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Serum cytokines, T lymphocyte subsets and STAT3 function in patients with herpes zoster as well as the intervention effect of mouse nerve growth factor

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ABSTRACT

Objective: To assess the levels of serum cytokines, T lymphocyte subsets and STAT3 in patients with herpes zoster as well as the intervention effect of mouse nerve growth factor. **Methods:** A total of 102 patients with herpes zoster were selected as observation group and received mouse nerve growth factor intervention, and 100 cases of normal people who received physical examination in our hospital during the same period as the healthy control group. The levels of serum Th1/Th2 cytokines, IgG subclass and complements and T lymphocyte subsets as well as STAT3 function of observation group before and after treatment and healthy control group were detected. **Results:** Serum IL-2 and γ-IFN levels of observation group after treatment were higher than those before treatment while IL-4, IL-5, IL-10 and TNF-α levels were lower than those before treatment (P<0.05); serum IgG1, IgG3, IgG4, C3 and C4 values of observation group after treatment were higher than those before treatment while IgG2 value was lower than that before treatment (P<0.05); CD3, CD4 and CD4/CD8 levels of observation group after treatment were higher than those before treatment while CD8 level was lower than that before treatment (P<0.05); STAT3, p-STAT3 and JAK2 expression levels of observation group after treatment were lower than those before treatment (P<0.05). **Conclusions:** There are abnormal immune system and STAT3 signaling pathway function in patients with herpes zoster, and mouse nerve growth factor intervention can restore multisystem balance and accelerate disease rehabilitation, and has positive clinical significance.

1. Introduction

Herpes zoster is common in clinical practice, it is caused by the varicella - zoster virus infection, the virus is neurotropic and incubates in neurons of dorsal root ganglion for a long time after infection, and when the resistance declines in patients, viruses begin to multiply and migrate along the nerve fibers to the skin, causing intense inflammation and perception of pain in the invaded nerves and skin[1,2]. Mouse nerve growth factor (mNGF) is the neurotrophin extracted from mice parotid gland, and it can repair and protect nerves. In the research, the levels of serum cytokines, T lymphocyte subsets and STAT3 in patients with herpes zoster as well as the intervention effect of mouse nerve growth factor were mainly assessed, hereby reported as follows.

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2. Materials and methods

2.1. General information

A total of 102 patients with herpes zoster were the observation group of the research, all patients had the typical symptoms and signs of herpes zoster, and the excluding criteria were: 1) those complicated with severe heart and lung dysfunction; 2) those complicated with malignant tumors; 3) those with autoimmune diseases; 4) those taking immune preparations in half a year; 5) pregnant women or breast-feeding women.

Relevant test indexes during treatment from September 2012 to March 2015 were reviewed. These 102 patients included 52 male cases and 50 female cases, they were 38-75 years old, the average was (58.12±8.43) years, the course of disease was 1-7 days and the average was (4.27±0.41) d. Another 100 normal people who

received physical examination in our hospital during the same period were the healthy control group, 54 cases were male and 46 cases were female, they were 35-72 years old and the average was (56.39±7.62) years. Differences in baseline information were not significant between two groups (P>0.05).

2.2. Mouse nerve growth factor intervention

Observation group received intramuscular injection of mNGF, 30 μg every time, making sure that mNGF bioactivity was above 1 5000 AU. Healthy control group did not receive any drugs or intramuscular injection treatment.

2.3. Observation indexes

A total of 2 mL peripheral venous blood was drawn from observation group before and after treatment, 2 mL fasting peripheral venous blood was drawn from control group in the morning, and enzyme-linked immunosorbent assay was used to detect the levels of serum cytokines, including Th1 and Th2 cytokine levels: interleukin-2 (IL-2), interferon- γ (IFN- γ), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10) and tumor necrosis factor- α (TNF- α). Levels of IgG subclass and complements were detected, including IgG1, IgG2, IgG3, IgG4, C3 and C4. Flow cytometer was used to detect levels of T lymphocyte subsets, including CD3, CD4, CD8 and CD4/ CD8 levels.

RT-PCR and Western-blot were used to detect serum STAT3, p-STAT3 and JAK2 expression levels and specific methods were as follows. RT-PCR: Trizol method was used to extract total RNA, ultraviolet spectrophotometer was used to detect RNA concentration, and TAKARA reverse transcription and RT-PCR kits were used for RT-PCR reaction. Primer Premier5.0 and Oligo6 software were used to design primers, and *GAPPDH* was selected as internal reference. Western-blot: BCA method was adopted, microplate reader was used to make standard curve and detect protein concentration, and anti-STAT3 antibody, anti-p-STAT3 antibody and anti-JAK2 antibody stayed overnight at 4 ℃. TBST wash was conducted for 3 times, second antibodies were incubated and set aside for 1 h at room temperature, and ImageQuant LAS 4000mini was used for instrument exposure.

