

Changes in Serum Cytokine Profile and Deficit Severity in Patients with Relapsing-Remitting Multiple Sclerosis

Georgi Slavov¹

¹ Department of Neurology, Medical University of Plovdiv, Plovdiv, Bulgaria

Corresponding author: Georgi Slavov, Department of Neurology, Medical University of Plovdiv, 15A Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria; Email: georgi.slavov.15130@gmail.com; Tel.: +359 885 247 929

Received: 15 May 2022 ♦ **Accepted:** 25 Oct 2022 ♦ **Published:** 31 Aug 2023

Citation: Slavov G. Changes in serum cytokine profile and deficit severity in patients with relapsing-remitting multiple sclerosis. *Folia Med (Plovdiv)* 2023;65(4):625-630. doi: 10.3897/folmed.65.e86576.

Abstract

Introduction: In experimental autoimmune encephalomyelitis, neurological deficit correlates with axonal loss and the CD8+ T cells are a likely mediator of axonal damage. In relapsing-remitting multiple sclerosis, there is a correlation of the immune inflammatory activity in the lesion foci with the axon transection.

Aim: To evaluate the changes occurring in the serum concentrations of TNF- α , IFN- γ , IL17, TGF- β 1, IL4, and IL10 during relapse and remission, and their correlations with the degree of neurological deficit.

Materials and methods: In an open, prospective, case-control study conducted between 2012 and 2014, we examined 86 people: 46 patients (33 women and 13 men) and 40 healthy individuals (20 women and 20 men).

Serum cytokine concentrations were analyzed using ELISA – once in the controls, twice in the patients during the relapse and remission of the condition.

The collected data were analyzed using SPSS 19.0. We used the Kolmogorov-Smirnov test, the independent sample *t*-test, the Spearman and Pearson correlation, the Mann-Whitney test, and regression analysis.

Results: Immune imbalance was found in the patients compared to the healthy controls in both relapse and remission. During the relapse, the IFN- γ levels were significantly increased compared to the levels in remission ($p=0.017$). During remission, the deficit was statistically significantly improved ($p<0.001$) and the anti-inflammatory IL4 and TGF- β 1 were increased compared to their levels in the exacerbation period ($p=0.006$ and $p=0.009$, respectively). There was a causal relationship between the serum concentrations of TNF- α and EDSS in relapse (Vanetto-significance). During this phase, the regression analysis established two factors that had statistically significant influence on the deficit severity – TNF- α and IL17 ($t=2.093$, $p=0.042$; $t=-2.140$, $p=0.038$).

Conclusions: IL17 and TNF- α serum concentrations are significant factors for the neurological deficit severity. The levels of IFN- γ , IL4, and TGF- β 1 during both periods are criteria for evaluation of the immune inflammatory activity.

Keywords

cytokines, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is an organ-specific disease with various patterns of myelin destruction in the central nervous system (CNS) and persistent immune imbalance in the periphery. Recent studies provide evidence of a causal relationship between intrathecal immunoglobulin synthesis and cerebral atrophy associated with axon loss.^[1] An immune mechanism of axonal transection is also assumed, but there is currently no conclusive evidence of direct disorders caused by specific immune mediators. In the experimental model (EAE), the deficit severity correlates with axon loss and the CD8+ T cells are a likely mediator of axonal damage.^[2-5] Experimental clinical results show that Th1-dependent synthesis of TNF- α , IFN- γ , and Th17-mediated production of IL17 and IL22 are inducers of the immune response while Th2-mediated secretion of IL4, IL5, IL6, and IL13 has anti-inflammatory effects. The peripheral imbalance is characterized by impaired function of the regulatory CD4+ CD25+ FoxP3 subpopulation producing the anti-inflammatory cytokines (cc) TGF- β 1 and IL10.^[6-11] Establishing scientific facts about the causal relationship between changes in the immune parameters and clinical indicators of disease activity is a prerequisite for achieving optimal suppression of the immune response by including targeted immunoeffective drugs.

AIM

The current study sought to assess changes in serum concentrations of TNF- α , IFN- γ , IL17, TGF- β 1, IL4, and IL10 during the relapse and remission phases, as well as their relationship to the severity of the neurological deficit.

Design

This open, prospective, case-control study was conducted between 2012 and 2014. Ethical approval was granted for this study by the Ethics Committee of the Medical University of Plovdiv (No. 3/05.07.2012).

