Increased mRNA expression of IL-23 in the peripheral blood of patients with multiple sclerosis

Ali Mehdizadeh1, Vahid Shaygannejad2*, Meysam Amidfar3, Seyed Javad Hasheminia4, Mohamad Mousaei Ghasroldasht5

1School of medicine, Isfahan University of medical sciences, Isfahan, Iran.  
2Isfahan neuroscience research center, alzahra research institute, Isfahan University of medical sciences, Isfahan, Iran.  
3Tehran University of medical sciences, Tehran, Iran.  
4Department of immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.  
5Biology department, shahid ashrafi Isfahan University, clinical laboratory, alzahra hospital, Isfahan, Iran.

ABSTRACT

Background: interleukin-23 (IL-23) is a member of the IL-12 cytokine family that has shown through enhancement of T helper type 17 (Th17) cells expansion could play an important role in the inflammatory autoimmune responses in multiple sclerosis (MS).

Methods: The objective of the present study is to measure the relative expression of IL-23 mRNA in the peripheral blood of 15 MS patients in comparison with 15 healthy control subjects.

Results: the relative gene expression level of IL-23 in the peripheral blood cells from MS Patients was significantly increased compared to healthy controls (p < 0.001).

Conclusions: Our findings revealed upregulated gene expression pattern of IL-23 in the peripheral blood of MS patients that may be a peripheral marker for diagnosis of MS and might be a novel and promising therapeutic target for MS.

Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory immune-mediated disease of the central nervous system (CNS) with unknown etiology and variable clinical profile1. It has demonstrated that production of cytokines by infiltrating inflammatory cells and resident cells in the brain is involved in directing and regulating the immune response and also mediating of tissue damage2. Cytokines and their receptors are important factors of immune system and play a role in pathologic hallmarks of MS3, 4. IL-23 is a heterodimeric cytokine which comprises two subunits including p19 and a common p40 subunit that shares with IL-125. It has demonstrated that a subset of inflammatory macrophages and dendritic cells express receptor complex of IL-236. IL-23 is constructed mainly by activation of dendritic cells (DCs) and phagocytic cells, preferentially activates memory rather than naive T cells, stimulates of INF-γ secretion from T cells and is involved in Th1-type immune responses7. It has found that IL-23 upon antigen-specific stimulation contribute to development and expansion of activated CD4+ cells that produce IL-17, IL-17F, IL-6, and TNF and plays a critical role in autoimmune inflammation8. Several studies suggest that the family members of IL-17 play an important role in autoimmune and inflammatory diseases9. IL-17 is produced by Th17 cells that...
are a subset of CD4+ T-cells and IL-23 increased number of IL-17-producing CD4+ cells\(^6\). Th17 cells and IL-23 are essential for the induction of experimental models of multiple sclerosis and increased expression of IL-23 and IL-17 has been reported in patients with MS\(^7,10-14\). Active and chronic active lesions in the multiple sclerosis have shown highly expression of proinflammatory cytokine IL-23 and activated macrophages/microglia and mature DCs have proposed as cellular sources of IL-23p19 in these lesions\(^12\). Production of p40 subunit of IL-23 and in vitro production of IL-23 by activated microglia support from this hypothesis that production of bioactive IL-23 plays an important role in the pathogenesis of MS\(^12,15\). IL-23 by modulation of the interaction of Microglia-T cell in multiple sclerosis may impact on the local inflammatory response in the brain\(^12\). It has suggested that correlation between expression of IL-23 and inflammatory and demyelinating lesions in MS that probably through recruitment and activation of some essential inflammatory cells that are responsible for chronic inflammation plays a crucial role in the immunopathogenesis of multiple sclerosis\(^12\). Targeting of IL-23 or IL-23-receptor has suggested as a probably promising therapeutic approach for a range of inflammatory autoimmune diseases\(^10\). In the present study, we examined mRNA expression level of IL-23 using Real-Time PCR technique in the peripheral blood of patients with multiple sclerosis and healthy control groups. We demonstrate herein that a high mRNA expression pattern of IL-23 in peripheral blood is a feature of MS.

