The serum levels and clinical significance of TNF-α, IL-4, IL-5, TGF and IFN-γ in patients with acute optic neuritis

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Abstract: Aim: To analysize the level of TNF-α, IL-4, IL-5, TGF and IFN-γ in acute optic neuritis patients. **Methods:** All patients were assigned to four groups, control, LogMAR>0.4, LogMAR<0.4 and glucocorticoid treatment group. The blood from all patients are obtained and analysized by ELISA. **Results:** IFN-γ was decreased significantly, which similar to control group level (P>0.05). IL-4 was higher in control group compared to other groups (P<0.05, P<0.01). For IL-10, TGF and RNF-a, there were similar level between glucocorticoid treatment and control groups (P>0.05), moreover, IL-10 in LogMAR>0.4 groups was also similar to control group (P>0.05). However, TGF and TNF-a in LogMAR>0.4 and LogMAR<0.4 groups, IL-10 in LogMAR<0.4 are higher compared to control groups (P<0.05,P<0.01). Compared to controls, patients showed significantly higher levels of IL-10 and TNF-a, increased IFN-γ/IL-4 (Th1/Th2) ratio (P<0.05). **Conclusion: O**ur research provide a theoretical basis for the treatment of acute optic neuritis.Additionally, both IL-4 and IL-10 might represent potential diagnostic markers for the disease.

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1. Introduction

Optic neuritis is a condition involving primary inflammation of the optic nerve. It may be associated with a variety of systemic autoimmune disorders, but the most common form,acute demyelinationg optic neuritis, is best known for its association with multiple sclerosis.(Dooley and Foroozan 2010)

Cytokines can be defined as regulatory polypeptides secreted by a wide variety of cells and exerting diverse effects in host defence and tissue repair. (Dinarello 2000; Tostes et al.,2012) Multiple sclerosis (MS) is considered to be at least partly caused by autoimmune reactions, and cytokines such as tumor necrosis factor (TNF)-a, interleukins (IL) and interferon (IFN)-γ have all been implicated in the pathogenesis.(Biswas et al.,2012; Josyula et al.,2012) Compelling evidence for a role of cytokines in the pathogenesis of MS comes from the demonstration of an increased relapse rate in MS patients treated with IFN-β.(Løken-Amsrud et al.,2012; Nakatsuji et al., 2012) Additional evidence that immunological reactions play a role in the pathogenesis of MS comes from the fact that recombinant IFN-β attenuates disease activity in relapsing- remitting MS.(Jensen et al.,2012) This is further supported by results obtained in the animal model of experimental allergic encephalomyelitis.(Berard et al.,2012)

In here, we studied the activation of the systemic acute phase response, since this is highly influenced by several inflammatory cytokines.

2.Objects and methods

2.1 Objects

Sixty cases of acute optic neuritis including outpatient and hospitalized patients were collected from our hospital from January, 2011 to December, 2011. 19 in male, 41 in female; aged from 22 to 56. The average age was 36. The disease course of 11 cases was less than 7 d. The course of 29 cases was 7-14 d, and the course of 20 cases was more than 20 d. In this study, patients met the following criterions: the onset of acute unilateral optic neuritis symptoms less than 8 weeks; aged from 18 to 45 years old; no neuritis history; except the multiple sclerosis (MS) symptom related to neuritis, patients had no other systemic diseases; patients had no treatment of MS or optic neuritis with glucocorticoid. The diagnostic criteria of acute optic neuritis were followings: 1) visual loss; 2) neuroretinal rim area; 3) visual field defects; 4) abnormal visual evoked potential; 5) normal optic disc or edema and hemorrhage. There were no liver or kidney diseases, tumors, infections or autoimmune diseases.

2.2 Research grouping

In our study, we found that the minimal visual acuity was more than 0.4 in some acute optic neuritis patients, and the visual acuity was improved rapidly after the treatment. The patients were divided into two groups: minimal visual acuity>0.4 (LogMAR>0.4, n=20, 12 in female, 8 in male) group; minimal visual acuity<0.4 (LogMAR<0.4, n=40, 29

in female, 11 in male)group; 20 healthy cases were chosen as control group. Thirty patients were chosen randomly to be treated with glucocorticoid. Firstly, methylprednisolone (250 mg/6 h) was intravenous injected for 3 days, then prednisone was administered orally (1 mg/kg/d for 11 days, 0.5 mg/kg/d for 22 days, 0.25 mg/kg/d for 44 days, 0.125 mg/kg/d for 88 days), the vision of patients could be recovered to 1.0 (LogMAR 0) within 3 months and no recurrence.

