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Changes of serum miR-21-5p level in patients with acute spinal cord injury treated with ganglioside and methylprednisolone pulse therapy and its correlation with therapeutic effect

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Abstract

Background: Acute spinal cord injury (ASCI) is a serious central nervous system condition that carries a high rate of disability and mortality, making it a primary focus of clinical research. The commonly used drugs for ASCI are ganglioside (GM-1) and methylprednisolone (MP). There are few studies available on the impact of GM-1 combined with MP pulse therapy on the clinical outcomes of patients with ASCI and the underlying mechanisms involved. *Methods:* In a randomized controlled trial, the 100 patients with ASCI who were treated at our hospital from 2021 to 2024 were selected for this study. They were arbitrarily separated into two equal cohorts, a control cohort and an observation cohort, each containing 50 patients. While the control cohort underwent treatment solely with GM-1, the observation cohort additionally received MP pulse therapy, both treatments administered over a span of six months. The general information of patients was recorded. The motor score, acupuncture pain score, light touch score, Barthel Index (BI) for evaluating daily living capabilities, American Spinal Injury Association (ASIA) neurological function score, and visual analogue scale (VAS) score were analyzed between the two groups before and after treatment to evaluate the recovery of neurological function. Additionally, quantitative real-time PCR (qRT-PCR) was used to assess the serum levels of miR-21-5p in both groups. Moreover, the concentrations of serum inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), along with nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), were quantified using ELISA. The *Pearson* method was employed to evaluate the correlation between miR-21-5p and the levels of CRP, TNF-α, IL-6, NGF, and BDNF. The receiver operating characteristic (ROC) curve was employed to evaluate the predictive ability of serum miR-21-5p regarding treatment effectiveness in patients with ASCI. *Results:* Three patients dropped out of the control group, and no patient dropped out of the observation group. There were no significant variations in the baseline data, index scores, or factor levels between the two groups prior to treatment ($P > 0.05$). After undergoing therapy, the observation group exhibited significantly higher BI index, ASIA motor and sensory scores, as well as increased in serum miR-21-5p levels, BDNF, and NGF compared to the reference group ($P < 0.05$). Furthermore, the observation group exhibited marked decreases in VAS ratings as well as serum CRP, TNF-α, and IL-6 levels, in stark contrast to the control group ($P < 0.05$). Furthermore, the level of miR-21-5p exhibited a negative correlation with the levels of CRP, TNF-α, and IL-6 ($P < 0.05$), while showing a positive correlation with the levels of BDNF and NGF ($P < 0.05$). The ROC curve analysis indicated that miR-21-5p possesses strong diagnostic potential for assessing ASCI efficacy, with an area under the curve (AUC) of 0.987, a sensitivity rate of 91.49%, and a specificity rate of 100.00%.
Conclusions: The combination of GM-1 and MP pulse therapy can significantly enhance neurological symptoms and facilitate the restoration of neurological function in patients with ASCI. This effect

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may be ascribed to the increased levels of miR-21-5p, BDNF, and NGF, along with a reduction in serum inflammatory factors.

Keywords: Acute spinal cord injury, ganglioside, methylprednisolone, inflammatory factors, miR-21-5p

INTRODUCTION

With the rapid development of infrastructure and construction industry, acute spinal cord injury (ASCI) has become one of the common clinical diseases. According to the WHO spinal cord injury report, there were 15.4 million patients with ASCI globally in 2021. In developed countries, the incidence of spinal cord injuries has reached 13 ~ 45 cases per million people, while in China, it has risen to 6.7 cases per 1,000 individuals.[1] More than 90% of these ASCI patients are due to the further deterioration of the damaged spinal cord due to the delay and non-standard subsequent treatment[2], so better treatment of ASCI patients is urgently needed. In addition to resource limitations, the time-consuming development of surgical procedures impose limitation on making surgical treatment more widely available. Pharmacological treatment has emerged as the preferred option for clinical management due to its effectiveness and rapid therapeutic action. According to the 2016 AOSpine (Arbeitsgemeinschaft fur Osteosynthesefragen Spine) practice guidelines, high-dose MP pulse therapy is recommended for ASCI patients within 8 h of injury.[3] Many animal studies have confirmed the positive effects of MP in clinical treatment[4], but the side effects of high-dose MP alone in clinical use are still a concern.[5] GM-1 exhibits neuroprotective properties for the central nervous system and has multiple roles in various neuropathological conditions.[6] Unfortunately, the understanding of GM-1 based on existing studies is still not adequate. In view of the lack of reports on the effect of the combination of GM-1 and MP pulse therapy on the changes of molecular indicators in patients with ASCI, this study intends to explore the effect of combined GM-1 and MP pulse therapy on the neurological symptoms and function of patients with ASCI.

