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Cytokine patterns after therapy with Anovex, Rebif, Betaferon and Cinnovex in relapsing–remitting multiple sclerosis Iranian patients

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Running title: Cytokine patterns in MS

Abstract

Background and aim: Several lines of evidence exist which suggest that changes in the expression of circulating cytokines are linked to the development or reoccurrence of multiple sclerosis (MS). This project aimed to evaluate the serum levels of relevant cytokines after therapy with IFN- β formulations in MS patients.

Material and methods: In this study, blood samples were collected from 70 MS patients undergoing four different types of IFN- β formulation treatment and 100 healthy controls. After 24 months of treatment the serum levels of IL-17A, IL-12 and IFN- γ and IL-10 in patients and healthy controls were analyzed by ELISA.

Finding: Our results showed that serum levels of IL-17A were significantly higher in patients treated with Cinnovex and Anovex when compared to healthy controls. Serum levels of IL-10 were significantly decreased after therapy with Cinnovex, whereas serum levels of IFN- γ were elevated. No difference in serum levels of IL-12 were detected in patients and controls.

Conclusion: Results of our study suggest that Cinnovex and Anovex modulate the immune system less than Rebif and Betaferon in MS patients and that an elevated dosage/regime of Cinnovex and Anovex may be required for better regulation of the immune system in MS patients.

Key Words: Multiple sclerosis, Betaferon, Rebif, Anovex, Cinnovex, cytokine.

Introduction:

Multiple sclerosis (MS) is a complex immune system related disorder [1]. The most prevalent symptoms of MS include the loss of myelin and central nervous system inflammation [2]. However, the mechanisms by which myelin degenerates in MS has yet to be clarified [3]. Current studies showed that several genetic and environmental parameters are involved in the pathogenesis and complications of MS [4]. Immune system related factors, including cytokines have recently been under scrutiny for their crucial roles in MS etiology [5, 6]. Elevated serum levels of IL-17A (as a marker for autoimmunity), IL-12 and IFN- γ (as Th1 cytokines) and declined levels of IL-10 (as T regulatory cytokines) were reported in MS patients [7, 8].

Four β -interferon (IFN- β) formulations; Cinnovex, Avonex Rebif (containing IFN- β 1a) and Betaferon (containing IFN- β 1b), have been used as first-line therapies for the treatment of relapsing–remitting MS (RRMS) in Iranian patients [9, 10]. Clinical presentation, multiplicity of MS plaques in the brain, plurality of the MS attacks and age of initiation of MS are the common factors which informed the choice of interferon

beta prescription in Iranian MS patients [9, 11, 12]. IFN- β 1a is a member of the interferon family used to treat MS and it is produced by mammalian cells whereas IFN- β 1b is produced in modified E. coli [13]. These products have been approved for the treatment of RRMS by the Iranian Health Ministry [9]. Several studies have shown that serum levels of inflammatory cytokines such as IL-17A, IL-12 and IFN- γ decreased after using IFN- β formulations, while, serum levels of IL-10 were increased [7, 14, 15].

Based on the observations reported above this study aimed to compare the serum levels of IL-17A, IL-12, IFN- γ and IL-10 in MS patients after using each of the IFN- β formulations.

Material and methods

Collection of samples:

Specimens were collected from 100 healthy controls and 36, 13, 12 and 9 RRMS patients that were treated for exactly 24 months with Cinnovex, Rebif, Avonex and Betaferon, respectively, from 2008 to 2009, at the Rafsanjan University of Medical sciences. The blood sampling was done after exactly 25 months of therapy (one month after the last treatment injection). The first treatment group received 30 μ g of Cinnovex (Cinnagen, Inc Tehran, Iran) injected intramuscularly once a week for a total of 24

