

## Research Article

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# Interleukin-33 plasma levels in patients with relapsing-remitting multiple sclerosis

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**Abstract:** Cytokines are implicated in the immunopathogenesis of multiple sclerosis (MS). Interleukin (IL)-33, one of the recently discovered members of the IL-1 superfamily, is a dual functional cytokine involved in various autoimmune disorders. In a case-control study, venous blood was collected from healthy subjects categorized as control group (n=44) and MS patients (n=44). All recruited patients were clinically diagnosed with relapsing-remitting MS (RRMS), including patients without treatment (new identified cases, n=16) and those treated with interferon beta (IFN- $\beta$ ) (n=28). The plasma levels of IL-33 in subjects were measured with ELISA. Significantly elevated IL-33 plasma levels were observed in RRMS patients ( $p=0.005$ ). Furthermore, IFN- $\beta$ -treated MS patients had lower levels of IL-33 compared to the untreated patients ( $p<0.001$ ). Increased IL-33 plasma levels in the patient group might be associated with development of MS. These results could contribute to our better understanding about the role of IL-33 in the immunopathogenesis of MS.

**Keywords:** cytokines; immunopathogenesis; interleukin-33 (IL-33); multiple sclerosis (MS); neuroinflammatory diseases.

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## Introduction

Multiple sclerosis (MS) is a neuroinflammatory disease of the central nervous system (CNS), identified by lesions in the brain's white matter and progressive disability in neuronal function (1). The age of onset of MS has been reported to be varying between 20 and 40 years (2). Some studies report that MS might slightly develop in either children or people above 60 years (3–5). Researchers have conflicting ideas on whether MS is a pathological disease, as MS presents with a variety of signs and symptoms (6, 7). Clinical investigations have demonstrated the role of immune cells in the progression of neural lesions during the acute early phase of MS, where relapses occur in the white matter of neurons and inside tracts in the gray matter, mainly because of inflammatory reactions. The main pathologic sign of MS is plaque in the demyelinated neurons, with immunocytological activity of resident immune cells during the course of the disease (8–11). Numerous clinical studies have shown that MS lesions are initiated by leukocyte cell types, including lymphocytes, macrophages and dendritic cells through various immunological mechanisms such as cytokine production (10, 12).

Cytokines have various functions in health and diseases, especially in MS (13–16). One of the most important cytokines that directly contributes to the immunopathogenesis of MS is IL-33 (17), a recently discovered cytokine, taking part in various inflammatory and autoimmune diseases like psoriasis, lupus erythematosus, ulcerative colitis and MS (17–20). ST2L, a receptor of IL-33, is expressed on the surface of various subsets of leukocytes and conducts signals to activate the MAPK and nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathways by TNF receptor-associated factor 6 (TRAF6) (21, 22). The IL-33/ST2 pathway is pivotal to inflammatory responses and autoimmune disorders (23). It has been reported that ST2L is principal for the inflammatory activity of IL-33 because of expression on several immune cells like endothelial cells, mast cells, macrophages, basophiles and dendritic cells (18, 19, 24–28).

Isfahan is one of the highest MS reported rate areas in Oceania and Asia (5, 29–33). Regarding the possible role of IL-33 in MS progression, we assessed IL-33 plasma levels in MS patients in comparison with healthy subjects.

## Materials and methods

### Patient selection

Venous blood samples (3–5 ml) were collected and plasma was isolated from 44 relapsing-remitting multiple sclerosis (RRMS) patients and 44 gender, race and age-matched healthy subjects with no previous neurological problem history. All patients were clinically diagnosed with RRMS according to the McDonald criteria (7) and no one used steroids or other medication to alleviate clinical symptom. The patient group was divided into two subsets, interferon beta (IFN- $\beta$ )-treated and newly diagnosed MS patients with no treatment initiation. None of our patients underwent clinical worsening in the preceding weeks. The study was approved by the Ethical Committee on Human Research in Isfahan University of Medical Sciences and informed consent was obtained from patients participating in this study. Details of data gathering and sampling have already been published (34, 35).