Clinically, spinal cord injury is categorized into two phases. Primary spinal cord injury results from mechanical damage to spinal cord cells due to external compression and impact, and secondary spinal cord injury due to the swift deterioration of the affected spinal cord, triggered by a cascade of biochemical reactions in nerve cells following the initial injury. Whereas the primary injury directly leads to structural disruption of the central nervous system, secondary spinal cord injury has become the primary emphasis of clinical management.[7,8] Secondary spinal cord injury has been shown to be due to acute cellular dysfunction and transmission of cell death, including neuronal immunity, inflammatory response, ischemia, cellular lipid peroxidation, and the dysregulation of neuronal microenvironment will affect normal central nervous system function.[9] Recent studies have confirmed that systemic immune and neuroinflammatory responses are key factors in determining secondary spinal cord injury[10], and immunosuppressive drugs can help in spinal cord recovery in ASCI patients.[11] Investigators generally believe that cellular inflammation is the decisive factor affecting the degeneration of nerve cells.[12] Numerous investigations have shown that the concentrations of pro-inflammatory cytokines IL-6 and TNF-α rise in patients with ASCI within hours of the injury. These cytokines promote the infiltration of microglia, neutrophils, and macrophages into the affected area, thereby exacerbating the inflammatory response.[10] In the study of pathological characteristics, the expression of CRP and IL-6 in plasma has a certain correlation[13,14], and IL-6 plays an important role in CRP transcription genes.[15] CRP is important not only as a marker of inflammation[16,17] but also as a predictor of neurologic recovery[14], since massive release of the inflammatory factor IL-6 stimulates hepatic production of CRP. Other investigators have found that nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have a vital function in neuronal regeneration and the restoration of neuronal activity.[18]

As a type of non-coding single-stranded RNA made up of 19 to 25 nucleotides, the untranslated region of miRNA plays an important role in influencing gene expression as an important factor controlling translation.[19] Previous studies have confirmed that miRNAs are closely related to cell regeneration, demyelination, and inflammatory response.[20] In SCI, miR-21-5p is considered to be a protective factor to reduce neuronal apoptosis[21], and it is the most significantly differentially expressed miRNA[22], so it became the target of this study. The central nervous fluid and blood of patients are considered as important sources of biomarkers[23]. In this study, fasting blood samples were obtained from ASCI patients to serve as a crucial source for measuring the miR-21-5p levels.

In this study, ASCI patients received treatment with GM-1 alongside MP pulse therapy, and the serum concentrations of miR-21-5p, CRP, TNF-α, IL-6, NGF, and BDNF were measured. Combined with the clinical manifestations of ASCI patients, restoration of neurological functionality was determined, and the predictive capability of miR-21-5p on the therapeutic effect of ASCI patients and the correlation between indicators were explored, so as to provide data support and theoretical support for clinical drug treatment of ASCI patients, and provide certain ideas for prognosis judgment.

METHODS

Study design

This was a randomized controlled trial formulated to assess the effect of GM-1 alone versus the combination of GM-1 and high-dose MP pulse therapy on clinical efficacy in patients with nonpenetrating ASCI. 100 patients who were encompassed by the initial screening were distributed randomly among two groups through sealed envelope selection method, with each group comprising 50 cases. The control group received intravenous injection of GM-1 300 mg at the first treatment and 100 mg each time thereafter for 6 days. In the observation group, MP 30 mg/kg (pulse therapy) was given within 15 minutes of the first treatment, and 45 minutes after the completion of the infusion, MP 5.4 mg/(kg·h) was injected intravenously for 23 hours. This was followed by an injection of GM-1 intravenously, which was administered in the same way as in the control group. To avoid adverse interactions that would arise from simultaneous administration of MP and GM-1, the first administration of GM-1 needed to be delayed until after the administration of MP. During the treatment, the subjects in both groups were given routine treatment, and no other psychotrophic drugs were given. All treatment protocols adhered to the guidelines established and approved by the hospital's ethics committee, and all procedures were carried out by the same team of treating physicians. The research was endorsed by all participants and their respective households.

