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Original Article

Relationship between Tumor Necrosis Factor-Alpha and Neuropeptide Y Expression and Neurological Function Score in Epileptic Children

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Abstract

Background: To observe the relationship between Tumor Necrosis Factor-alpha (TNF-α) and Neuropeptide Y (NPY) expression and neurological function score in epileptic children.

Methods: Fifty-four epileptic children diagnosed and treated in Xuzhou Children's Hospital, China from Feb 2017 to Mar 2018 were collected and included in a research group (RG), while 30 healthy children who underwent physical examination at the same time were included in the control group (CG). ELISA was used to detect the expression of TNF-α and NPY in the serum of children in the two groups, and those before treatment were compared. The National Institute of Health stroke scale (NIHSS) and Hamilton Anxiety (HAMA) scores before and after treatment were observed, and Pearson correlation was used to analyze the relationship between the expression levels of TNF-α and NPY in the serum as well as NIHSS and HAMA scores.

Results: The expression levels of TNF-α and NPY in the serum of children in the RG were significantly higher than those in the CG (P<0.001). The expression level of TNF-α was positively correlated with the NIHSS and HAMA scores (r=0.748, P<0.001) (r=0.772, P<0.001). The expression level of NPY was positively correlated with the NIHSS and HAMA scores (r=0.768, P<0.001) (r=0.643, P<0.001).

Conclusion: TNF-α and NPY are highly expressed in epileptic children and are positively correlated with neurological function score.

Keywords: Tumor necrosis factor-alpha; Neuropeptide Y; Epileptic children; Neurological function

Introduction

Epilepsy is a neurological disease that occurs at least twice within 24 h without cause (1). Around the world, about 80% of epilepsy patients come from low-and middle-income countries, and the prevalence rate, morbidity and mortality are higher than those in high-income countries (2-4), which may be related to the high incidence of perinatal adverse events, head injuries and parasitic infections (5-7). According to 2010 statistics, it was estimated that at least 65 million people worldwide sufferred from epilepsy (3). The incidence of epilepsy in children is between 41-187/100,000, and the incidence and prevalence rate are also relatively high in underdeveloped countries, with focal seizures accounting for the main proportion (8). A Norwegian study showed that epilepsy is most common in children, 1 in 150 children will be diagnosed with epilepsy before the age of 10, and the incidence of infants is the highest (9). Patients with severe epileptic sei-

zures and poor compliance will have higher mortality (10). Epilepsy patients usually have multiple conditions (such as stroke, depression or developmental delay) at the same time, which will complicate their epilepsy treatment, damage their physical and mental health and lead to early death (11, 12). Therefore, epilepsy has become the focus of improving health quality in today's society, and searching for effective prevention and treatment has become the current research focus.

Currently NPY and its Y2 receptor have become targets for gene therapy of epilepsy (13). NPY belongs to the neuroendocrine peptide NPY family, which plays a neuroprotective role, increases nutritional support, reduces excitatory toxicity, regulates calcium homeostasis and reduces neuroinflammation (14). NPY also plays an important regulatory part in immune function and inflammatory response of the central nervous system, such as regulating chemotaxis, phagocytosis of immune cells and production and release of cytokines (15). NPY could prevent excessive production of IL-1β and TNF-α and inhibit excitability by inhibiting microglial reactivity, thus protecting neurons (16). In the research of Han and others (17), pro-inflammatory factor TNF-α was highly expressed in epileptic rats, and inflammatory reaction participated in the pathophysiological mechanism of epilepsy. TNF-α and NPY were closely related to the occurrence and development of epilepsy, so we speculated that there was also some relationship between the two factors and the neurological function of children.

To sum up, this study will detect the expression of TNF-α and NPY in epileptic children and study the relationship between the neurological function scores of the two, providing valuable reference for clinical prevention and treatment of epilepsy.

Materials and Methods

Clinical data

Fifty-four epileptic children diagnosed and treated in Xuzhou Children's Hospital, China from Feb 2017 to Mar 2018 were collected and included in the research group (RG) of this study, including 29 males and 21 females, and 30 healthy children undergoing physical examination at the same time were included in the normal control group (NCG), including 16 males and 14 females. All testing indexes in the healthy children laboratory were normal.

This study was approved by the Medical Ethics Committee of our hospital. The informed consent was obtained from the patients'guardians.

Inclusion and exclusion criteria

Inclusion criteria: All children were diagnosed as epilepsy by electroencephalogram and MRI. The diagnostic criteria referred to the relevant guidelines formulated by the International League against Epilepsy in 2010 (18). The clinical data of the children were perfect, no antiepileptic drug treatment was carried out, and the patients' guardians were informed and signed an informed consent form.

Exclusion criteria were as belows: age < 1 yr, liver and kidney dysfunction, brain injury, poor compliance, malignant tumor, infection.