Cohort

Eighty-six subjects were studied: 46 patients (33 women and 13 men) and 40 healthy people (20 women and 20 men).

Inclusion criteria

Age from 18 to 50 years; patients with relapsing-remitting type of MS, consecutive attacks, degree of neurological deficit 1.5-5.0 EDSS.

Exclusion criteria

Primary and secondary progressive course, acute and chronic infections, disease modifying drug therapy in the

year preceding the date of registration, corticosteroid treatment 3 months before the first test, and treatment with immunosuppressive drugs.

MATERIALS AND METHODS

Clinical

The McDonald's (2010) criteria for MS diagnosis, Expanded Disability Status Scale (EDSS) for determining the degree of neurological deficit. The relapse is defined as the manifestation of new neurological signs or worsening of old ones lasting >24 hours, aggravation of EDSS by ≥ 0.5 in the absence of fever and after a period of 30 days improvement, or stable condition after another attack. Patients with relapse were hospitalized in the Clinic of Nervous Diseases at St George University Hospital, Plovdiv. Treatment with methylprednisolone (Sopharma) 500 mg i.v. in the morning, at a course dose of 2500 mg.

Laboratory

The serum levels of TNF- α , IFN- γ , IL17, TGF- β 1, IL4, and IL10 in the patients were studied twice, during relapse and during remission, at least two months after the relapse. The cytokines of the control group were studied once. The serum cytokine concentrations in pg/ml were determined with ELISA. Venous blood taken routinely was used in the study. The obtained serum was stored at -20°C until the test was performed. The serum levels were determined with enzyme-linked immunosorbent assay with original ELISA kits (eBioscience, Austria, catalogue No. BMS2082). Each sample was examined by duplicate analysis. They were read serially by an ELISA-reader (Sirio-microplate reader SEAC-Italy) at $\lambda=450$ nm (reference $\lambda=620$ nm). The concentration of each cytokine was calculated by plotting a standard curve.

Statistical analysis

Data were analyzed using SPSS 19.0. The following methods were used: the Kolmogorov-Smirnov test to determine the distribution, the independent samples *t*-test, the paired *t*-test, the Spearman correlation, the Mann-Whitney test, and regression analysis.

RESULTS

The clinical characteristics of the cohort are presented in **Table 1**.

The two groups were age-matched ($t=1.76$, $p>0.05$). The proportion of patients with MS onset between 17 and 30 years of age (52%) was the largest. The majority of patients had had multiple sclerosis for up to 5 years (57%). All pa-

Table 1. Clinical characteristics of the cohort

Indicator Groups	N	Sex		Years Mean ± SEM	Years of disease	Years at first manifestations
		F n	M n			
Controls	40	20	20	34.67±1.15	-	-
Patients	46	33	13	37±1.83	7.17±1.17	30±1.29

tients had another relapse by the end of the first month, with the greatest proportion being those registered by the end of the third week (76.08%, n=35). The average severity of neurological deficit in relapse was 2.36±0.11, in remission - 1.64±0.11. The neurological deficit significantly decreased during remission compared to that in relapse ($p<0.001$).

Tables 2 and 3 present the results of the studied cytokines during the two periods after comparison with the controls.

Significantly low levels of TNF- α , IL10, and IFN- γ were found in the patients during both periods; significantly higher levels of TGF- β 1 were found during remission compared to the controls. The cytokine changes during both periods are presented in **Table 4**.

During remission, the concentrations of IL4 and TGF- β 1 increased statistically significantly, and the mean IFN- γ levels decreased. Correlation analysis and single-factor linear regression were used to analyze the relationship between the changes in the studied cytokines and the deficit severity during the two phases.

During the relapse, we found a weak statistically significant correlation between TNF- α levels and the deficit severity ($r=0.301$).

During exacerbation, IL17 and TNF- α affected the neurological deficit severity.