Patient selection

Inclusion criteria: A total of 100 ASCI patients hospitalized in the Department of Spinal Cord Trauma of our hospital from 2021 to 2024 were chosen as the study participants. Based on the results of Lau's study[24], the study subjects were 18-70 years old, male and female, within 8 h of trauma and without drug treatment, so as to reduce drug residual effects. All regulations and ethical guidelines will be strictly followed during the research process.

Exclusion criteria: In view of the significant chance of suicide in patients with bipolar disorder and schizophrenia, patients exhibiting moderate to severe depressive symptoms will be excluded, and timely psychiatric treatment should be initiated. Patients with cardiovascular and cerebrovascular diseases, autoimmune diseases, gastrointestinal bleeding, diabetes, and diseases of liver and kidney function were excluded based on the guidelines for the treatment of Spinal Cord Injury (IANR/CANR).[25] Patients who were allergic to basic drug components such as GM-1 or glucocorticoid MP were not included in this comparative study. Physical examination, laboratory analysis (blood, urinalysis) and exclusion of pregnant women or patients with other neurological deficits are also required to reduce the influence of individual differences. As this study did not involve any other intervention, subjects who did not cooperate with this trial were excluded. In addition, ASCI patients with adverse events during treatment were promptly terminated and given corresponding treatment measures. ASCI patients with adverse events were not included in the results analysis.

ASIA stage

The sensory and motor activities of the injured spinal cord were scored based on American Spinal Injury Association (ASIA)[26]: palpation and acupuncture sensation were performed on 28 key points in the nerve distribution area of T1-S5, and acupuncture pain and light touch sensation on both sides of the patient's body before and after treatment were evaluated. A total score of 224 points was generated according to the sensitivity of each nerve distribution area (each discrimination value was 0-2 points).

Motor function was scored as follows: Muscle strength testing was performed on 20 sarcomere on both sides of the patient's body. The assessment score for each sarcomere ranged from 0 to 5, with a total score of 100. The revival of spinal cord function was evident in the combined motor responses, acupuncture and light touch sensation scores. The higher the rating, the more favorable the rehabilitation of spinal cord function.

Scores on the Barthel index (BI) stage

BI of activities of daily living including quality of life, including eating, dressing, bathing, toileting, walking, and walking up and down stairs, were assessed before and after treatment on the basis of a previous study.[27] The scoring criteria were: (> 60) good quality of life; (40-60) with living disorders, some of which could not take care of themselves; (< 40) severe life disorder, completely unable to take care of themselves. If BI score was higher than 60, the treatment effect was considered obvious.

Visual analogue scales (VAS) stage

According to the VAS, the pain degree of patients was scored regularly, and the assessment time was consistent with the ASIA assessment period. The scoring criteria were as follows: (0-4) no or mild pain; (5-7) Average pain; (8-10) Intense or intolerable pain. The VAS score was repeated three times for each patient, with an interval of 15 minutes. The maximum VAS score was regarded as the degree of pain, and the smaller the score was, the less the pain was.

Extraction and quantitative real-time PCR of miR-21-5p

The measurement was performed according to the previous research method.[28] For miR-21-5p expression level determination, fasting venous blood (5 ml) was collected from the ASCI patients prior to treatment and again the following day after 6 months of treatment. After collection, the blood was left for 2 hours, and the centrifuge parameters were adjusted to 3000 rpm at a temperature of 4°C for 5 minutes. The separated peripheral blood was placed in the refrigerator at -80°C. Mirna-21-5p was isolated from serum samples extracted using mir Vana™ PARIS miRNA isolation kit (Ambion Inc, Austin TX, USA). The isolated miR-21-5p was then reverse transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Forster City, CA, USA). Finally, qRT-PCR reactions were performed for miR-21-5p using the TaqMan MicroRNA PCR kit (Applied Biosystems, Forster City, CA, USA) on an ABI PRISM 7900HT sequencing system. miR-21-5p primer sequence design as follows, positive: 5'-ACACTCCAGCTGGGTAGCTTATCAGAC TGA-3, the reverse: 5'-TGGTGCGTGGAGTC G-3; U6, forward: 5'-CTCGCTTCGGCAGCA CA-3', reverse: 5'-AACGCTTCACGAATTTG CGT-3'. The reaction conditions of PCR enhancement in quantitative real-time PCR (qRT-PCR) were divided into two steps: first, prepurification at 95°C for 15min; The second step was denaturation at 95°C for 10s. The second process was repeated 40 times, with annealing and extension occurring at a low temperature of 60°C for 30 s. The relative expression targeted miR-21-5p levels was evaluated by referring to the $2^{-\Delta\Delta Ct}$ cycle threshold method (Ct).[29,30]