months. The second group received 44 µg of Rebif (Ares-Serono, Geneva, Switzerland) subcutaneously three times a week for 24 months. The third group received 30 µg of Avonex (Biogen Inc., Cambridge, MA, USA) intramuscularly once a week for 24 months and finally, the fourth treatment group received 8 million IU (250 µg) of Betaferon (Schering, Inc Berlin, Deutschland) injected subcutaneously every other day for a total of 24 months. An expert neurologist confirmed the occurrence of MS, according to clinical and paraclinical findings (MRI study, oligoclonal bands in CSF, and evoked potentials) based on McDonald's criteria [16]. The patient groups had the same sex, similar age, duration of disease and socio-economical status. Assessment of socio-economic conditions were measured based on the level of education (diploma: weak, under graduate: moderate and post graduate: high) and monthly income (under \$250: weak, \$250-\$1000: moderate and more than \$1000: high). Healthy control cases were selected from the Rafsanjane population with matched sex, age and socio-economical status. All collection of samples and patient interviews had been pre-approved by the Rafsanjan University of Medical Science's ethical committee. Consent forms were filled out by both patients and controls prior to blood collection being performed.

Detection the serum level of cytokine:

The serum levels of IL-17A, IFN- γ , IL-12 and IL-10 were measured by ELISA (eBioscience, ESP) in patients and healthy controls immediately after blood collection. Assays were performed according to the manufacturer's guidelines. The sensitivity of kits was ± 2 pg/ml and the coefficient variation (CV) for inter- or intra-assay was determined to confirm the assessment reliability.

Statistical analysis:

The differences in variables were analyzed by one-way analysis of variance (ANOVA). When P values were less than 0.05 the data was considered significant

Results

Our results showed that the difference was not significant between groups in relation to age, sex and socio-economic condition (Table 1).

The data shows that the serum levels of IL-17A were 9.73 ± 1.25 , 8.94 ± 1.88 , 12.5 ± 1.94 , 8.36 ± 2.33 and 4.43 ± 1.88 pg/ml in MS patients that were treated by Cinnovex, Rebif, Avonex and Betaferon and healthy controls, respectively (Figure 1). Statistical analysis showed that the serum level of IL-17A was significantly higher in patients treated with Cinnovex and Anovex compared to healthy controls ($p < 0.001$) but the differences between Rebif ($p = 0.162$) and Betaferon ($p = 0.544$) treated groups and

healthy controls were not significant (Figure 1), statistical analysis also showed that the serum levels of IFN- γ were significantly higher in the Cinnovex treated group (11.16 ± 1.83) than healthy controls (3.41 ± 1.83) ($p=0.001$) (Figure 1). Serum levels of this cytokine did not vary in other treated groups in comparison to controls (Figure 1).

Our results also showed that differences between groups regarding the serum level of IL-12 were not significant (Figure 1).

The data shows that serum levels of IL-10 were 3.37 ± 2.8 , 6.54 ± 4.36 , 4.75 ± 4.20 , 14.84 ± 5.76 and 11.54 ± 4.36 pg/ml in MS patients that were treated by Cinnovex, Rebif, Avonex and Betaferon and healthy controls, respectively (Figure 1). Statistical analysis showed that the difference was significant only between the Cinnovex treated group and healthy controls ($p=0.047$).

Discussion:

To minimize the potential effects caused by demography and socio-economics differences in our study, we matched our patient and control groups. Previous studies have shown that expression of cytokines affects the pathogenesis of MS [3]. Results of our study showed that the serum levels of IL-17A and IFN- γ were increased in Cinnovex treated patients, while

serum levels of IL-10 were decreased. Elevated serum levels of IL-17A were also detected in the Avonex treated population.