**Table 1:** Comparison of mean IL-33 plasma levels in different factors within each group.

Groups	Factors	Mean $\pm$ SEM <sup>a</sup>	p-Value <sup>b</sup>
Case	Sex		
	Male (n = 6)	106.91 $\pm$ 64.08	0.688
	Female (n = 38)	195.28 $\pm$ 131.18	
	Age (years)		0.417
	15–25 (n = 4)	33.39 $\pm$ 4.22	
	26–35 (n = 17)	66.20 $\pm$ 24.51	
	36–45 (n = 13)	97.29 $\pm$ 63.69	
	Greater than 45 (n = 10)	57.07 $\pm$ 23.60	
	History of viral disease		0.261
	Yes (n = 10)	69.03 $\pm$ 20.10	
No (n = 34)	75.79 $\pm$ 26.87		
Family history of MS		0.630	
Yes (n = 11)	43.91 $\pm$ 14.69		
No (n = 33)	84.37 $\pm$ 27.68		
Control	Sex		0.808
	Male (n = 6)	22.50 $\pm$ 6.32	
	Female (n = 38)	24.30 $\pm$ 3.38	
	Age (years)		0.816
	15–25 (n = 1)	40.49 $\pm$ 0	
	26–35 (n = 12)	21.84 $\pm$ 2.34	
	36–45 (n = 29)	26.28 $\pm$ 10.69	
Greater than 45 (n = 2)	19.73 $\pm$ 4.95		

<sup>a</sup>Mean  $\pm$  SEM (pg/ml).

<sup>b</sup>p-Values are two-tailed.

**Table 2:** Mean IL-33 plasma levels in MS and control groups.

	Control (n = 44)	Case (n = 44)	p-Value <sup>a</sup>
Total	24.05 $\pm$ 3.02 <sup>b</sup>	74.25 $\pm$ 21.15	0.005
Treated (n = 28)	–	55.87 $\pm$ 24.26	0.031 <sup>c</sup>
Untreated (n = 16)	–	84.76 $\pm$ 9.35	0.018 <sup>c</sup>

<sup>a</sup>p-Values are two-tailed.

<sup>b</sup>Data are shown as mean  $\pm$  SEM (pg/ml).

<sup>c</sup>Compared with control group.

### Cytokine ELISA

The plasma levels of IL-33 were measured by an enzyme-linked immunosorbent assay (ELISA) kit (BOSTER Immunoleader, CA, USA) according to the manufacturer's instructions.

### Statistical analysis

In order to check the normal distribution of plasma levels, a Kolmogorov-Smirnov Z-test was performed. Assessment of association between mean plasma levels and various subjects' factors was carried out by ANOVA and independent *t*-tests in the control subjects and Mann-Whitney and Kruskal-Wallis tests in the patient group (Table 2). The comparison of IL-33 plasma concentrations between the case and healthy groups was evaluated by the Mann-Whitney test. Data was demonstrated as mean  $\pm$  SD for age and sex (Table 1) and mean  $\pm$  SEM for comparison of IL-33 plasma level with various subjects' characteristic factors (Table 2). *p*-Values of less than 0.05 were considered as statistically significant and all *p*-values were two-tailed. Statistical analysis was executed using SPSS version 16.

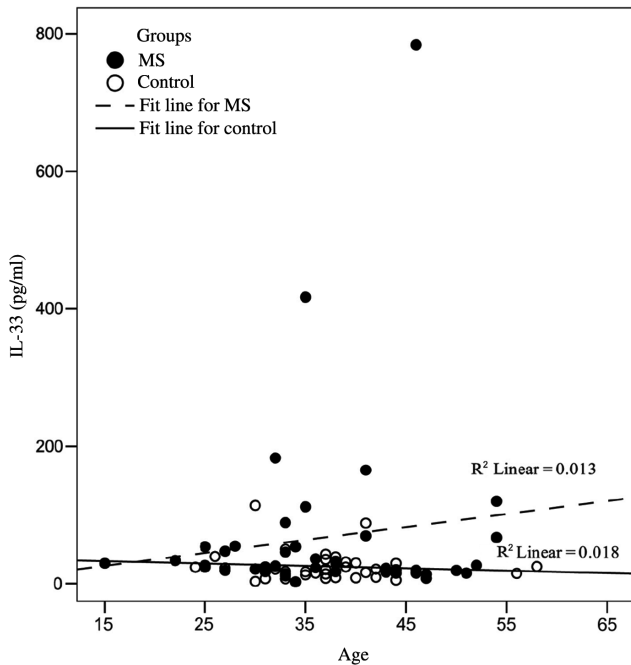
## Results

### Main characteristics of patients and healthy controls

Information on age and gender of patients and healthy controls showed that both groups were matched for gender and age (*p* > 0.05).