ELISA assay

Serum CRP (Shanghai Fusheng Industrial Co., LTD., product number: FS-E6542) and TNF-α (Wuhan Lingjie Biotechnology Co., LTD., product number: LJS-E-00110), IL-6 (Wuhan Lingjie Biotechnology Co., LTD., Product number: LJS-E-00140), BDNF (Shanghai Keyshun Biotechnology Co., LTD., product number: KS016032) and NGF (Shanghai Jianglai Biotechnology Co., LTD., product number: JL18187) expression levels. The operating requirements of the kit were followed, the OD value at 450nm wavelength was recorded, and the levels of the measured factors were quantified according to the standard curve.

Statistical analysis

Data presentation includes Mean±standard deviation (Mean±SD) and were found to follow a normal distribution. Student's t-test (two-tailed) was utilized to compare the data between groups, while the *Pearson* correlation coefficient was employed to assess correlations within various groups. $P < 0.05$ was statistically significant. Explore the predictive capability of miR-21-5p expression level for the treatment outcomes of spinal cord injury based on the area under the receiver operating characteristic curve (AUC). All statistics and plots were performed by GraphPad Prism 9.5 software (Chicago, Illinois, USA).

RESULTS

Demographic characteristics of ASCI patients

One hundred patients with ASCI were included in the research and randomly partitioned into 50 control groups and 50 observation groups, among which 3 patients with ASCI had adverse reactions. The mean age of the control and observation group was 46±9.32 and 46±9.76 years, respectively, and the difference observed between the two groups failed to reach statistical significance ($P > 0.05$). Comparison between groups of ASCI

patients showed no significant variations regarding gender, age, accident causes, and timing ($P > 0.05$). (Table 1)

Occurrence of adverse reactions

Among the 100 ASCI patients involved in the study, there was one case each of pulmonary infection, urinary tract infection, and deep vein thrombosis in the lower limbs, with no occurrences of gastrointestinal bleeding or glaucoma complications. The occurrence of complications did not exhibit a statistically noteworthy variation among the two groups ($P > 0.05$). Table 2.

Comparison of ASIA sensory and motor function of ASCI patients

Based on the ASIA neurological function scores before and after treatment, two groups demonstrated marked enhancements in sensory perception and motor capabilities compared to their prior-treatment scores ($P < 0.05$). The combination of GM-1 and high-dose MP pulse therapy demonstrated a more effective enhancement in sensory and motor functions than GM-1 alone, which was statistically meaningful ($P < 0.05$). Table 3.

Comparison of BI and VAS in ASCI patients

Prior-treatment, there were no notable statistical variations observed in the BI and VAS scores when comparing the control group with the observation group ($P > 0.05$). After treatment, both the BI and VAS scores showed significant improvement and reduction, respectively, both groups showed changes from their respective baseline measurements ($P < 0.05$). When compared to the control group, the combination of GM-1 and MP was found to substantially enhance the standard of living and alleviating pain in the affected areas ($P < 0.05$). Table 4.

Comparison of serum NGF and BDNF levels in ASCI patients

After treatment, the serum BDNF protein levels of the two groups were increased to 4.72±0.34 ng/L and 5.50±0.23 ng/L, and the NGF expression levels were increased to 519.98±32.89 ng/L and 542.24±21.92 ng/L, respectively. After treatment, the combination of GM-1 and MP significantly increased the serum levels of NGF and BDNF ($P < 0.05$). Table 5.