Sample collection and detection

Altogether 3ml of blood from vein of the selected children was taken on an empty stomach within 24 h of epileptic seizure and early morning after treatment, while the same blood sample was taken from the control group (CG). Then, it was left at room temperature for 30 min, centrifuged at 3000rpm/min for 10 min, and the supernatant was taken and placed in a refrigerator at -80 ℃ for freezing, waiting for centralized detection.

A blank hole, a standard product hole and a sample hole to be tested were arranged, the standard product with a concentration of 0 was added into the blank hole, the standard product hole was added with the standard product, and the sample hole was firstly added with the sample to be tested. Sample diluent and Horseradish Peroxidase (HRP) labeled detection antibody were added to all micropores except the sample hole. They were fully washed to remove unbound biotinylated antibody, HRP labeled avidin was added, and TMB substrate was added for color development

after washing again. TMB turned blue under catalysis and turned yellow under the action of acid. The absorbance (OD value) was measured by microplate reader at 450 nm wavelength, and the corresponding concentration was converted from the standard curve.

Outcome measures

Main outcome measures: Neurological function was scored by Neurological Deficit Scale (NDS) (19) before and after treatment respectively; the higher the score was, the more serious the nerve injury was. The Hamilton Anxiety Scale (HAMA) (20) was used to score anxiety before and after treatment; the higher the score was, the more serious the anxiety was.

The expression levels of TNF-α and NPY in the serum of the children in the RG and the children in the CG before and after treatment were observed. The NIHSS and HAMA scores of the children before and after treatment were observed. Pearson correlation was used to analyze the relationship between the expression levels of TNF-α and NPY in the serum and NIHSS and HAMA scores.

Secondary outcome measures: The correlation between TNF-α and NPY expression in serum of children was observed and compared.

Statistical analysis

In this study, SPSS 20.0 (Shanghai Cabit Information Technology Co., Ltd., China) software package was used to carry out statistical analysis on the collected data, and Prism 7 (Shenzhen Qiruitian Software Technology Co., Ltd., China) was used to draw the picture of them. The counting data were expressed by the rate (%), the chi-square test was used for comparison and expressed by χ^2, and the measurement data were expressed by (Meas±SD). The comparison of normal distribution data between the two groups used independent-samples *t* test, and comparison between the two groups before and after treatment used paired *t* test and expressed by *t*. Pearson correlation analysis was used for correlation analysis. A *P* value lower than 0.05 was considered to be a statistical difference between the two groups.

Results

Comparison of clinical data of children in the two groups

More details about baseline data were shown in Table 1.

Table 1: Comparison of clinical data of children in two groups

Factor		*Research group (RG) (n=54)*	*Control group (CG) (n=30)*	*t/χ^2 value*	*P value*
Age (yr)		7.1±2.3	6.4±3.2	1.159	0.250
Gender					
	Male	31 (57.41)	16 (53.33)	0.130	0.719
	Female	23 (42.59)	14 (46.67)		
BMI (kg/m^2)		21.82±2.36	22.04±2.48	0.493	0.623
Place of residence					
	Cities and towns	30 (55.56)	18 (60.00)	0.156	0.693
	Countryside	24 (44.44)	12 (40.00)		
Total cholesterol (mmol/L)		4.38±0.83	4.08±0.67	1.678	0.097

Fasting blood glucose (mmol/L)		5.91±1.33	5.60±1.74	1.157	0.250
Low density lipoprotein cholesterol (mmol/L)		1.14±0.41	1.21±0.23	0.856	0.394
Course of disease (yr)		3.8±0.8	0 (0.00)		
Seizure frequency (min)		15.4±3.7	0 (0.00)		
Seizure types					
	Focal	39 (72.22)	0 (0.00)		
	Comprehensive	15 (27.78)	0 (0.00)		

Expression of TNF-α and NPY in the serum of children in the two groups before and after treatment

ELISA results showed that the expression level of TNF-α in the serum of children in the RG (4.91±0.56) was significantly higher than that in the CG (1.32±0.28) (P<0.001), and the expression level of NPY in the serum of children in the RG (196.28±15.74) was significantly higher than that in the CG (113.24±11.38) (P<0.001). After treatment, the levels of TNF-α (1.58±0.53) and NPY (121.83±13.55) in the serum of the children in the RG decreased, with statistical differences compared with those before treatment (P<0.001), but there was no statistical difference (P>0.05) compared with the CG, as shown in Fig. 1.