The key roles of IL-17A, IL-12 and IFN- γ as inducers and IL-10 as an inhibitor of autoimmunity are well established [17, 18]. Several studies showed that serum levels of inflammatory cytokines were significantly increased in MS untreated patients [7, 8, 14]. Interestingly, previous studies showed that using IFN- β formulations, as first line therapies for the treatment of RRMS, could lead to decreased serum levels of inflammatory and increased levels of anti-inflammatory cytokines in treated patients compared to untreated patients [7, 8]. If elevated serum levels of IL-17A, IL-12 and IFN- γ are expected in untreated patients then based on the results reported here it could be concluded that Cinnovex and Anovex modulate the immune system less than Rebif and Betaferon in our studied MS patients, meaning that Rebif and Betaferon are more effective at returning patients' levels of these cytokines to those observed in healthy patients. In addition, CinnoVex had the least effect of the treatments in returning IL-10 serum levels back up to those seen in healthy controls. Furthermore, we concluded that an elevated dosage/regime of Cinnovex and Anovex may be required for better regulation of immune system if they are to be used more effectively as first line therapeutics in our population. Our results showed that Cinnovex

and Anovex had a same effect but differ from the results obtained from Rebif and Betaferon treatment. This may be related to this fact that Cinnovex and Avonex are expressed in Chinese hamster ovary (CHO) cells, therefore they are folded, glycosylated and phosphorylated in the same fashion as they would be in human cells. Betaferon and Rebfin are expressed in bacteria and therefore will have different post-translational modifications, additional studies would be required to determine if these differences have physiological significance in patient treatments.

To our knowledge, this is the first study that showed evaluated serum levels of four cytokines (three inflammatory and one anti-inflammatory) in four IFN- β formulation groups, simultaneously. However, Szczuciński A et al., showed that serum level of CCL2 (MCP-1) chemokines were also increased after two years of Rebif treated of MS patients [19]. Losy J et al., reported that serum levels of IL-12 were higher in IFN- β 1a treated patients than healthy controls, but this was not observed until after 6 months of therapy with IFN- β 1a [15]. Another investigation demonstrated that serum levels of TGF- β were decreased after therapy with IFN- β 1a [14]. Their study also showed that serum level of IL-10 did not vary between patients and controls [14]. In apparent contradiction to our data, Carrieri PB et al., showed that serum levels of IL-10 but not IL-12 were increased in IFN- β 1a treated MS

patients [7]. Previous studies have also shown that the expression of matrix metalloprotease-1 (MMP-1) was increased in MS [20]. In addition, an investigation on the USA population showed that serum levels of tissue inhibitor of MMP-1 (TIMP-1) were increased in IFN- β 1a treated patients [20]. Losy J et al., showed that serum level of MCP-1 and MIP-1 α chemokines did not vary in MS patients after IFN- β 1a therapy [21].

The discrepancies between our results and those of other groups could be explained by the different genetic background of our study population. Furthermore, our Cinnovex treated group was larger than those used by other groups giving this current study greater statistical power thereby identifying significant changes in serum levels of cytokines that other studies may not have been able to detect.

The data would suggest that we do not have the complete picture in terms of the modulation of the immune system in response to MS or therapeutic regimes and additional research is required. Due to the complex nature of RRMS, other aspects of the immune system need to be examined when patients are treated with IFN- β containing formulations.

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Variant	Healthy control	Cinnovex treatment Patient	Rebif treatment Patient	Avonex treatment Patient	Betaferon treatment Patient
Age (year)	35.1 ± 3.4	36.3 ± 3.1 (p=0.9)	35.8 ± 3.3 (p=1.0)	33.7 ± 3.7 (p=0.38)	34.2 ± 4.3 (p=0.9)
Sex					
Female	79 (79%)	27 (75%) (p=0.7)	10 (77%) (p=0.9)	9 (75%) (p=0.7)	7 (78%) (p=0.8)
Male	21 (21%)	9 (25%) (p=0.6)	3 (23%) (p=0.3)	3 (25%) (p=0.6)	2 (22%) (p=0.7)
Socio-economic status					
Weak	22 (22%)	9 (25%) (p=0.5)	3 (23%) (p=0.8)	3 (25%) (p=0.5)	2 (22%) (p=1.0)
Medium	47 (47%)	16 (44 %) (p=0.2)	6 (46%) (p=0.8)	6 (50%) (p=0.1)	4 (44%) (p=0.2)
High	31 (31%)	11 (31%) (p=1.0)	4 (31%) (p=1.0)	3 (25%) (p=0.1)	3 (34%) (p=0.6)