2.4. Statistical methods

Data obtained in the research was analyzed by SPSS23.0 software, measurement data was in terms of mean±sd, comparison between two groups was performed by t test, and P<0.05 was set as the standard of statistical significant differences.

3. Results

3.1. Th1/Th2 factor levels

IL-2 and γ -IFN levels of observation group before treatment were lower than those of healthy control group while IL-4, IL-5, IL-10 and TNF- α levels were higher than those of healthy control group (P<0.05), and serum IL-2 and IFN- γ levels after mouse nerve growth factor treatment were higher than those before treatment while IL-4, IL-5, IL-10 and TNF- α levels were lower than those before treatment (P<0.05), shown in Table 1.

3.2. Levels of IgG subclass and complements

Detection of levels of IgG subclass and complements showed that IgG1, IgG3, IgG4, C3 and C4 values of observation group before treatment were lower than those of healthy control group while IgG2 value was higher than that of healthy control group (P<0.05), and IgG1, IgG3, IgG4, C3 and C4 values of observation group after mouse nerve growth factor treatment were higher than those before treatment while IgG2 value was lower than that before treatment (P<0.05), shown in Table 2.

Table 1
Serum Th1/Th2 factor levels in patients with herpes zoster (ng/L).

Groups	Th1		Th2			
	IL-2	IFN- γ	IL-4	IL-5	IL-10	TNF- α
Observation group						
Before treatment	46.27±4.07	81.49±7.66	79.56±8.34	101.37±12.41	89.67±8.14	76.43±7.11
After treatment	92.15±8.75	157.48±14.25	65.24±7.11	79.64±8.54	61.35±7.29	50.39±4.81
Control group	132.48±12.09	201.47±23.56	53.28±5.94	64.38±7.12	45.38±4.11	34.28±3.45
t	11.384	14.394	8.293	9.283	7.293	8.092
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2
Serum IgG subclass and complement levels in patients with herpes zoster (g/L).

Groups	IgG subclass				Complements	
	IgG1	IgG2	IgG3	IgG4	C3	C4
Observation group						
Before treatment	6.14±0.59	4.63±0.53	0.76±0.08	0.30±0.04	1.08±0.12	0.23±0.03
After treatment	6.89±0.65	3.76±0.42	1.12±0.13	0.41±0.05	1.37±0.15	0.42±0.04
Control group	7.27±0.78	3.42±0.35	1.27±0.14	0.45±0.04	1.54±0.13	0.48±0.05
t	5.384	5.093	5.182	5.384	5.782	5.913
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

3.3. T lymphocyte subsets

The results below showed that CD3, CD4 and CD4/CD8 levels of observation group before treatment were lower than those of healthy control group (P<0.05), the abnormalities of above indexes were improved after treatment, CD3, CD4 and CD4/CD8 levels of observation group after treatment were higher than those before treatment while CD8 level was lower than that before treatment (P<0.05), shown in Table 3.

3.4. STAT3 function

The results showed that STAT3, p-STAT3 and JAK2 expression levels of observation group were higher than those of healthy control group (P<0.05), and after mouse nerve growth factor treatment, STAT3, p-STAT3 and JAK2 expression levels in patients with herpes zoster were lower than those before treatment (P<0.05), shown in Table 4.

4. Discussion

Herpes zoster is an infectious skin disease caused by the varicella-zoster virus, and the virus is mostly activated and spreads after fatigue, drinking and stress stimulation, causing inflammation change in skin dominated by peripheral nerve. Herpes zoster nerve injury can leave severe pain, which is characterized by burning, tingling, cutting pain and a variety of other types, severely increases the pain and lowers the quality of life[3]. mNGF is separated from mouse submandibular gland, and has potent trophism on normal cells in the nervous system. mNGF has about 90% homology with human nerve growth factor, and has a good recovery effect on damaged nerves. There are serious local nerve damage and the resulting persistent pain in herpes zoster patients, and mNGF application is expected to repair the damaged nerves and fundamentally relieve patients' suffering[4,5]. In the research, mNGF was applied to herpes zoster patients, and the changes of immune function and related signaling pathway function before and after treatment were mainly compared. Under normal circumstances, Th1/Th2 is in balance in the body, and Th1 and Th2 cells secrete a variety of cytokines respectively and form complex network, completing immune regulation of the body. Th1 cells mainly mediate cellular immune response, and are mostly associated with inflammation and tissue damage. Th2 cells mainly mediate humoral immunity, are related to the sustained and chronic infection, and play an important role in suppressing the immune response and reducing excessive damage[6]. There is negative feedback effect between Th1 and Th2-type immune response, thus maintaining the body's immune balance, and once this balance is broken, the body will be in pathological state. Th1 cells mainly include IL-2 and IFN-γ, and Th2 cells mainly include IL-4, IL-5, IL-10 and TNF-α. In the research, detection of the levels of Th1 and Th2 cells showed that IL-4, IL-5, IL-10 and TNF-α levels of observation group were higher while IL-2 and γ-IFN levels were lower, indicating that Th2 cells were dominant in patients with herpes zoster[7,8]. After mouse nerve growth factor intervention, IL-4, IL-5, IL-10 and TNF-α levels of observation group decreased while IL-2 and γ-IFN levels increased, indicating that it could effectively recover the Th1/Th2 balance in patients with herpes zoster and promote disease recovery.

