Elevated IL-38 Serum Levels in Newly Diagnosed Multiple Sclerosis and Systemic Sclerosis Patients

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Highlights of the Study

- Levels of interleukin (IL)-38 were higher in new untreated cases of multiple sclerosis and systemic sclerosis than in treated patients and healthy controls.
- IL-38 may be involved in the feedback loop to compensate the effects of secreted pro-inflammatory cytokines in these diseases.

Keywords

Interleukin-38 · Multiple sclerosis · Systemic sclerosis · Autoimmune diseases

Abstract

Objective: Interleukin (IL)-38 is a newly discovered member of the IL-1 cytokine family with a proposed anti-inflammatory profile. We studied the probable role of this cytokine in the pathogenesis of two autoimmune diseases: multiple sclerosis (MS) and systemic sclerosis (SSc). **Subjects and Methods:** A total of 87 MS patients and 86 SSc patients (40 new and recently untreated cases and 46 treated cases) were selected for this study. Eighty-seven and 80 age- and sex-matched healthy subjects were included as controls for MS and SSc, respectively. Clinical and paraclinical features of the patients were recorded at the time of sampling. Serum IL-38 was

measured by ELISA. **Results:** Levels of serum IL-38 did not significantly differ between the total MS or SSc patients compared to controls. However, levels of IL-38 were significantly higher in newly diagnosed patients of MS (206.43 ± 38.97 pg/mL, $p < 0.0001$) than in those previously treated (158.04 ± 39.45 pg/mL). Similarly, new/recently untreated cases of SSc patients showed increased IL-38 levels (185.19 ± 36.27 pg/mL, $p = 0.001$) compared to treated patients (166.82 ± 33.08 pg/mL). IL-38 levels in newly diagnosed MS patients ($p = 0.007$) and new/recently untreated SSc patients ($p = 0.032$) were significantly higher than those in healthy controls. **Conclusion:** The higher serum levels of IL-38 in new or recently untreated cases of MS and SSc patients than in treated patients and healthy controls suggest the possible role of this cytokine in the development of these diseases or as part of a feedback loop to attenuate the inflammatory conditions in early stages of these diseases.

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Introduction

The interleukin (IL)-1 cytokine family comprises 11 members with different pro-inflammatory or anti-inflammatory profiles [1]. The newest member of this family, IL-38, previously named IL-1HY2, has been suggested to function in an anti-inflammatory manner. This viewpoint originates from the reported sequential homology of this cytokine with IL-36Ra and IL-1Ra, two other members of the IL-1 cytokine family with proven anti-inflammatory properties [2]. Growing evidence has illustrated the role of IL-38 in the pathological pathways of several diseases; polymorphisms of the gene encoding this cytokine have been shown to be associated with ankylosing spondylitis [3], psoriatic arthritis [4], and rheumatoid arthritis [5]. The anti-inflammatory characteristics of IL-38 have been clarified in more detail in a study on its role in allergic asthma, which showed that IL-38 inhibits the induced expression of some inflammatory cytokines and chemokines such as IL-6 and CCL-5 and also antagonizes the activation of p38, mitogen-activated protein kinase, and nuclear factor- κ B signaling pathways [6]. Considering the anti-inflammatory profile of IL-38 and its reported roles in the context of some immune-mediated disorders, we aimed to investigate the levels of this cytokine in some autoimmune diseases (AIDs).

We have previously reported an anti-inflammatory role of IL-38 in Behcet's disease [7] and vitiligo. In this study, we examined the levels of this cytokine in multiple sclerosis (MS) and systemic sclerosis (SSc). Both of these diseases are caused by a variety of genetic and environmental triggers, and despite major differences in pathogenesis, they have common immune system abnormalities, and T cells play a key role in both the diseases [8]. MS is a chronic inflammatory disease of the central nervous system with a more frequent presentation in women than men [9]. MS is subdivided into 4 different stages as relapsing-remitting MS, primary progressive MS, secondary progressive MS, and progressive relapsing MS [10]. A hallmark event of the disease pathogenesis is immune-mediated infiltration of effector T cells in the central nervous system. The secretion of cytokines by these cells could lead to neuroaxonal damage [11]. SSc is an autoimmune disorder, with inflammation and fibrosis of the skin and internal organs which lead to severe clinical and histological injuries. The disease comprises two different types according to the extent of skin involvement, as diffuse cutaneous and limited cutaneous SSc types [12]. The etiopathogenesis of the disease is not clearly identified; genetic susceptibility in accordance with environmental

triggers such as viral infections has been reported to be involved [13]. T cells play critical roles in the pathogenesis and the onset of fibrosis in this disease, especially by secretion of profibrotic cytokines such as IL-6 and transforming growth factor- β [14]. We have focused on IL-38, a newly discovered cytokine of the IL-1 family whose potential role in the pathogenesis of MS and SSc is unknown.