The serum miR-21-5p, CRP, TNF-α and IL-6 levels in ASCI patients

Compared with before treatment, the changes in inflammatory factor levels after treatment were statistically significant in the two groups of the comparisons ($P < 0.05$). After therapeutic intervention, the observation group demonstrated a notable elevation in serum miR-21-5p concentrations, accompanied by marked reductions in CRP,TNF-α, and IL-6 levels, in contrast to the control group ($P < 0.05$). Table 6.

Correlation analysis of miR-21-5p expression level with CRP, TNF-α and IL-6

In this study, we evaluated the associations between the levels of miR-21-5p in serum and

Table 1: Demographic characteristics of ASCI patients

General Characteristics		Control group (n=47,%)	Observation group (n=50,%)	*t*	*P* value
Sex	Male	30 (63.8)	34 (68.0)	0.4291	0.6688
	Female	17 (36.2)	16 (32.0)		
Age (years) Mean±SD		46±9.32	46±9.76		
Cause of injury	Road accidents	21 (44.7)	22 (44.0)	0.4901	0.6252
	Falling accidents	15 (31.9)	14 (28.0)		
	Extreme sports	6 (12.8)	7 (14.0)		
	Violence	5 (10.6)	6 (12.0)		
	Others	0 (0.0)	1 (2.0)		
Unmedicated time after trauma (h)	Maximum	7.99	7.80	1.0454	0.2985
	Minimum	1.50	1.24		
Mean±SD		5.21±1.79	5.60±1.80		

Note: SD: Standard deviation.

Table 2: Adverse reactions in ASCI patients

Item	Number of adverse reactions					
	N	Pulmonary (*n*=50,%)	Urinary system infection (*n*=50,%)	DVT (*n*=50,%)	Gastrointestinal hemorrhage (*n*=50,%)	Glaucoma (*n*=50,%)
Control group	47	1 (2.0)	1 (2.0)	1 (2.0)	0	0
Observation group	50	0	0	0	0	0

Note: N: No adverse reaction; DVT: Deep venous thrombosis of lower extremity.

Table 3: AISA sensory and motor function scores were evaluated before and after treatment

	Mean±SD		
	Time	Control group (n=47)	Treatment group (n=50)
ASIA Sensory Function Score (0-224)	Prior treatment	121.02±15.72	117.93±17.36
	After treatment	168.51±11.29*	192.65±14.27*#
ASIA Motor Function Score (0-100)	Prior treatment	49.64±8.02	47.97±5.64
	After treatment	64.00±5.88*	75.90±2.60*#

Note: *: Compare with the control group, $P < 0.05$; #: Compared with the treated control group, $P < 0.05$.

Table 4: BI and VAS scores were recorded before and after treatment

	Mean±SD		
	Time	Control group (n=47)	Observation group (n=50)
BI (0-60)	Prior treatment	33.92±4.85	35.74±6.07
	After treatment	44.02±4.98*	51.64±3.28*#
VAS (0-10)	Prior treatment	3.81±0.63	3.95±0.72
	After treatment	1.77±0.20*	1.21±0.20*#

Note: *: Compare with the control group, $P < 0.05$; #: Compared with the treated control group, $P < 0.05$.

Table 5: The nerve factors levels in serum of patients before and after treatment

	Mean±SD		
	Time	Control group (n=47)	Observation group (n=50)
BDNF(ng/L)	Prior treatment	2.95±0.36	3.15±0.45
	After treatment	4.72±0.34*	5.50±0.23*#
NGF(ng/L)	Prior treatment	427.34±30.05	431.38±35.64
	After treatment	519.98±32.89*	542.24±21.92*#

Note: *: Compare with the control group, $P < 0.05$; #: Compared with the treated control group, $P < 0.05$.

three indicators of inflammation, as well as two neurotrophic factors, BDNF and NGF, in patients with ASCI following 6 months of treatment. After the combination of GM-1 and MP pulse therapy, a notable inverse relationship was observed between the concentrations of miR-21-5p in serum and the CRP levels ($R^2 = 0.7991$, $P < 0.0001$). Similarly, a strong negative correlation was noted between serum miR-21-5p and TNF-α levels ($R^2 = 0.8257$, $P < 0.0001$) and IL-6 levels ($R^2 = 0.7650$, $P < 0.0001$) among ASCI patients ($P < 0.05$). On the contrary, a notable positive correlation was

