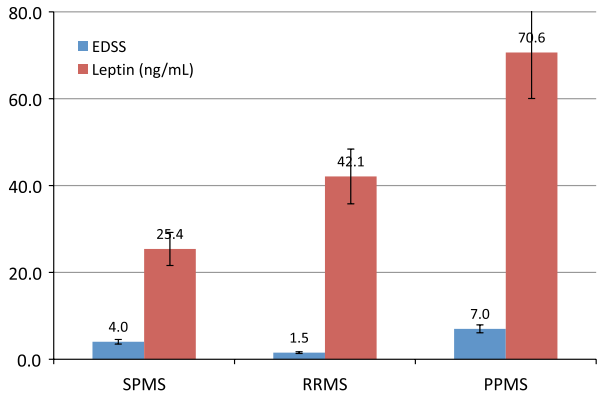

Figure 4 Serum leptin level and EDSS in different MS subtypes. Serum leptin levels were higher in RRMS and PPMS. Correlation between leptin levels and EDSS were observed in SPMS and PPMS but not in RRMS.

the PPMS and SPMS groups, which means patients with higher EDSS in these two groups, but not RRMS, also had elevated leptin levels in their serum (Fig. 4).

Discussion

In order to shed light on the correlation of leptin hormone with autoinflammatory diseases of the CNS, including MS, NMO and OSMS, leptin serum levels were measured and analyzed in different patient groups and matched healthy controls.

Leptin belongs to the family of long-chain helical cytokines, such as IL-6 and IL-12, and its main receptor (OB-Rb) is a member of the class I cytokine receptor superfamily.[7,21] Increasing evidence suggests that leptin, through both direct and indirect effects, could play an important role in induction and progression of animal models of autoimmunity and MS.[21–24] Our data provided more evidence for the pro-inflammatory role of leptin in the MS disease spectrum.

Leptin concentration, unaffected by BMI and age, was higher in females than in males ($P < 0.001$; Table 1, Fig. 3). Surprisingly, whereas the female-to-male ratio of leptin concentration was approximately 1.7 in healthy persons, MS and OSMS groups, it was 7.4 in the NMO group. As female sex hormones are known to affect B cell biology and thus enhance the possibility of autoantibody production, it seems that leptin can induce a B cell response in NMO, which leads to the production of autoantibodies.[25] Furthermore, some studies showed gender effects on the disease course and history of NMO. Weinshenker et al. showed that female sex portends a 10-fold risk of developing a relapsing disease course, but does not otherwise influence the clinical phenotype, attack severity or recovery, or disease-related mortality.[26,27] In contrast, females show higher leptin levels compared with age- and BMI-matched males, showing a gender effect, most probably as a result of sex hormones, in leptin production.[28] Because of the effect of leptin in pro-inflammatory responses, the higher leptin serum level in NMO females might imply a difference in immune response and disease course in them compared with male NMO, which needs to be explored (Table 2).

Previously, Matarase et al. described an inverse correlation between leptin concentration and CD4+CD25+T_{reg} cells in MS patients. This data suggested that leptin might act as a negative regulator for the proliferation of T_{reg} cells leading to imbalanced cell-based immunity and susceptibility to autoimmunity.[29,30] The present investigation resulted in similar conclusions that increased levels of leptin could be indicative of MS progression.

Our data are in line with the results of Alatab et al., and are also confirmed by the results of Martins et al., showing elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor-α and IL-1 in non-relapsing MS.[31,32] Patients with more progressive forms of MS (PPMS and SPMS), which are mostly severe and drastic, have even higher levels of leptin compared with the other forms, suggesting leptin is exacerbating the disease. As of yet, the differences between OSMS, NMO and CMS remain controversial; the diagnosis of different demyelinative involvement of the optic nerve(s) and spinal cord is questionable and challenging. Some authors categorize OSMS as a subtype of MS, and others regard OSMS, NMO and MS as distinct types of neuroinflammatory diseases.[33,34] Variable leptin serum levels seen in different patient groups in the present study showed that the immunological pathways involved in these diseases seem to be at least partly distinct.

Previous studies reported different cytokine profiles in NMO and MS patients, and we are for the first time reporting leptin levels in patients diagnosed with NMO.[35] Furthermore, studies after discovery of anti-aquaporin-4 (NMO-IgG) in sera of NMO patients showed that humoral immunity and complement-dependent cytotoxicity (CDC) are highly activated in this disease.[17,36,37] Also, data provided by Agrawal et al. showed that leptin can stimulate B cells to secrete pro-inflammatory cytokines, such as IL-6, through JAK2/STAT3 and p38MAPK/ERK1/2 signaling cascades.[38] Such a significant difference between leptin concentrations in NMO compared with other patient groups is

indicative of the leptin role in the pathological immune response contributing to NMO. The underlying mechanisms of our observation need to be further examined.

In conclusion, we found elevated leptin serum levels in patients with MS, consistent with previous observations. Here we report, for the first time, the leptin concentration in patients suffering from NMO and OSMS. Because there is a significantly higher level of leptin in patients with the primary progressive form of MS as well as NMO compared with other groups, the mechanism of action of leptin in these diseases requires investigation.

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Disclosure

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