In IgG subtypes, IgG1, IgG3 and IgG4 are induced and produced by IL-4 from Th2 reaction, and IFN-γ from Th1 reaction promotes IgG2 expression. The above study has shown that there are increased Th1 cellular immune function, decreased Th2 cellular immune function and Th1/Th2 imbalance in patients with herpes zoster, and in the research, serum IgG2 level of observation group was higher before treatment while IgG1, IgG3 and IgG4 levels were lower, consistent with the existing conclusions[9]. And after mouse nerve growth factor intervention, IgG2 level of observation group decreased while IgG1, IgG3 and IgG4 levels increased, and the overall IgG levels in the body tended to be normal. Study has shown that the complement system is activated in herpes zoster patients, C3

Table 3

Serum T lymphocyte subset levels in patients with herpes zoster.

Groups	CD3	CD4	CD8	CD4/ CD8
Observation group				
Before treatment	53.28±5.02	31.48±3.29	29.76±2.65	1.09±0.12
After treatment	61.59±5.83	35.21±3.49	27.03±2.41	1.18±0.12
Control group	63.27±5.99	38.27±3.45	25.14±2.39	1.29±0.13
t	8.293	7.283	6.293	5.182
P	<0.05	<0.05	<0.05	<0.05

Table 4

STAT3-related function levels in patients with herpes zoster.

Groups	mRNA			Protein		
	STAT3	*p–STAT3*	*JAK2*	STAT3	p-STAT3	JAK2
Observation group						
Before treatment	98.26±10.37	112.64±13.28	93.55±10.27	114.57±13.85	127.49±14.36	86.49±8.52
After treatment	76.95±8.34	89.54±8.32	67.88±6.12	90.57±8.53	85.38±9.29	67.47±5.32
Control group	65.38±5.92	71.29±8.64	58.37±5.42	78.15±7.09	71.23±6.54	58.36±6.01
t	8.394	10.273	8.034	9.183	11.274	9.493
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

and C4 lysis are accelerated, and the levels are reduced, becoming one of the characteristics of herpes zoster[10]. In the research, after observation group accepted mouse nerve growth factor intervention, C3 and C4 levels increased, indicating that after intervention, the excessive complement lysis in patients was suppressed.

T lymphocytes are the important groups in the body's immune system, and normally T lymphocyte subsets in body are in balance to ensure the normal immune function. T lymphocyte number imbalance or dysfunction can lead to abnormal function of the immune system and increased risk of infection. Researches have confirmed that there is the cellular immune dysfunction in patients with herpes zoster, especially the function inhibition in the aspect of the immune response[11,12]. $CD4^+$T cells are the main cells involved in the body's immunity, belonging to the helper T cells; $CD8^+$T cells belong to cytotoxic cells. Above research results showed that CD3, CD4 and CD4/CD8 levels in patients with herpes zoster were lower, indicating that there was disorder of cellular immune function in patients. After treatment with mouse nerve growth factor, it was found that CD3, CD4 and CD4/CD8 levels of observation group increased while CD8 level decreased, indicating the application of mouse nerve growth factor could effectively relieve the patient's immune inhibition, restore the balance of immune cell number and function, and strengthen the antiviral effect.

JAK/STAT is the signaling pathway with cytokine stimulation discovered in recent years, and it plays an important role in inhibiting inflammatory response, neurodegeneration, neuropathic pain and other aspects[13]. It is currently believed that the STAT family, especially STAT3, is one of the key links in immune regulation and stress response. Researches have confirmed that JAK/STAT signaling pathway is activated in neuropathic pain, and herpes zoster patients are also accompanied with significant neuropathic pain[14,15]. Some scholars put forward the conjecture that JAK/STAT signaling pathway is involved in herpes zoster pain, the mRNA and protein expression of the JAK/STAT signaling pathway downstream of JAK2, STAT3 and p-STAT3 were also detected in the research, and the results showed that STAT3, p-STAT3 and JAK2 expression levels in patients with herpes zoster reduced after treatment, indicating that JAK/STAT signaling pathway was involved in the occurrence of herpes zoster pain, and mouse nerve growth factor intervention could inhibit the function of JAK/STAT signal pathway and down-regulate the expression levels of related factors.

To sum up, it is concluded as follows: there are abnormal immune system and STAT3 signaling pathway function in patients with herpes zoster, and mouse nerve growth factor intervention can restore multisystem balance and accelerate disease rehabilitation, and it’s worth popularization and application in clinical practice in the future.

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