Table 6: The relative expression of serum miR-21-5p levels and inflammatory factors in ASCI patients before and after treatment

	Mean±SD		
	Time	**Control group (n=47)**	**Observation group (n=50)**
miR-21-5p (Ct)	Prior treatment	1.87±0.35	1.21±0.30
	After treatment	3.78±0.76*	4.16±0.59*#
CRP (ng/L)	Prior treatment	11.26±2.87	10.35±2.92
	After treatment	6.94±1.09*	4.26±1.71*#
TNF-α (ng/L)	Prior treatment	50.99±12.24	53.47±10.65
	After treatment	32.17±5.72*	25.82±6.09*#
IL-6 (ng/L)	Prior treatment	55.95±7.45	55.21±7.43
	After treatment	40.84±7.48*	33.12±4.65*#

Note: *: Compare with the control group, $P < 0.05$; #: Compared with the treated control group, $P < 0.05$.

discerned between the miR-21-5p and BDNF levels in serum ($R^2 = 0.8248$, $P < 0.0001$), as well as between serum miR-21-5p and NGF levels ($R^2 = 0.8269$, $P < 0.0001$) in ASCI patients ($P < 0.05$). Figure 1.

The predictive capability of miR-21-5p expression level for clinical efficacy in ASCI patients

We combined the ASIA Sensory Function Score (An increase of 60 points or more is considered to be a recovery of sensory function), ASIA Motor Function Score (An increase of 20 points or more in score is considered to be an improvement in motor function), BI score (An increase of 15 points or more in Barthel Index score indicates that the patient's ability to perform activities of daily living has increased as a result of spinal cord recovery) and VAS score (A decrease in score of 1.5 or less means pain relief) as parameters for spinal cord recovery analyses, and analysed the predictive capability of miR-21-5p in peripheral venous blood in assessing the outcome of patients with ASCI by means of receiver operating characteristic (ROC) plot. The results are shown in Figure 2. The AUC predicted by miR-21-5p was 0.987 [95%CI (0.9672, 1.000)], the sensitivity was 91.49%, the specificity was 100.00%, and the threshold was 2.60 Ct. Thus, miR-21-5p exhibited high levels of sensitivity and specificity. The findings suggested that plasma miR-21-5p had an important clinical predictive value for spinal cord recovery in ASCI patients after treatment.

DISCUSSION

After spinal cord injury, immune responses, neural-cell edema, dysregulation of intracellular calcium, excess accumulation of glutamate, and ischemic cellular injury all cause apoptosis.[10] During immunization, astrocytes are stimulated by the growth factor IGF-1 to form glial scars[31], which in turn impede axonal regeneration of nerve cells[32], leading to cavitation of the spinal cord.[33] Nerve tissue relies on high metabolic level to ensure cell axon elongation and information transmission.[34] Barriers not only hinder the information transmission and communication between the central nervous system, but also accelerate the apoptosis of central nervous cells. Therefore, improving the microenvironment of residual nerve cells is the key to the recovery of nerve function. As an important component of cell membrane, GM-1 can cross the cell barrier and reverse the changes of Na^+/K^+-ATPase and Mg^{2+}-ATPase induced by ischemia, thereby promoting axon regeneration and transmission, thereby alleviating cell apoptosis.[35,36] Other scholars have observed that MP treatment reduces astrocytes in the injured spinal cord and maintains information transmission between nerve cells.[37] In this study, the combination of GM-1 and MP or GM-1 alone significantly improved the index symptom scores, suggesting that GM-1 can advance the nerve cell function of ASCI patients, and the combination of GM-1 and MP is more effective.

