CLINICAL AND LABORATORY STUDY OF PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES IN WOMEN WITH MULTIPLE SCLEROSIS

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system characterised with a complex system of interactions between proinflammatory and anti-inflammatory cytokines in its course.

AIM: The aim of the present study was to investigate the serum levels of cytokines TNF-α, IFN-γ, IL-4 and IL-10 in female patients with MS and healthy individuals, the changes occurring in the relapse and remission phases of the disease and their correlation with the severity of the neurological deficit.

PATIENTS AND METHODS: Thirty-five women with relapsing-remitting MS were examined. The patients' age ranged between 18 and 50 years and MS was verified clinically and by magnetic resonance imaging according to the McDonald criteria. Thirteen of the patients were treated with interferon-β-1b. The serum concentrations of TNF-α, IFN-γ, IL-4 and IL-10 were determined twice – in relapse and in remission – using an enzyme-linked immunosorbent assay (ELISA). The control group consisted of 35 age-matched healthy females.

RESULTS: The comparison of cytokine serum concentrations during the two phases of the disease showed significant elevation of the TNF-α serum levels in the relapse phase and of IL-4 – in the remission phase. The comparison between the patients and the healthy control subjects demonstrated statistically significant lower concentrations of TNF-α in remission patients and higher concentrations of IL-10 in relapse patients. The patients with interferon-β-1b treatment showed different profile of cytokine secretion from the patients without interferon-β-1b treatment. Interferon-β-1b-treated patients showed significantly lower serum levels of TNF-α and IFN-γ during the relapse phase and higher TNF-α and IL-10 serum levels during the remission phase compared with the untreated patients.

CONCLUSIONS: Serum levels of TNF-α and IL-4 objectively reflect the immune response during relapse and remission of the disease. The severity of neurological deficit as estimated with the expanded disability status scale (EDSS) does not depend on the serum levels of TNF-α, IL-10 and IFN-γ in the two phases of MS.

Key words: multiple sclerosis, cytokines, interferon-β-1b

INTRODUCTION

Multiple sclerosis is an immunological organ-specific disease with persisting immunological dysbalance in the patient serum and cerebrospinal fluid. Experimental and clinical studies have revealed different profiles of cytokine secretion during relapse and remission of MS. It is thought that it is the Th1-dependent secretion of TNF-α, IFN-γ, IL-12 that triggers the autoimmune reaction, while the Th2 synthesis of IL-4, IL-6, and IL-10 has anti-inflammatory effects. There is a large body of evidence showing that some mediators of inflammation, which include cytokines, can protect the neurons from degeneration at some phases of the disease.

The studies of cytokine profiles in MS patients and the correlations with the clinical picture and progress of the disease have yielded contradictory results. Finding what roles play each of the immune components of the MS pathological process is of crucial importance if we want to determine the “targets” for the drugs and the intensity of their therapeutic effect determined very accurately.

The aim of the present study was to investigate...
the serum levels of cytokines TNF-α, IFN-γ, IL-4 and IL-10 in female patients with MS and healthy individuals, the changes which occur during the relapse and remission phases of the disease and their correlation with the severity of the neurological deficit.

PATIENTS AND METHODS

PATIENTS
We conducted an open, prospective and randomized study at the Department of Neurology, Medical University, Plovdiv between 2003 and 2007. The study included 35 female patients with MS and a control group of 35 healthy women aged between 18 and 50 years.

Inclusion criteria
1. Gender – females;
2. Age – between 18 and 50 years;
3. Relapsing-remitting MS diagnosed according to the McDonald criteria (2000) and verified by magnetic resonance tomography.

Exclusion criteria
1. Endocrinological, renal, hepatic and immunological disorders;
2. Acute and chronic infections;
3. Therapy with immunosuppressive drugs;
4. Therapy with corticosteroids three months before the first examination.