Table 4. Changes in mean serum cytokine concentrations during the periods of relapse and remission

Cytokines pg/ml	Clinical period	U	P
IL17	Relapse	0.53	>0.05
	Remission		
IL4	Relapse	2.73	0.006
	Remission		
IL10	Relapse	0.25	>0.05
	Remission		
TNF- α	Relapse	0.49	>0.05
	Remission		
TGF- β	Relapse	2.74	0.009
	Remission		
IFN- γ	Relapse	2.39	0.017
	Remission		

DISCUSSION

During both relapse and remission, impaired immune tolerance was found in the patients compared to the healthy people: significantly low levels of TNF- α , IFN- γ , IL10 in both phases and TGF- β 1 increase during remission. Other

Table 2. Comparison of the pro-inflammatory cytokines levels during the two clinical periods with those in the controls

Groups	N	IL17		TNF- α			IFN- γ			
		Mean/SD pg/ml	U	P	Mean/SD pg/ml	U	P	Mean/SD pg/ml	U	P
Controls	40	44.908/19.765			4.447/2.434			5.065/4.777		
Patients in relapse	46	58.346/48.973	-0.77	>0.05	3.493/2.553	-2.40	<0.05	1.969/2.117	-3.68	<0.001
Patients in remission	46	53.670/39.776	-1.36	>0.05	3.327/1.925	-2.49	<0.05	1.145/1.142	-5.04	<0.001

Table 3. Comparison of the anti-inflammatory cytokines levels before the two clinical periods with those in the controls

Groups	N	IL4		IL10			TGF- β			
		Mean/SD pg/ml	U	P	Mean/SD pg/ml	U	P	Mean/SD pg/ml	U	P
Controls	40	15.148/19.204	-	-	2.631/1.466	-	-	0.504/0.2778	-	-
Patients in relapse	46	11.506/7.372	-0.33	>0.05	1.666/1.461	-3.55	<0.001	0.540/0.190	-1.50	>0.05
Patients in remission	46	16.554/15.227	-1.05	>0.05	1.744/1.664	-2.97	<0.01	0.714/0.405	-2.09	<0.05

Table 5. Single-factor regression linear model for EDSS and IL17 during relapse

Model	Indicator						
	Non-standardized coefficients		Standardized coefficients	t	P	95.0% Confidence interval for B	
	B	Std. Error	Beta			Lower	Upper
1 (Constant)	2.633	0.167		15.797	0.000	2.297	2.969
IL17 in relapse	-0.005	0.002	-0.307	-2.140	0.038	-0.009	0.000

a. dependent variable: EDSS in relapse

Table 6. Single-factor regression linear model for EDSS and TNF- α during relapse

Model	Indicator						
	Non-standardized coefficients		Standardized coefficients	t	P	95.0% Confidence interval for B	
	B	Std. Error	Beta			Lower	Upper
1 (Constant)	2.050	0.182		11.256	0.000	1.683	2.417
TNF- α in relapse	0.088	0.042	0.301	2.093	0.042	0.003	0.174

a. dependent variable: EDSS in relapse

authors have found high concentrations of TGF- β 1, IFN- γ , TNF- α , and IL6 before exacerbation, and suggest compensatory excess activity or fluctuations in antimyelin reactivity.^[12,13] We assume that the obtained results also reflect this aspect of cytokine secretion. Pro-inflammatory IFN- γ dominates during the deficit aggravation phase. Several studies have identified IFN- γ as a marker for assessing the Th1-induced pro-inflammatory activity.^[14-18] Some experimental findings contradict this hypothesis. In monkeys treated with IFN- γ – reduction of inflammation. Reyers et al. assume anti-inflammatory effects and synergistic suppressive action with IFN- β on the progression of the experimental model.^[19] The result obtained for a significant increase in IFN- γ during the relapse, we evaluate as a sign of pro-inflammatory activity. During remission, the deficit severity significantly decreases, and IL4 and TGF- β 1 increase. Numerous reports confirm the anti-inflammatory profile of these cytokines: higher IL4, TGF- β mRNA serum levels in patients with mild deficit compared to cases of severe disability, negative correlation between serum TGF- β 1 concentrations and magnetic resonance imaging.^[20-22] In this study, we have established a causal relationship between TNF- α levels and the deficit severity during relapse. Two factors with statistically significant effect on the deficit severity were found during the same clinical phase – TNF- α and IL17. Other observations during the period of disease activity establish high concentrations of TNF- α in the cerebrospinal fluid of the patients, compared to the healthy, high levels of ICAM1 and TNF- α receptors in the cerebrospinal fluid of patients.^[23] A study of a therapeutic intervention that blocks the TNF- α effects shows deterioration of the disease, which suggests another aspect of its action – neuroprotective/anti-inflammatory.^[24] We evaluate the obtained results as a sign of pro-inflammatory

effects. The information about the immunomodulatory potential of IL17 is not definitive. In animals with suppressed IL17 expression, the model has a reduced deficit. In newborn mice treated with myelin proteins and increased IL17 secretion, the experimental model did not develop.^[25-28] Other results show pro-inflammatory effects: elevated IL17 concentrations in the patients compared to the control, significantly higher proportion of ThIL17 lymphocytes during exacerbation compared to remission.^[28-31] According to the contemporary notion of plasticity/ instability of the differentiation program into T-regulatory or Th17 pro-inflammatory phenotype, the low levels of TGF- β 1 in the presence of IL6, IL21, IL23 rearrange the program to polarization into Th17 phenotype.^[32,33] This study did not find significant differences between the changes in IL17 during the two phases and compared to the controls.