Subjects and Methods

Selection of MS Patients and Healthy Controls

A total of 87 MS patients (69 females and 18 males; mean age 32.50 ± 6.92 years; range 19–52 years) were enrolled in this study. MS diagnosis was based on the Modified McDonald Criteria for MS [15] by an expert neurologist. Patients admitted to the Department of Neurology, Namazi Hospital, Shiraz, were involved in our study. Forty-four patients were newly diagnosed, and 43 patients had a history of previous treatment with prednisolone, methotrexate, intravenous immunoglobulin, and/or interferon beta. Furthermore, 87 healthy individuals (70 females and 17 males; mean age 38.7 ± 8.81 years; range 24–60 years) were inducted as controls. Healthy controls were subjects with no current acute or chronic diseases and no history of AIDs, and had been referred to the laboratory for routine blood tests.

Selection of SSc Patients and Healthy Controls

A total of 86 diagnosed patients (79 females and 7 males; mean age 42.24 ± 11.23 years; range 18–69 years) referred to the Outpatient Clinic of Rheumatology Department, Hafez Hospital, Shiraz, were involved in this study. Diagnosis was based on the American College of Rheumatology criteria for the classification of SSc [16]. Eighty age- and sex-matched healthy volunteers who were referred to the laboratory for routine blood tests and had no current acute or chronic disorders and no history of AID were considered as the control group (age range 19–68 years, mean age 41.06 ± 11.26 years). The patients were categorized into two subtype groups as diffuse and limited scleroderma; among them, 40 patients were newly diagnosed or had not received any medication for at least 3 months (new/recently untreated), and 46 patients were recently treated with prednisolone, methotrexate, and/or cyclophosphamide.

Characteristics of both groups of MS and SSc patients regarding laboratory and clinical data were recorded at the time of sampling. The study protocol was approved by the Ethics Committee of the National Institute for Medical Research Development (971223). Informed consent was obtained from all the subjects. Demographic and clinical features of the patients with MS and SSc are shown in Tables 1 and 2, respectively.

Estimation of Cytokine Levels

Three milliliters of whole blood was obtained from every subject and centrifuged at 4,000 rpm for 15 min to collect the serum. The samples were kept at -70°C until use. IL-38 was estimated by ELISA (R&D Systems, Minneapolis, MN, USA) using the manufacturer's instructions. In brief, anti-IL-38 monoclonal antibodies were pre-coated on to a microplate. Serum samples (100 μL) were added to each well after which 100 μL of the working solution of

Table 1. Demographic and clinical features of patients with MS

Characteristic	Previously treated patients	New cases	Total patients
Patients, <i>n</i>	43	44	87
Age, years	33.63±6.89	31.4±6.85	32.64±7.11
Gender: female/male	34/9 (79.1/20.9)	35/9 (79.5/20.5)	69/18 (79.3/20.7)
Disease onset, years	30.31±7.49	31.39±6.8	30.98±7.38
Disease duration, years	1.71±3.05	–	1.71±3.05
Family history of other AIDs	Yes: 1 (2.4) No: 41 (97.6)	Yes: 2 (4.5) No: 42 (95.5)	Yes: 3 (3.5) No: 83 (96.5)
Family history of MS	Yes: 2 (4.8) No: 40 (95.2)	Yes: 2 (4.7) No: 41 (95.3)	Yes: 4 (4.7) No: 81 (95.3)
RRMS	25 (58.1)	41 (93.2)	66 (75.9)
PPMS	8 (18.6)	3 (6.8)	11 (12.6)
SPMS	10 (23.3)	–	10 (11.5)
EDSS	3.05±2.13	1.58±1.37	2.33±1.9
Progression index [EDSS/duration, years]	1.6±1.33	–	1.6±1.33

Data are presented as mean ± SD, or *n* (%). AIDs, autoimmune diseases; EDSS, expanded disability status scale; PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis; MS, multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis.

streptavidin-horseradish peroxidase-conjugated antibodies were added, and then the optical density of each well was determined using a microplate reader at 450 nm.