The serum levels of TNF-α, IFN-γ, IL-4 and IL-10 were measured twice:
• during the relapse phase defined as the appearance of new symptoms or deterioration of already existing ones, lasting more than 24 hours and at least a 30-day period of improvement or stabilization of the neurological status after the previous relapse.
• during the remission phase (at least 2 months after the previous exacerbation).

METHODS

Clinical tests
• McDonald criteria (2000) for diagnosis of MS;
• Expanded Disability Status Scale (EDSS) to determine the severity of the neurological deficit.

Laboratory tests
Serum TNF-α, IFN-γ, IL-4 and IL-10 levels of the MS patients and the control subjects were measured twice; venous blood for examination was taken using ordinary procedures. The obtained serum was stored at -70°C until the analysis was carried out. The laboratory analyses of MS patients were performed during relapse and remission. For the control group the cytokine serum levels were estimated as the mean of two measurements. The TNF-α, IFN-γ, IL-4 and IL-10 serum levels were determined by ELISA using kits of BIOSOURCE Europe S.A. The absorption, which is proportional to the cytokine concentration, was measured colorimetrically using TECAN ELISA reader at 450 and 630 nm. The concentration of each of the cytokines was calculated with the help of a standard curve.

STATISTICS
Statistical analysis were performed using SPSS 13.0; the level of significance was accepted at p < 0.05. We used the Independent Samples t-test, the paired-samples t-test, the Wilcoxon signed-rank test and correlation analysis.

The study was approved by the Scientific Ethics Commission of the Medical University, Plovdiv, Proceedings №3/28.03.2005.

RESULTS
The clinical characteristics of the study contingent are presented in Table 1.

Thirteen of the study patients (37.14%) were treated with interferon-β-1b – group A, unlike the rest 22 patients (62.86%) included in group B.

The mean level of neurological deficit as estimated with the EDSS was significantly lower in remission compared with the one in relapse p < 0.001 (t = 9.62).

The results of the investigated immune indicators (serum cytokines) are presented in Fig. 1.

The changes of the cytokine serum levels were analyzed using the Independent Samples t-test. The comparison between the results of the patients and the healthy controls showed statistically significant lower concentrations of TNF-α in the patients during remission (p < 0.05, t = 1.99) and higher concentrations of IL-10 during relapse (p < 0.01, t = 3.18). We noticed the following tendencies: higher mean values of IFN-γ and IL-10 in the patients in remission compared with the controls. During the two phases of clinical manifestations the changes of the cytokine levels were analyzed using the Wilcoxon signed-rank test. The following statistically significant correlations were found: significant elevation of IL-4 levels (p < 0.05) during remission compared to the levels during relapse and of TNF-α levels (p < 0.01) during exacerbation compared to the levels during remission (Fig. 1).

Tables 2 and 3 present the results from the investigation of the cytokine levels in group A.
(interferon-β-1b-treated patients) and group B (interferon-β-1b non-treated patients) analyzed using the paired-samples t-test.

The IFN-γ levels were significantly elevated in group A during remission compared with the relapse levels (p < 0.05, t = 2.19). The changes in TNF-α, IL-4 and IL-10 levels during the two periods of clinical manifestations could not reach statistical significance.

In group B statistically significant elevation of TNF-α (p < 0.05, t = 2.19) and of IL-10 (p < 0.05, t = 2.31) were found during exacerbation compared to the remission levels.

The concentrations of the investigated cytokines in group A and group B during the different phases of disease are compared in Figs 2 and 3.

The group A patients had significantly lower concentrations of TNF-α (p < 0.05, t = 4.20) and IFN-γ (p < 0.01, t = 6.56) during relapse and elevated concentrations of TNF-α (p < 0.05, t = 4.42) and IL-10 (p < 0.05, t = 4.07) during remission in comparison with group B patients.

The correlation analysis revealed moderate positive correlation between the degree of disability as estimated with the EDSS in remission and the concentration of IL-4 during the same period (p < 0.05, r = 0.38).