CONCLUSIONS

The IL17 and TNF- α serum concentrations are factors for the neurological deficit severity and further studies are needed to clarify their role.

The levels of IFN- γ , IL4, and TGF- β 1 during both periods are criteria for evaluation of the immune inflammatory activity.

Acknowledgements

The authors have no support to report.

Funding

The authors have no funding to report.

Competing Interests

The authors have declared that no competing interests exist.

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Изменения цитокинового профиля сыворотки крови и выраженность дефицита у пациентов с рецидивирующе-ремиттирующим рассеянным склерозом

Георги Славов¹

¹ Кафедра неврологии, Медицинский университет - Пловдив, Пловдив, Болгария

Адрес для корреспонденции: Георги Славов, Кафедра неврологии, Медицинский университет - Пловдив, бул. „Васил Априлов“ № 15А, 4002 Пловдив, Болгария; Email: georgi.slavov.15130@gmail.com; тел.: +359 885 247 929

Дата получения: 15 мая 2022 ♦ **Дата приемки:** 25 октября 2022 ♦ **Дата публикации:** 31 августа 2023

Образец цитирования: Slavov G. Changes in serum cytokine profile and deficit severity in patients with relapsing-remitting multiple sclerosis. Folia Med (Plovdiv) 2023;65(4):625-630. doi: 10.3897/folmed.65.e86576.

Резюме

Введение: При экспериментальном аутоиммунном энцефаломиелите неврологический дефицит коррелирует с потерей аксонов, а CD8+ Т-клетки являются вероятным медиатором повреждения аксонов. При рецидивирующе-ремиттирующем рассеянном склерозе имеется корреляция иммуновоспалительной активности в очагах поражения с перерезкой аксона.

Цель: Оценить изменения сывороточных концентраций TNF- α , IFN- γ , IL17, TGF- β 1, IL4 и IL10 в период обострения и ремиссии и их взаимосвязь со степенью неврологического дефицита.

Материалы и методы: В открытом проспективном исследовании случай-контроль, проведённом в период с 2012 по 2014 г., обследовано 86 человек: 46 больных (33 женщины и 13 мужчин) и 40 здоровых лиц (20 женщин и 20 мужчин).

Концентрацию цитокинов в сыворотке крови анализировали с помощью ELISA – однократно в контроле, двукратно у больных в период рецидива и ремиссии состояния.

Собранные данные были проанализированы с помощью SPSS 19.0. Мы использовали тест Колмогорова-Смирнова, t-тест для независимых выборок, корреляцию Спирмена и Пирсона, тест Mann-Whitney и регрессионный анализ.

Результаты: Иммунный дисбаланс был обнаружен у пациентов по сравнению со здоровым контролем как при рецидиве, так и при ремиссии. Во время рецидива уровни IFN- γ были значительно повышены по сравнению с уровнями в период ремиссии ($p=0.017$). В период ремиссии дефицит статистически значимо уменьшался ($p<0.001$), а противовоспалительные IL4 и TGF- β 1 повышались по сравнению с их уровнями в периоде обострения ($p=0.006$ и $p=0.009$ соответственно). Существовала причинно-следственная связь между концентрациями TNF- α в сыворотке крови и EDSS при рецидиве (значимость Vanetto). На этом этапе с помощью регрессионного анализа были установлены два фактора, оказывавших статистически значимое влияние на выраженность дефицита – TNF- α и IL-17 ($t=2.093$, $p=0.042$; $t=-2.140$, $p=0.038$).

Заключение: Концентрации ИЛ-17 и ФНО- α в сыворотке крови являются значимыми факторами тяжести неврологического дефицита. Уровни IFN- γ , IL4 и TGF- β 1 в оба периода являются критериями оценки иммуновоспалительной активности.

Ключевые слова

цитокины, рассеянный склероз