### Characteristic features of subjects and correlation with IL-33 plasma level

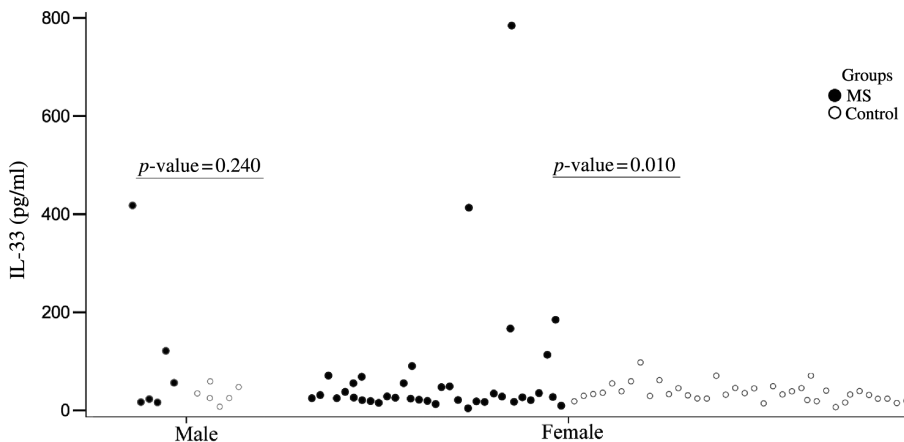
The association between various characteristic features of patients and the healthy group and IL-33 plasma levels was assessed. As shown in Table 1, no factors were correlated with IL-33 level.



**Figure 1:** Plasma IL-33 levels based on age in the case and control groups.

The alterations of IL-33 in various age ranges in MS patients and healthy subjects are shown in Figure 1. According to the Pearson correlation coefficient analysis, the relationship between mean IL-33 plasma level and increase in age was weak and direct in the MS group and was feeble and inverse in the control group ( $\rho_{(MS)} = 0.11$  and  $\rho_{(Control)} = 0.13$ ).

Also, after separating the subjects based on gender, we observed a significant difference in female plasma IL-33 levels between both healthy and patient sub-groups ( $p = 0.01$ ), but not in the male subsets (Figure 2).



**Figure 2:** Plasma IL-33 levels in each of the case and control groups based on gender.

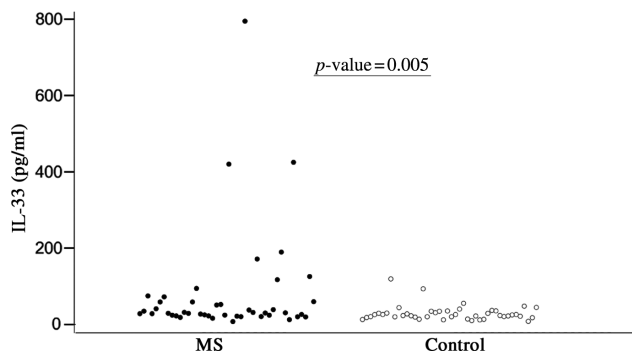
### Increased plasma levels of IL-33 in RRMS patients

The plasma levels of IL-33 were assessed in RRMS patients and in the control group. From Table 2 and Figure 3, the mean plasma levels of IL-33 in the RRMS patients were significantly higher than in the healthy subjects ( $p < 0.05$ ). We also classified case groups into the two subgroups treated and untreated patients, and after final assessment, the results revealed a significant difference in IL-33 levels between the control group and both subgroups of MS patients. Besides, the mean levels of IL-33 in treated patients were significantly lower than those in the new diagnosed cases ( $p > 0.001$ ).

### Discussion

Cytokines have a crucial role in the immunopathogenesis of MS. Many studies have demonstrated increased infiltrating leukocytes in the brain, which consequently lead to CNS lesions through inflammatory cytokines and activated transcription factors like NF- $\kappa$ B (36, 37), signal transducer and activation of transcription 1 (STAT1) (37–39) and STAT6 (40, 41). The provocative cytokines result in the over-expression of inflammation-mediated genes and accordingly release toxic molecules, which increase demyelination (42–44). Activation of NF- $\kappa$ B and Toll-like receptor signaling pathways stimulates TNF- $\alpha$  or IL-1 $\beta$ , which mediates IL-33 transcription (21, 27, 45, 46).