**DISCUSSION**

The immune response to the myelin antigens in MS is still not entirely clear. TNF-α is given as one of the major pro-inflammatory cytokines in demyelinating disorders; it is considered to possess myelinotoxic effects. The present study revealed statistically significantly lower levels of TNF-α in remission and elevated levels of IL-10 in relapse in the MS patients compared with healthy individuals.

**Table 1. Clinical characteristics of studied contingent**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (years) mean ± SE</th>
<th>Duration of the disease (years) mean ± SE</th>
<th>EDSS mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>35</td>
<td>34.80 ± 1.53</td>
<td>5.66 ± 0.91</td>
<td>3.63 ± 0.25</td>
</tr>
<tr>
<td>Controls</td>
<td>35</td>
<td>30.45 ± 1.01</td>
<td>-</td>
<td>2.26 ± 0.22*</td>
</tr>
</tbody>
</table>

* p < 0.001.

![Figure 1. Serum cytokine concentrations of studied contingent.](image-url)
The significant decrease of TNF-α levels in remission which become lower than those of controls is probably an expression of abating autoimmune demyelination during clinical improvement. It is supposed that the elevated IL-10 serum levels during the relapse phase result from the activation of the regulatory mechanisms of the body which suppress autoimmune reactions. On the other hand several experimental studies reported exacerbation of the disease clinical manifestations after administration of IL-10 in doses higher than the physiological ones. The significant elevation of IL-10 in patients during remission, which was found in the present study and compared to the healthy controls supported this point of view. The revealed tendency of increased secretion of mediators with the opposite effect (IFN-γ and IL-10) in remission patients compared with the controls showed persisting regulatory dysbalance in the profile of the cytokines from the Th1/Th2 system. This hypothesis was supported by the significant elevation of the IL-10 serum levels during relapse in the interferon-β-1b non-treated patients. Similar data were reported by K. Hohnoki et al., M. C. Rodriges-Sanz, et al. and de Andres S, et al.

The significant elevation of IL-4 plasma levels in all patients in remission compared with that in exacerbation is probably an indication of a shift from a Th1 to a Th2 immune response. The results of our study are consistent with the conclusion F Weber makes that a shift of the immune reaction from type Th1 to type Th2 should result in suppression of the inflammatory process in the central nervous system. The hypothesis of pro-inflammatory pathogenic effect of TNF-α and IFN-γ is based on clinical evidence: statistically significant elevation of TNF-α during relapse and increased frequency of disease exacerbations after intravenous treatment with IFN-γ. The significant elevation of TNF-α serum levels during exacerbation we found in this study in comparison with the levels in remission

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>MS phase</th>
<th>n</th>
<th>Mean ± SE pg/ml</th>
<th>SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>relapse</td>
<td>13</td>
<td>21.72 ± 3.18</td>
<td>11.48</td>
<td>1.53</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>13</td>
<td>19.63 ± 3.68</td>
<td>13.25</td>
<td>2.19</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>relapse</td>
<td>13</td>
<td>2.86 ± 0.81</td>
<td>2.92</td>
<td>0.13</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>13</td>
<td>4.35 ± 0.89</td>
<td>3.21</td>
<td>2.74</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>IL-10</td>
<td>relapse</td>
<td>13</td>
<td>6.70 ± 1.85</td>
<td>6.68</td>
<td>0.72</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>13</td>
<td>7.03 ± 1.69</td>
<td>6.12</td>
<td>3.28</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>IL-4</td>
<td>relapse</td>
<td>13</td>
<td>2.36 ± 0.60</td>
<td>2.17</td>
<td>0.75</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>13</td>
<td>3.14 ± 0.86</td>
<td>3.11</td>
<td>2.31</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
confirms the role of this cytokine as a factor triggering the immune inflammatory process. There are some difficulties in the clinical interpretation of the changes we found in the IFN-γ concentrations during the two phases of the disease. We established significant differences in all patients individually in the changes of IFN-γ and a tendency towards elevation of its plasma levels during remission compared with the relapse phase. Y. Miyazaki et al. reported that interferon-β-1b exerted a significant suppressive effect on the IFN-γ synthesis by the peripheral myelin-reactive CD4+ CD28+ T cell subpopulations, but had an insignificant effect on the production of IFN-γ by the CD4+ CD28- T lymphocyte branches. We suggest that the results of the present study reflect such an aspect of dysbalance of the cytokine-regulatory system. During remission interferon-β-1b-treated patients demonstrated statistically significantly elevated IL-10 plasma levels compared with this indicator in non-treated patients for the same clinical period. We accept that these results are associated with the increased synthesis of IL-10 by the auto-reactive T lymphocytes under the influence of interferon-β-1b which was experimentally proved by Zang YC, et al. and S Chabot and VW Yong in their studies.