Statistical Analysis

Statistical analysis was performed by SPSS v.25 software (SPSS, Inc., Chicago, IL, USA). The normal distribution of data was evaluated using the Kolmogorov-Smirnov test. We used nonparametric tests including the Kruskal-Wallis test for multiple comparisons and Mann-Whitney U tests for comparison of IL-38 levels between two groups. Pearson's correlation test was used to assess any correlation between quantitative variables. $p < 0.05$ was considered significant.

Results

IL-38 Levels in MS Patients and Related Healthy Controls

IL-38 levels were not significantly different between the patient (166.94 ± 43.44 pg/mL) and control (171.55 ± 40.96 pg/mL) groups ($p = 0.17$, Fig. 1). IL-38 levels did not differ between female (163.14 ± 39.57 pg/mL) and male patients (181.49 ± 54.8 pg/mL, $p = 0.15$) or female (172.70

± 44.28 pg/mL) and male (167.21 ± 25.27 pg/mL) controls ($p = 0.88$).

Levels of IL-38 were significantly different between patients who were newly diagnosed (206.43 ± 38.97 pg/mL) and those who were previously treated (158.04 ± 39.45 pg/mL, $p < 0.0001$). Furthermore, these 2 groups of patients showed significant differences in IL-38 levels compared to the same age- and sex-matched groups of control (172.62 ± 43.18 pg/mL and 166.91 ± 30.17 pg/mL, respectively; $p = 0.007$) (Fig. 2).

We analyzed the relationship between IL-38 levels of the patients and their clinical features. No significant relationship was found between parameters such as disease onset, disease duration and family history of MS or other AIDs, and IL-38 serum levels in every patient group (newly diagnosed and previously treated) and between males and females of these groups.

Among the patients, 76.4% presented with relapsing-remitting, 12.4% had primary progressive, and 11.2% were in the secondary progressive course of disease at the time of sampling. IL-38 levels did not differ between these groups of patients (169.43 ± 47.89 pg/mL, 168.24 ± 24.69

Table 2. Laboratory and clinical characteristics of patients with SSc

Variable	Treated patients	New/recently untreated patients	All patients
Patients, <i>n</i>	46	40	86
Age, years	42.7±12.06	41.73±10.32	42.24±11.23
Gender			
Male	2 (4.3)	5 (12.5)	7 (8.1)
Female	44 (95.7)	35 (87.5)	79 (91.9)
Disease onset, years	37.06±13.07	36.74±10.15	36.92±11.75
Disease duration, years	5.01±5.73	5.01±4.35	5.01±5.12
Family history of other AIDs, <i>n</i> (%)	15 (37.5)	9 (23.1)	24 (30.4)
Disease subtype			
Limited	19 (41.3)	21 (52.5)	40 (46.5)
Diffuse	27 (58.7)	19 (47.5)	46 (53.5)
Capillaroscopy			
Active	10 (45.5)	16 (47.1)	26 (46.4)
Late	4 (18.2)	8 (23.5)	12 (21.4)
Early	7 (31.8)	10 (29.4)	17 (30.4)
Skin manifestations			
Rodnan skin score	16.04±7.57	17.78±7.05	16.85±7.34
Calcinosis	6 (22.2)	1 (2.5)	7 (13.8)
Telangiectasia	12 (44.4)	12 (49.2)	24 (47.1)
Raynaud's phenomenon, years	6.06±6.4	4.72±4.58	5.36±5.44
Vascular involvement	38 (82.6)	37 (92.5)	75 (87.2)
Joint involvement (FTP)			
Normal	40 (87)	35 (87.5)	75 (87.2)
Moderate	4 (8.7)	3 (7.5)	7 (8.1)
Severe	2 (4.3)	2 (5)	4 (4.7)
Organ involvement			
Muscle	9 (19.6)	5 (12.5)	14 (16.3)
Gastrointestinal	31 (72.1)	27 (67.5)	58 (69.9)
Cardiac	10 (27)	7 (17.5)	17 (22.1)
Lung	28 (68.3)	15 (38.5)	43 (53.8)
FVC, %	76.31±21.06	69.97±20.09	74.03±20.74
FVC <80% of predicted	19 (59.4)	11 (61.1)	30 (60)
ESR, mm/h	22.64±24.45	21.24±22.82	21.94±23.41
CRP >3, mg/L	4 (13.3)	6 (22.2)	10 (17.5)

Data are presented as number, frequency (%), and mean ± SD. AIDs, autoimmune diseases; FTP, finger to palm; FVC, forced vital capacity; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MTX, methotrexate; SSc, systemic sclerosis.

pg/mL, and 149.08 ± 19.79 pg/mL, respectively, $p = 0.4$). No significant association with expanded disability status scale was detected. The progression index (expanded disability status scale /duration) which was only available in 13 patients showed no correlation with IL-38 levels in previously treated patients.