In the current study, we compared the plasma levels of IL-33 in RRMS patients with those in the healthy subjects. We did not find any significant association among various factors of MS patients and healthy control groups and



**Figure 3:** Comparison of IL-33 plasma levels in RRMS patients and healthy subjects.

mean IL-33 plasma levels. To the best of our knowledge, this is the first study describing the relationship between IL-33 and various features of investigated subjects.

Many findings have proved the involvement of immunity and imbalance between pro- and anti-inflammatory cytokines in the pathogenesis of MS (19, 47, 48). IL-33 has a contradictory function in immunity. It is well known that IL-33 induces mRNA expression of pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1 $\beta$  in the microglia. Binding of IL-33 to its receptor (ST2) induces activation of NF- $\kappa$ B and plays a critical role in the development of experimental autoimmune encephalomyelitis (EAE) (49–51). On the other hand, ST2 deficiency in dendritic cell surfaces leads to exacerbation of EAE (52). Furthermore, in response to IL-33, dendritic cells inhibit EAE (53). Interestingly, ST2 deficiency in BALB/C mice abrogates resistance to EAE (52). These are conflicting examples about the role of IL-33 in the immune system. Although clinical studies have shown that administration of IL-33 causes development of inflammation in the EAE model (54), other investigations have declared that IL-33 is not a necessary element for EAE pathogenesis (55). This is another evidence for the dual role of IL-33.

Previous studies have mentioned that IFN- $\beta$  treatment could significantly suppress IL-33 plasma levels (56). In accordance with them, our assessments revealed that plasma levels of IL-33 in IFN- $\beta$ -treated patients were significantly lower than in untreated patients ( $p < 0.01$ ). It could be noticed that IFN- $\beta$  therapy in RRMS patients can modulate MS development, which would be a favorite future therapeutic target for MS patients (57, 58). Today, it has been admitted that IFN- $\beta$  can be used as disease-modifying therapy agent that, due to its anti-inflammatory properties, can boost remyelination indirectly. While there are many new medications to treat MS patients, such as glatiramer acetate, or various corticosteroids, IFN- $\beta$  is still one of the most commonly used drugs as a desirable

and safe first-line treatment approach (59). Furthermore, follow-up studies have reported that using IFN- $\beta$  can be beneficial for decreasing severity of disease (60).

It has been shown that IL-33 can biologically mediate several significant immune disorders, including rheumatoid arthritis, asthma, inflammatory bowel disease, dermatitis, cardiovascular disease and autoimmune hepatitis (18, 19). Moreover, the role of IL-33 in the immune system and the CNS is noticeable. In the immune system, the serum level of IL-33 that is elevated in response to the systemic inflammation results in the activation of various immune cells like dendritic cells, macrophages and lymphocytes. On the one hand, this initiation causes down-regulation of systemic inflammation. In the CNS, IL-33 stimulates vascular permeability in the blood-brain barrier (BBB) toward infiltrating immune cells, which consequently causes BBB disruption. Entry of immune cells into the CNS is responsible for beginning inflammation and promoting CNS resident immune cells like astrocytes to produce IL-33. It mediates infiltrating immune cells and/or CNS resident cells via binding to ST2 receptor, which causes cytokine and antibody generation and may modulate MS development. IL-33 also has a pivotal role in myelin damage/repair through its direct effect on oligodendrocytes (17) and glial activation, particularly astrocytes, and on induction of microglia proliferation, which causes the production of pro- and anti-inflammatory cytokines at the same time (61, 62).

Scientific attention has recently been directed toward the evaluation of IL-33 in the brain and immune-related diseases (63). There is enough evidence to show upregulated IL-33 expression by astrocytes and peripheral leukocytes in MS patients (64). Although it has been found that IL-33 induces pro-inflammatory type-2 responses, the anti-inflammatory properties of this cytokine have been thought to be beneficial in autoimmunity (65, 66) as well as in many other disorders (67).

In conclusion, in agreement with previous studies, our data confirmed that IL-33 levels were significantly higher in RRMS patients than in healthy controls, which suggested that IL-33 is a pro-inflammatory cytokine that might be involved in the pathogenesis of MS. Furthermore, IFN- $\beta$  can modulate IL-33 production in RRMS patients and could be a new promising target for therapeutic strategies.

## Highlights

- IL-33 is an important cytokine playing a critical role in autoimmune diseases, especially MS.

- Our assessment revealed a significant increase in IL-33 plasma levels of MS patients.
- There was a reduction in IL-33 levels in IFN- $\beta$ -treated patients compared with untreated patients.

**Conflict of interest statement:** The authors declare no conflicts of interest.

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