Clinically, it is difficult to judge the degree of symptoms and the recovery of neurological function in ASCI patients by neurological function examination alone[38], and measurement values are needed as an important basis for the recovery of the nervous system. Different inflammatory factors interact with each other. After a large

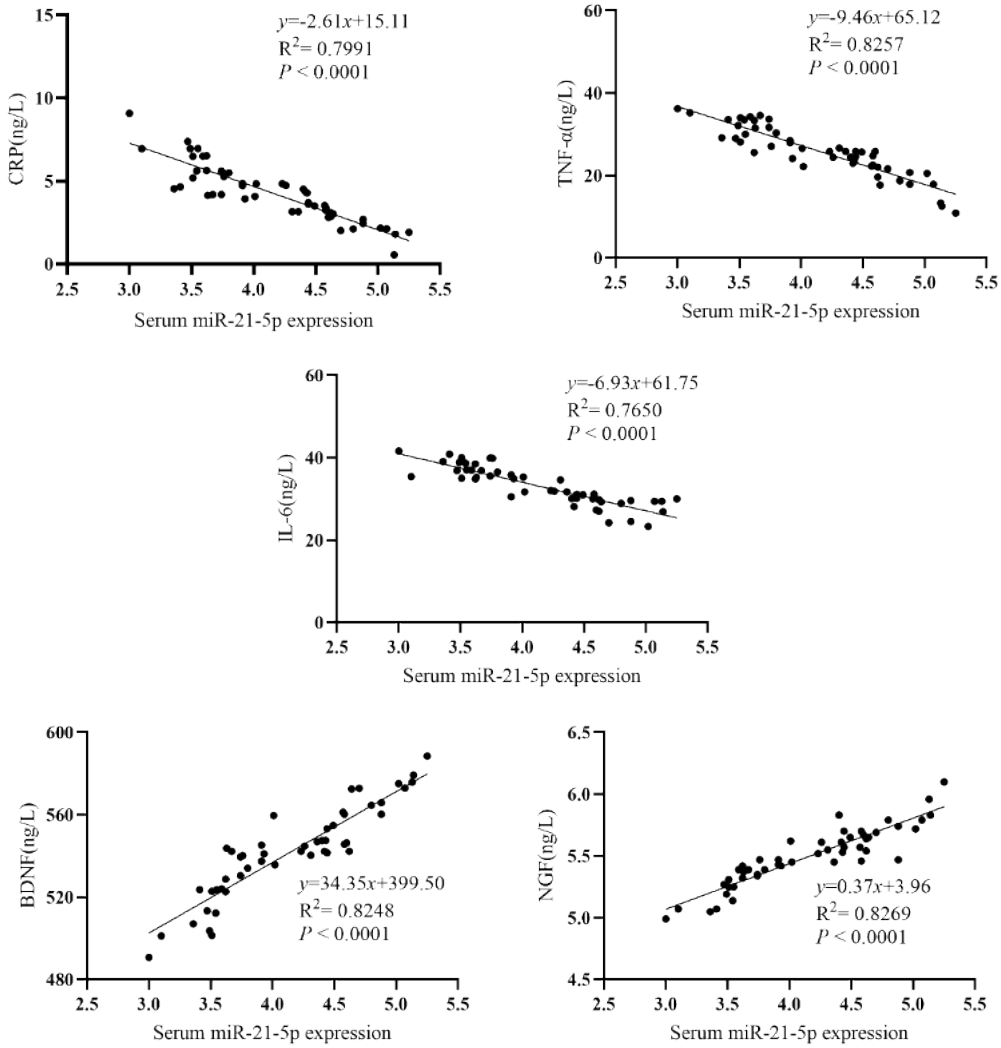

Figure 1. *Pearson* correlation between miR-21-5p and CRP, TNF-α, IL-6, BDNF and NGF in patients after treatment

amount of IL-6 is released in the injured nerve cells, the TNF-α and CRP levels are increased.[13,39] The inflammatory cytokine TNF induces the formation of adhesion molecules ICAM-1 and VCAM-1 in the spinal cord barrier. Additionally, TNF can cooperate with leukocytes to activate inflammatory mediators in the central nervous system, worsening secondary damage to the spinal cord.[31] The findings from this study indicated that GM-1 and MP significantly reduced the expression of inflammatory mediators CRP, TNF-α, and IL-6 in the serum of ASCI patients, which verified that CRP, TNF-α, and IL-6 low levels exerted a beneficial influence on the regeneration and functional recovery of nerve cells. MiRNA significantly contribute to the regulation of inflammatory responses and pathological immune reactions.[40] miR-21-5p controls astrocyte activation and reduces inflammatory response of nerve cells through the protein kinase B (AKT)/phosphatidylinositol 3-hydroxykinase (PI3K) pathway.[41,42] Based on the ROC, the AUC of serum miR-21-5p in ASCI patients after treatment was 0.987, which was highly predictive of spinal cord recovery in ASCI patients after treatment. Based on this, we further examined the impact of serum miR-21-5p expression on the inflammatory factors CRP, TNF-α, and IL-6 levels in patients with ASCI. The findings substantiated the elevation in the expression level of miR-21-5p significantly inhibits the inflammatory factor CRP TNF-α, and IL-6. BDNF was first identified from the pig brain in 1982 and was found to play a pivotal role in the process of nerve cell growth, repair and