We found no causal relationship between the...
severity of disability (EDSS) and the level of TNF-α, IL-10 and IFN-γ during the two phases of clinical manifestations. That is why we suppose that the regulatory cytokine dysbalance and EDSS reflect the degree of immunological inflammation with different specificity. Literature data show direct correlation between the severity of neurological deficit and the loss of axons, but the axonal lesions are of different mechanisms: immunological-inflamatory, degenerative (Wallerian degeneration) and ischemic. 4

CONCLUSIONS
The serum levels of TNF-α and IL-4 objectively reflect the activity of the immune response during relapse and remission of MS.

The severity of neurological deficit as estimated by EDSS does not depend on the serum levels of TNF-α, IFN-γ and IL-10 during the two periods of MS.

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Клинико-лабораторное исследование провоспалительных и антивоспалительных цитокинов у женщин с множественным склерозом

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Резюме

Введение: Множественный склероз (МС) представляет собой аутоиммунное, демиелинизирующее заболевание центральной нервной системы в ходе которого развивается сложная сеть взаимодействий между провоспалительными и антивоспалительными цитокинами.

Цель: Исследовать сывороточные уровни TNF-α, IFN-γ, IL-4 и IL-10 у женщин с МС и у здоровых лиц, а также изменения, наступающие во время приступа и во время ремиссии заболевания и их связь с тяжестью неврологического дефицита.

Материал и методы: Обследовано 35 женщин в возрасте от 18 до 50 лет с приступно-ремитентным, клинически и резонансно подтвержденным МС по критериям Mc Donald. 13 женщин лечены интерфероном β-1b. Сывороточные концентрации TNF-α, IFN-γ, IL-4 и IL-10 определены двукратно – во время приступа и во время ремиссии с помощью энзимосвязанного иммуносорбентного теста (ELISA). Контрольная группа включает 35 здоровых женщин того же возрастного интервала.

Результаты: При сравнении сывороточных концентраций цитокинов во время обоих фаз заболевания устанавливаются значимо более высокие уровни TNF-α во время приступа и IL-4 – во время ремиссии. При сопоставлении результатов пациенток и здоровых женщин зарегистрированы статистически значимо более низкая концентрация TNF-α у больных во время ремиссии и более высокая концентрация IL-10 во время приступа. У пациенток с и без интерферона β-1b наблюдается различный профиль секреции цитокинов. Пациентки, леченные препаратом интерферон β-1b, имеют значительно более высокие TNF-α и IFN-γ во время приступа и более высокие TNF-α и IL-10 в состоянии ремиссии по сравнению с нелеченными.

Выводы: Сывороточные уровни TNF-α и IL-4 объективно отражают активность иммунной реакции во время двух периодов клинических проявлений МС (приступ и ремиссия). Тяжесть неврологического дефицита, оцененная с помощью EDSS, не зависит от сывороточных уровней TNF-α, IL-10 и IFN-γ во время двух периодов клинических проявлений.