IL-38 Levels in SSc Patients and Related Healthy Controls

We did not find any differences between the total SSc patients (175.37 ± 35.61 pg/mL) and control group

(170.84 ± 41.72 pg/mL) in IL-38 serum levels ($p = 0.16$). IL-38 levels were also not different between female (174.65 ± 34.96 pg/mL) and male patients (183.41 ± 44.55 pg/mL, $p = 0.69$), or female (171.63 ± 43.29 pg/mL) and male (162.66 ± 18.56 pg/mL) control subjects ($p = 0.87$). IL-38 levels in treated patients (166.82 ± 33.08 pg/mL) were significantly different from those in new untreated cases (185.19 ± 36.27 pg/mL, $p = 0.001$); IL-38 levels were also significantly different between new untreated cases (185.19 ± 36.27 pg/mL) and age- and sex-matched group of controls (173.4 ± 46.53 pg/mL, $p = 0.032$) (Fig. 3). IL-

Fig. 1. IL-38 serum level differences between total MS patients and healthy controls. Data are represented as mean $\pm$ SD. IL-38, interleukin-38; MS, multiple sclerosis.

Fig. 2. IL-38 serum level differences between newly diagnosed MS patients and previously treated patients and their age- and sex-matched control groups. Data are represented as mean $\pm$ SD. *** $p < 0.0001$; ** $p < 0.007$. IL-38, interleukin-38; MS, multiple sclerosis.

Fig. 3. IL-38 serum level differences among total patients, treated patients, and new/recently untreated cases of systemic sclerosis and their age- and sex-matched control groups. Data are represented as mean $\pm$ SD. * $p = 0.032$; ** $p = 0.001$; IL-38, interleukin-38; ns, nonsignificant.

38 levels were not significantly different between treated patients and age- and sex-matched controls ($p = 0.8$).

After stratification of the patients based on limited and diffuse disease subtypes, we could not identify any significant differences in the IL-38 serum levels between these subtypes in groups of total patients (177.58 ± 39.49

pg/mL vs. 172.82 ± 30.84 pg/mL, $p = 0.88$), treated ones (170.93 ± 37.63 pg/mL vs. 160.98 ± 25.08 pg/mL, $p = 0.43$), and new/recently untreated cases (187.03 ± 41.15 pg/mL vs. 183.53 ± 32.17 pg/mL, $p = 0.92$). Furthermore, in total ($p = 0.69$), treated ($p = 0.22$), and new/recently untreated patients ($p = 0.91$), no significant difference in

Fig. 4. Correlation of IL-38 serum levels with age of total SSc patients (a) and treated ones (b). IL-38, interleukin-38; SSc, systemic sclerosis.

IL-38 in relation to gender and family history of other AIDs ($p \geq 0.5$) was observed. On the other hand, a significant negative correlation between age and IL-38 serum level in total patients ($p = 0.004$, $r = -0.3$) and treated ones ($p = 0.043$, $r = -0.3$) was observed, so that the level of this cytokine diminished with increased age of the patients in the above-mentioned groups (Fig. 4). This correlation was not observed in new/recently untreated cases ($p = 0.19$). It should be noted that no significant correlation between age of healthy controls and IL-38 serum levels was found ($p = 0.18$). We analyzed the relationship of IL-38 levels of patients with their clinical features. No significant difference in IL-38 levels in total, treated, and new/recently untreated patients in relation to skin score and involvement of different organs such as skin ($p \geq 0.49$), vascular ($p \geq 0.24$), lung ($p \geq 0.26$), gastrointestinal ($p \geq 0.23$), cardiac ($p \geq 0.38$), and muscle ($p \geq 0.14$) was found. We also could not observe any significant relation of serum IL-38 levels to the clinical parameters such as serum erythrocyte sedimentation rate ($p = 0.68$) and CRP ($p = 0.29$) in total, treated, and new/recently untreated patients.