Table 1 Demographic and socioeconomic conditions of MS patients and controls

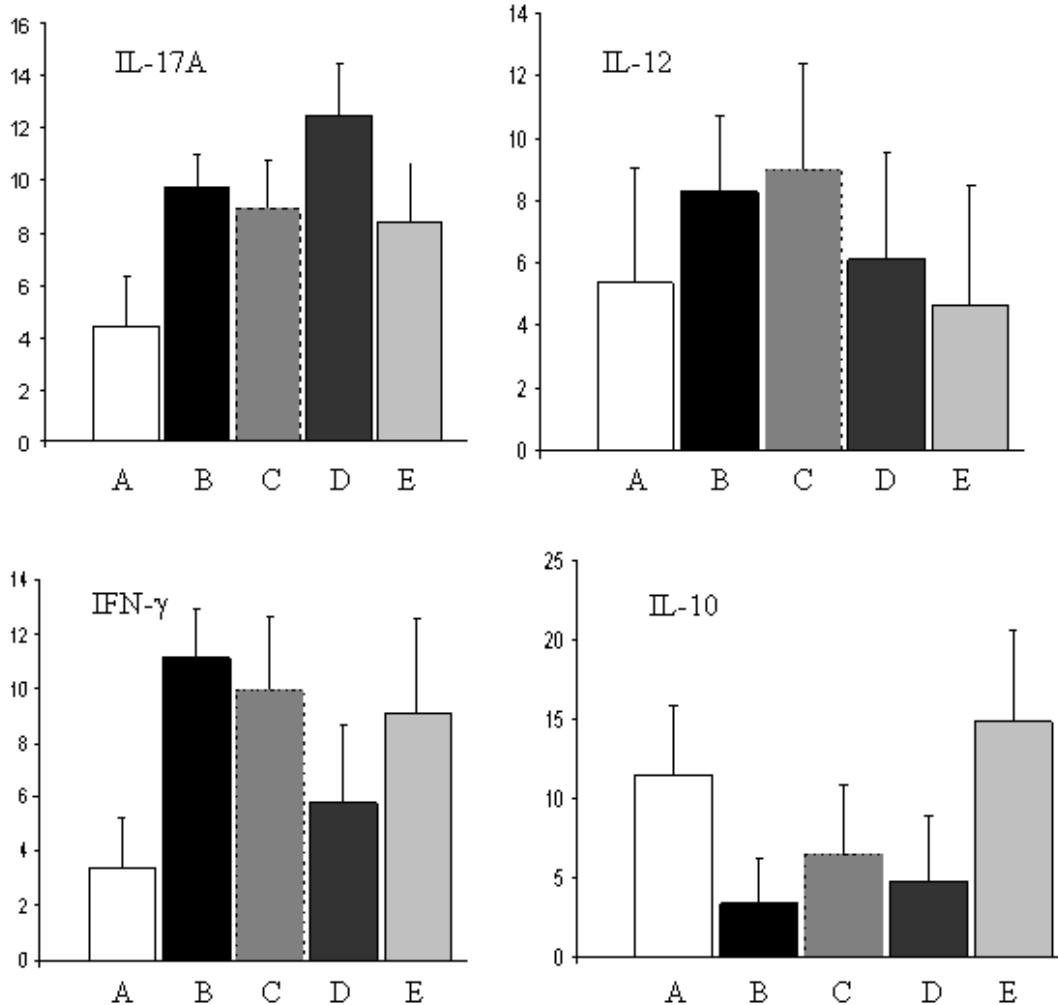


Figure 1. Serum levels of IL-17A, IL-12, IFN- γ and IL-10 in MS and healthy controls after IFN- β formulation treatment. Results are shown as mean \pm standard error. Columns A are the Healthy controls and columns B, C, D and E are the Cinnovex, Rebif, Anovex and Betaferon treated MS patients, respectively.