### Material and methods

Fifteen patients with clinically definite MS (9 women, 6 men) from the MS Clinic of Kashani University Hospital at the Isfahan University of Medical Sciences and 15 healthy control subjects (8 females, 7 males) were included in this study. Severity of illness was evaluated using expanded disability status scale (EDSS)\(^16\) and disease type was defined as relapsing-remitting (RR), primary progressive (PP) or secondary progressive (SP). All of patients were diagnosed according to the revised McDonald criteria\(^17\), and MS clinical forms were determined according to the classification of Lublin & Reingold\(^18\). Ten patients (66.7%) suffered from relapsing-remitting MS; three patients (20%) were in the primary progressive phase of the disease and two patients (13.3%) were in the secondary progressive phase of the disease. None of the Patients was received any immunomodulatory drugs such as methylprednisolone or interferon-(IFN-β) for at least 4 weeks prior to inclusion, and had not had an acute exacerbation prior to inclusion. The Ethical Committee on Human Research, Isfahan University of Medical Sciences approved protocol of study and written informed consent was obtained from all participating prior to entering the study. Patients with infection diseases or other autoimmune diseases, cardiovascular or renal disease, cancer and blood systemic disorders were excluded from study. Clinical and demographic features of participants have been summarized in Table 1.

### Statistical analysis

The normal distribution of clinical variables and gene expression data were checked using Kolmogorov–Smirnov test. Two-tailed t-test, chi-square test, Kruskal-Wallis test and Mann–Whitney test were used for comparison of study groups by SPSS20. The relationships between IL-23 mRNA and clinical characteristics were examined with Pearson’s correlation coefficient. \(p<0.05\) was considered as statistical significant.

### Results

Demographic characteristics didn’t show significant difference between two groups. Two-tailed t-test showed significantly higher relative levels of IL-23 mRNA (7.98 ± 1.61, \(t=-16.693, p<0.001\)) in peripheral blood of patients with multiple sclerosis in compared to healthy controls (Figure 1). We didn’t find significant correlation between mRNA expressions of IL-23 and severity of illness in MS patients (\(rs=0.065, p=0.817\)). A significant negative correlation was found between severity of illness and age in patients with MS (\(rs=-0.731, p=0.002\)). Multiple sclerosis

| Table 1: Demographic characteristic of participants |
|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| Characteristics             | Controls, N= 15             | MS group, n= 15               | t-test/ X2                  | Significance, p          |
| Age (SD), y                  | 46.40 (18.34)              | 36.60 (6.33)                 | t(df = 28) = 1.95           | 0.061                     |
| Gender, M/F                 | 7/8                         | 6/9                          | \(X^2 = 0.136\) (Fisher exact) | 0.713                     |
| EDSS score                  | -                           | 6.66 (1.23)                 |                             |                           |

Venous blood samples (5 ml) were collected from both control and patient groups and Total RNA was extracted from whole blood using using High pure RNA isolation kit according to the manufacturer’s instructions (Qiagen, USA). Spectrophotometry showed optical density ratio of 260 nm to 280 nm (1.8−2.1) for extracted RNA confirming purity and quantity of extracted RNA in optimal range. Complementary DNA (cDNA) was synthesized with oligo (dT) primer using a reverse transcription kit (Fermentaz, Germany). IL-23 mRNA expression was quantified by real-time PCR using Prime Q-Mastermix reagent kit (2X, Real-time PCR with SYBR Green I) (GenetBio, republic of Korea). All reactions were performed at triplicate. The relative gene expression of the IL-23 was normalized to GAPDH as the reference gene.
patients showed no significant correlation between age and IL-23 mRNA level (rs=0.148, p=0.599). Two-tailed t-test revealed no significant difference in the mRNA expression level of IL-23 between male and female (p =0.493).

A Kruskal-Wallis test showed that there was a statistically significant difference in the relative levels of IL-23 mRNA between three groups ($\chi^2=7.33$, df=2, $p=0.026$).

Mann–Whitney test showed significantly higher relative levels of IL-23 mRNA in peripheral blood of RRMS patients in compared to PPMS patients ($Z=-2.20$, $p=0.028$). Relative levels of IL-23 mRNA in peripheral blood didn’t show a significant difference between RRMS patients and SPMS patients ($Z=-1.937$, $p=0.053$) as well as between PPMS patients and SPMS patients ($Z=-0.577$, $p=0.564$).

**Discussion**

In the present study we found higher mRNA expression of IL-23 gene in peripheral blood of MS patients in compared to control subjects. Our results are in agreement with findings reported by several studies indicating increased plasma and mRNA expression levels of IL-23 in MS patients\(^{12, 14, 15, 20}\). It has found that MS patients show increased secreted levels of IL-23 from monocyte-derived Dendritic Cells (DCs) and also mRNA overexpression of both IL-23p19 and IL-12/23p40\(^{14}\). It has postulated that IL-23 by affecting of immune system through polarization of the innate immune response toward a Th1 bias may plays an important role in the pathogenesis of Multiple sclerosis as a Th1 type T cell mediated inflammatory autoimmune and demyelinating disease of the CNS\(^{14, 21}\).