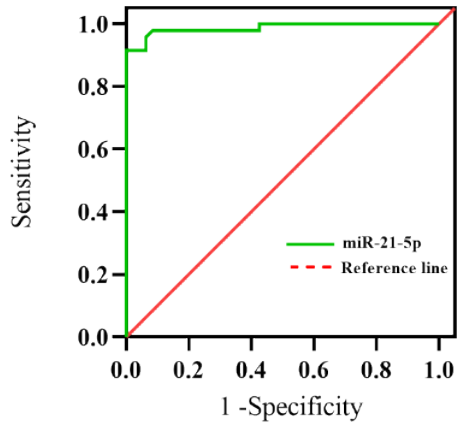

Figure 2. Assessment of the receiver operating characteristic (ROC) curve pertaining to miR-21-5p levels in individuals with ASCI

regeneration.[43,44] BDNF, a neuronal regulatory protein for the central nervous system[45], activates the transmembrane tyrosine kinase B (Trk-B) receptor[46], triggering cellular pathways that alter specific neuronal functions.[47] Previous studies have confirmed that the synaptic response of nerve cells in mice with low BDNF secretion is significantly weakened.[48] Other studies have shown that the decrease in the secretion of inflammatory factors including CRP, TNF-α, and IL-6 leads to the increase in the release of the pro-regenerative factors BDNF and NGF[49], and NGF can activate the MAPK/ERK and PI3K/AKT signaling pathways to achieve axonal regeneration and differentiation of nerve cells.[50] In this research, serum BDNF and NGF levels were measured both before and after treatment. The results indicate that GM-1 and MP shock therapy significantly increased serum BDNF and NGF levels in patients with ASCI before and after treatment. The results indicated that the combination of GM-1 and MP treatment significantly elevated the BDNF and NGF levels in patients with ASCI. Combined with the molecular data of the above studies, the improvement of BI index, VAS score and ASIA pathological pain in ASCI patients was observed. It is reasonable to speculate that GM-1 combined with MP pulse therapy can reduce the levels of inflammatory factors CRP, TNF-α and IL-6 by increasing the level of miR-21-5p, and then increase the release of pro-regenerative factors BDNF and NGF, finally alleviate neurological dysfunction, improve clinical neurological symptoms, and play a better therapeutic effect on ASCI patients. The combined use of GM-1 and MP to detect serum miR-21-5p in patients with injured spinal cord repair will also be a promising direction for research.

This study has certain limitations. The sample size is relatively small, which may bias the results and adversely affect the extrapolation and reliability of the conclusions. Individual differences in the underlying conditions of patients may also interfere with the generalisability of the study results. For this reason, it is advisable to further expand the sample size and develop precise testing indicators to more comprehensively assess the efficacy and safety of the combination therapy, so as to conduct more accurate ROC analyses.

In conclusion, this study indicate that combining GM-1 with MP pulse therapy significantly improves spinal cord function in ASCI patients compared to GM-1 administration alone, potentially offering valuable insights for the treatment of ASCI patients during the acute injury phase (< 8 h). In addition, the combination of the two drugs has a better effect on increasing miR-21-5p and reducing the level of inflammatory factors, which may be one of the important reasons for the significant curative effect. This study provides certain data for the changes in body characteristics of ASCI patients and the elucidation of the treatment mechanism, which can provide certain directions for subsequent clinical medication. However, the number of patients included in this study is limited, and the SCI patients were not strictly graded for further effect exploration. It is essential to conduct extensive and thorough clinical research in the future.

DISCLOSURE

Ethics: This study was approved by Ethics Review Committee of Hainan Provincial People's Hospital. Ethical approval No. Med-Eth-Re [2018]

01. We secured a signed informed consent form from every participant.

Data availability: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Financial support: None

Conflicts of interest: None

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