2.3 Detection methods

Total 3 ml venous blood was collected form fasting patients; serum was separated within 2 h and frozen in -70°C refrigerator. The TNF-α, IL-4, IL-5, TGF and IFN-γ levels were determined by double-antibody sandwich ELISA (Jingmei Biological Engineering Co., Ltd).

2.4 Statistical analysis

Data were presented as mean ± standard deviation, the difference between groups were compared using t test. Multivariate forward stepwise logistic regression analysis was performed to identify variables that had indepen-dent associations with acute optic neuritis. $P<0.05$ was statistically significant.

3. Results

3.1 General data

As show in table 1, general data,including sex,course of disease,blood sugar,white blood cell, cholesterol and Triglyceride, had no statistical differences between LogMAR>0.4 and LogMAR<0.4 ($P>0.05$).

Table 1. General data of patients with optic neuritis.

	control（20）	LogMAR>0.4（20）	LogMAR<0.4（40）	glucocorticoid treatment(30)	P
sex（F/M）	12/8	12/8	29/11	20/10	0.079
Age	37.2±16.4	39.2±17.3	35.8±14.6	38.8±12.6	0.112
course of disease(dyas)	-	10.35±9.6	32.35±21.6	129.11±21.22	-
blood sugar（mg/dL）	84.1±3.5	85.1±2.9	84.±3.2	86.3±4.2	0.887
white blood cell（K/UL）	5.5±0.3	5.7±0.4	5.6±0.5	5.4±0.3	0.098
cholesterol	163.4±8.4	173.4±9.1	180.1±7.1	175±6.4	0.56
Triglyceride	100.2±20.4	91.6±12.8	87.4±11.5	90.9±15.7	0.543

3.2 TNF-α，IL-4，IL-10，TGF，IFN-γ expression

As shown in table 2, in glucocorticoid treatment group, IFN-γ was decreased significantly,which similar to control group level($P>0.05$). However, compared to the control group, the IFN-γ expression was higher in LogMAR>0.4($P<0.05$) and LogMAR<0.4($P<0.01$). IL-4 was higher in the control group compared to other groups($P<0.05$,$P<0.01$). For IL-10,TGF and RNF-a, there was similar level between glucocorticoid treatment and control groups($P>0.05$), among IL-10 in LogMAR>0.4 groups was also similar to control group($P>0.05$). However, TGF and TNF-a in LogMAR>0.4 and LogMAR<0.4 groups,IL-10 in LogMAR<0.4 were higher compared to control groups($P<0.05$,$P<0.01$).

Table 2. TNF-α，IL-4，IL-10，TGF，IFN-γ expression in four groups.

	LogMAR>0.4	LogMAR<0.4	control	glucocorticoid treatment
IFN-γ	187.19±77.92a	450.76±216.19b	57.38±24.29	73.70±21.86c
IL-4	53.67±13.57b	46.79±22.04b	153.26±36.39	56.28±16.32a
IL-10	61.02±16.15c	237.16±93.03b	57.02±19.36	59.11±21.22c
TGF	312.51±223.73a	334.56±188.28a	421.46±206.02	418.93±166.19c
TNF-α	187.19±77.92a	450.76±216.19b	57.38±24.29	73.7±21.86c

aP<0.05,VS control;bP<0.01,VS control; cP>0.05,VS control

3.3 Th1/Th2 cytokine ratio

As shown in Fig 1, IFN-g/IL-4 ratio was remarkably elevated in LogMAR>0.4, LogMAR<0.4 and glucocorticoid treatment patients with acute optic neuritis ($p < 0.05$).

3.4 Associations between cytokine levels and acute optic neuritis

As shown in table 3,multivariate analysis revealed that lower levels of IL-4 (less than 100 pg/ml) and higher levels of IL-10 (greater than 100 pg/ml) were independent predictors of acute optic neuritis with odds ratios of 0.038(95% CI, 0.003-0.015; $p<0.001$) and 1.56 (95% CI,0.98-2.35; $p = 0.034$), respectively. In contrast, serum levels of IFN-γ, TGFand TNF-a

did not show independent associations with acute optic neuritis.