Discussion

AIDs often have no known cause, but genetic predisposition and environmental factors play a role in their occurrence [17, 18]. Immune system dysregulation and, as a result, activation of different immune cells and their

effector functions such as secretion of anti-inflammatory and/or pro-inflammatory cytokines cause the subsequent immunopathological phenomena and clinical manifestations of the disease. Various studies have focused on the investigation of the exact roles of cytokines in AIDs [19, 20].

MS is a neuro-inflammatory autoimmune disorder of young adults. It is well-documented that Th1 and Th17 cells and various cytokines secreted by different immune cells are the main causes of tissue damage [11, 21]. Previous studies have indicated that the level of some pro-inflammatory cytokines such as IL-1, IL-12, IL-17, IL-22, and tumor necrosis factor- α is elevated in MS, whereas anti-inflammatory cytokines such as IL-4 and IL-10 are decreased [22].

In our study, we compared the serum levels of IL-38 between patients and healthy controls; no significant difference in this cytokine was detected between these two groups. Our patient group comprised two subgroups: newly diagnosed cases and previously treated MS patients. IL-38 levels were significantly higher in the sera of new cases than in the treated patients. The lower levels of this cytokine in treated patients could be the result of drugs that may lead to decreased secretion of cytokines. On the other hand, when we compared the IL-38 levels in new patients with their age- and sex-matched healthy controls, we found higher levels of this cytokine in patients than in controls. Elevated levels of IL-38 in newly diagnosed patients than in their related healthy controls may suggest a role for this cytokine in disease pathogen-

esis, or, on the other hand, may suggest the involvement of IL-38 in the feedback loop to compensate for the effects of secreted pro-inflammatory cytokines. Levels of the anti-inflammatory cytokine IL-37, another member of the IL-1 cytokine family, are increased in MS; it is suggested that IL-37 may be part of a feedback loop to ameliorate the inflammation seen in this disease [23]. Interestingly, other anti-inflammatory cytokines such as IL-4 and IL-10 are decreased in MS [22, 24]. This might be attributed to Th1/Th17 dominance in the immunopathogenesis of MS, leading to upregulation of pro-inflammatory cytokines and downregulation of anti-inflammatory ones.

SSc is a chronic and multisystem AID with major characteristic skin and organ fibrosis as the result of vasculopathy, action of autoantibodies, and deposition of excessive collagen [13]. Both innate and adaptive immune systems have been shown to contribute to the pathogenesis of SSc; cytokines such as IL-1 α , IL-1 β [25], IL-18, IL-13, and IL-4 [26] produced by various immune cells have pro-inflammatory and pro-fibrotic effects. We report here that levels of IL-38 were significantly higher in new untreated patients than in treated patients and also than in healthy control subjects. The increased levels of IL-38 in new scleroderma patients may suggest a role for this cytokine in the pathogenesis of the disease or may reflect the attenuation of inflammatory conditions that emerge at the onset of the disease. It is also possible that the reduction in IL-38 in treated patients could be due to the suppressive effect of treatment with steroids such as prednisolone; steroids have been shown to suppress most cytokines, but there are no reports on the effects of steroids on IL-38 levels. Other members of the IL-1 cytokine family such as IL-1 α , IL-1 β , IL-18, and IL-33 [27] have been demonstrated to play a role in the pathogenesis of scleroderma.

We did not find any relationship between IL-38 levels and clinical features of both diseases such as disease subtypes, activity, organ involvement, or laboratory indices such as erythrocyte sedimentation rate and C-reactive protein. Thus, our data do not indicate a role for IL-38 in disease manifestations of SSc and MS. Limitations of this study include the low number of patients and that some clinical data such as the relapse/flare condition of the patients at the time of sampling were not available.

Conclusion

In this study, we found that the serum levels of IL-38 were higher in new cases of MS and new/recently untreated SSc patients than in treated patients and healthy con-

trols. This may suggest a role for IL-38 in the development of these diseases, or a role in the feedback loop to attenuate the inflammatory conditions in the early stages of the diseases.

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Statement of Ethics

Subjects (or their parents or guardians) have given their written informed consent and the study protocol was approved by the institute's committee on human research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M. Zarrabi performed the analysis and drafted the manuscript. M. Nazarinia and A. Rahimi Jaberi referred the patients. N. Gholijani helped in designing the experiments. Z. Amirghofran conceived the original idea, supervised the project, and provided critical revision of the manuscript.

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