Proinflammatory cytokine IL-23 plays a key role in chronic inflammatory autoimmune responses and IL-23-deficient mice have shown a resistance to induction of experimental autoimmune encephalomyelitis (EAE)\(^{16}\). In addition, it has found that MS patients have significant higher plasma level of IL-23 when compared to healthy subjects\(^{20}\). A significantly higher serum and CSF levels of IL-23 and positive correlation of IL-23 with IL-17 and with the poor outcome of MS have been reported in MS patients, which suggest IL-23 as a probably sensitive and stable marker for development of MS and therapeutic targets for MS\(^{24}\). The ability of IL-23 in expansion of T helper type 17 (Th17) cells plays a crucial role in the development and the maintenance of autoimmune inflammation\(^{25}\).

Stopping of organ-specific autoimmune inflammation by specific inhibition of the IL-23/IL-17 immune axis in the mouse suggest that specific blockade of the IL-23 immune pathway in humans may be an efficient and undamaged treatment for inflammatory diseases mediated by immune system dysfunction\(^{26}\). Moreover, MS patients have demonstrated increased amounts of IL-12p40 in the nervous system and increased production of IL-12 by PBMCs\(^{27, 28}\). Analysis of mRNA expression levels of IL-23 in PBMCs of untreated MS patients showed increased mRNA expression of IL-23 that treatment with IFN-β induced suppression of IL-23 mRNA levels\(^{15}\). Investigation on the expression of IL-23 in brain tissues of MS patients using in situ hybridization (ISH) and immunohistochemistry showed highly expression of IL-23p19 in active and chronic active lesions\(^{15}\). It has demonstrated that activated macrophage/microglia produce p19 subunit of IL-23 in the lesions in multiple sclerosis\(^{12}\). In addition, reported findings related to production of p40 subunit in these lesions as well as in vitro evidence about production of IL-23 in activated microglia, powerfully verifies this hypothesis that production of bioactive IL-23 by microglia plays a critical role in the pathogenesis of MS\(^{12, 15}\). Moreover, active and chronic active lesions have clearly demonstrated highly mRNA and protein expression of p19 subunit of IL-23 in macrophages/microglia in, but silent lesions and normal brain sections showed low or even undetectable expression\(^{12}\). Association between IL-23 expression and inflammatory and demyelinating lesions suggesting IL-23 as a probably crucial factor that plays an important role in the immunopathogenesis of multiple sclerosis through recruitment and activation of variety of inflammatory cells involved in the induction of chronic inflammation\(^{15}\). Collected data in mice confirm that in the development of EAE the IL-23 plays an important role rather than IL-12\(^{10, 29}\). It has found that therapeutic effect of IL-23 induced by anti-IL-23p19 inhibited relapse of disease during active EAE\(^{26}\). Some mechanisms of action have suggested for anti–IL-23p19 therapeutic inhibition of EAE relapse including inhibition of epitope spreading to additional encephalitogenic epitopes such as proteolipid protein178–191 (PLP178–191) and recruitment of newly activated pathogenic T cells and reduction of the number of inflammatory myeloid cells invading the CNS and diminishing the levels of monokines secreted by
It has been suggested that reduced number of inflammatory myeloid cells that invade the CNS and diminish the concentration of monokines produced by dendritic cells and macrophage such as IL-1, IL-6, and TNF implicate in the mechanism of action of anti–IL-23p19 therapy\textsuperscript{26}. The pathological hallmarks of EAE and MS have attributed to this myeloid cell invasion and production of proinflammatory factors\textsuperscript{26}. It has proposed that the inhibition of IL-23 using designing drugs that target the IL-23p19 or IL-23 receptor might be a novel and promising therapeutic strategy in treatment of autoimmune inflammatory diseases such as multiple sclerosis\textsuperscript{25, 26}. The main finding of our study is that untreated MS patients have increased expression of IL-23 mRNA in the peripheral blood that is agreement with findings reported by previous studies\textsuperscript{12, 14, 19, 20}. Most of these studies measured the common IL-12p40 subunit shared by IL-12 and IL-23. It has demonstrated that patients with both RR and SP MS show overexpression of IL-23p19 and p40, but not IL-12p35 mRNA and also increased IL-23 production compared to healthy subjects\textsuperscript{14}. Accordingly, increased mRNA levels of IL-23 have been found in MS\textsuperscript{12, 13}. It has demonstrated that IL-23 deficient mice are resistant to EAE animal models and untreated MS patients have shown increased levels of IL-23 mRNA that treatment with IFN-β result in suppression of IL-23 mRNA\textsuperscript{10, 19}. In conclusion, we have presented findings that peripheral blood from patients with multiple sclerosis exhibit higher mRNA expression of proinflammatory cytokine IL-23 when compared to healthy controls. These findings support the role of IL-23 in the pathogenesis of multiple sclerosis as an inflammatory autoimmune disease and may have implications as a specific peripheral marker for MS.

References