Table 3.Associations between acute optic neuritis and serum cytokine levels

	Odds ratio	95%CI	P value
IL-4			
(<100pg/ml versus >=100pg/ml)	0.038	0.003-0.015	<0.001
IL-10			
(>100pg/ml versus <=100pg/ml)	1.56	0.98-2.35	0.034

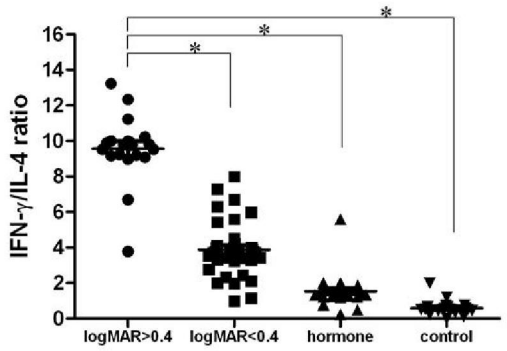

Fig 1. Determination of IFN-g/IL-4 ratio.*p < 0.05 versus the control.

4. Discussion

Th cells can be divided into Th0, Th1 and Th2 cells according to the secretion of cytokines. (Kemp 2000) Th0 cells are the precursor cells of Th1 and Th2 cells. Th1 cells secrete IL-2, IFN-γ, TNF-β and IL-17. They mediate cellular immune responses and cause the generation of proinflammatory cytokines, which play an important role in the induction of autoimmune diseases.(Viallard et al.,1999) Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. These cytokines mediate humoral immunity, produce IgE, activate eosinophils and cause the production of anti-inflammatory cytokines, which play a leading role in allergic reactions.(Wong et al.,2001; Zhu and Paul 2008)Th1/Th2 cells and their secreted cytokines are maintained in a state of dynamic equilibrium in normal situation, which is important for immune homeostasis. In here, the results showed that the IFN-γ levels were significantly increased and IL-4 levels were significantly decreased in serum of RA patients, suggesting the cytokines produced by Th1/Th2 cells appeared imbalance in MS patients. Therefore, recovery of Th1/Th2 cell balance may be a new approach for the treatment of acute optic neuritis.(Tsakiri et al.,2012)

TNF-α is a proinflammatory cytokine produced by macrophages.Low concentrations of TNF-α affect local leukocytes and vascular endothelial cells to induce inflammatory response. However, high concentrations of TNF-α could lead to systemic reactions, such as fever, disseminated intravascular coagulation and even cachexia.(Romero et al.,2010) TNF-α level is significantly increased in relapsing stage of MS, and decreased in remission stage. (Titelbaum et al.,2005) TNF-α level is also increased in CSF and peripheral blood of MS patients, and is associated with clinical status. Our results suggested that TNF-α might be related to the immunopathological process of MS and play an important role in the demyelination. Therefore, TNF-α could be used as a secondary indicator of the evaluation of MS, and a predictor of recurrence. IFN-γ is produced mainly by activated T cells and NK cells, which can activate macrophages, anti-virus response, promote the MHC molecule expression and antigen presentation, and inhibit Th2 cells.(Zloza et al.,2012) T cells can secrete IFN-γ after antigen exposure in MS, IFN-γ promote macrophages to produce IL-1 and IFN-γ, IL-1 promote astrocytes to express TNF-α. Benvenuto R et al showed that CSFT cell clone could produce lots of IFN-γ and TNF-α compared to peripheral blood cells in MS patients, which could promote vascular endothelial cells to express certain adhesion molecules.(Benvenuto et al., 1992) It has proved that IFN-γ is administered to MS patients could cause exacerbation, which suggest that IFN-γ might be related to immunopathological processes of MS. IFN-γ also play an important role in the demyelination, and an be used as a predictor of recurrence. IL-10 is an endogenous early inflammatory cytokine inhibitor produced by TH cells, which plays an important role in remission and stability of MS. It has been reported that suppression of IL-10 mRNA expression could be detected in peripheral blood mononuclear cells of acute relapsing stage in MS patients. Liu Z et al reported that IFN-γ therapy could lead to an increase of IL-10 secretion in CD4 T lymphocytes and mononuclear cells. (Liu et al.,2008) The above reports and our study suggest that IL-10 could be used as an indicator of MS relief.

The IFN-γ produced by Th1 cells was significantly increased and IL-4 produced by Th2 cells was significantly decreased, suggesting that the cytokines secreted by Th1/Th2 cells appeared significant imbalance, and the cytokines secreted by Th1 cells appear significant advantages in MS patients.

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