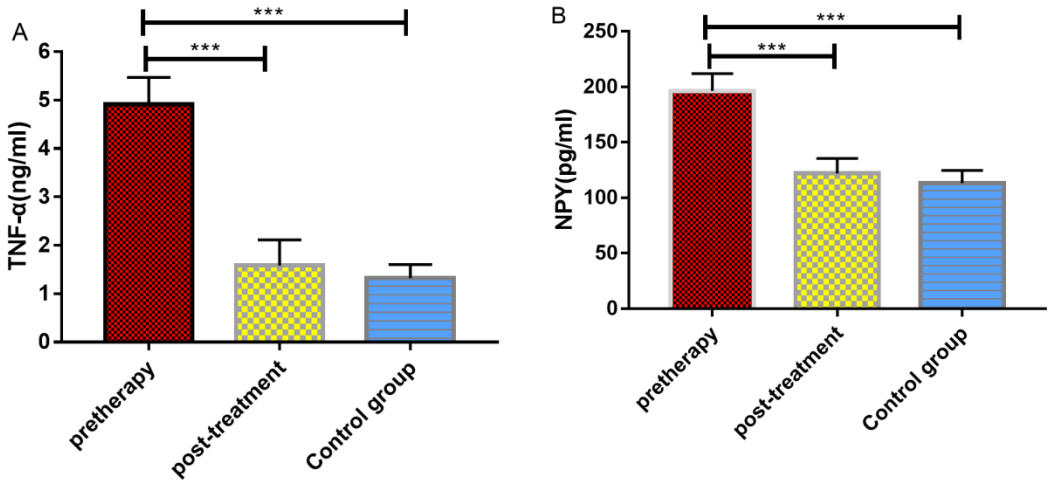

Fig. 1: Expression of TNF-α and NPY in the serum of children in the two groups before and after treatment

A. The expression level of TNF-α in the serum of children in the RG (4.91±0.56) before treatment was significantly higher than that in the CG (1.32±0.28), and TNF-α (1.58±0.53) in the serum of children in the RG decreased after treatment, which was significantly different from that before treatment.

B. The expression level of NPY in the serum of the children in the RG before treatment (196.28±15.74) was significantly higher than that in the CG (113.24±11.38). After treatment, the NPY in the serum of the children in the RG decreased (121.83±13.55), which was significantly different from that before treatment.

*** indicates P<0.001

Correlation between TNF-α and NPY expression in the serum of children before treatment

Pearson correlation analysis was used to analyze the correlation between TNF-α and NPY expression in the serum of children before treatment. It was found that there was a positive correlation between TNF-α and NPY expression (r=0.751, *P*<0.001), and scatter diagrams were drawn. From Fig. 2, we can see that the serum NPY level has a significant upward trend with the increase of TNF-α.

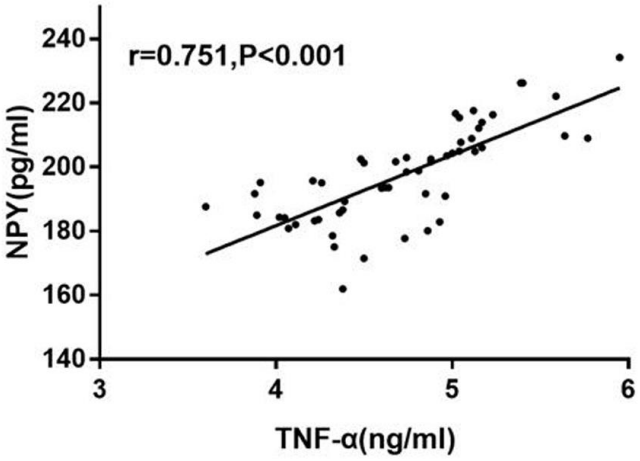

Fig. 2: Scatter diagrams of correlation between TNF-α and NPY expression in the serum of children before treatment
TNF-α and NPY expression are positively correlated (r=0.751, *P*<0.001)

NIHSS and HAMA scores of children before and after treatment

According to statistics of the NIHSS and HAMA scores of children before and after treatment, the NIHSS score of children before treatment (16.75±2.35) was significantly higher than that after treatment (11.88±2.07) (*P* < 0.001), and the HAMA score of children before treatment (15.18±8.54) was significantly higher than that after treatment (11.52±6.83) (*P* < 0.001), as shown in Table 2.

Table 2: NIHSS and HAMA scores of children before and after treatment

Group	*NIHSS score*	*HAMA score*
Before treatment	16.75±2.35	15.18±8.54
After treatment	11.88±2.07	11.52±6.83
t value	11.430	2.460
P value	<0.001	0.016

Correlation between TNF-α, NPY expression and NIHSS, HAMA score in the serum of children

Pearson correlation analysis was used to analyze the correlation between the expression of TNF-α and NPY in the serum as well as NIHSS and HAMA scores before treatment. It was found that the expression of TNF-α was positively correlated with NIHSS and HAMA scores (r=0.748, *P*<0.001) (r=0.772, *P*<0.001). NPY expression was positively correlated with NIHSS and HAMA scores (r=0.768, *P*<0.001) (r=0.643,

P<0.001), and scatter diagrams were drawn. From Fig. 3, we can see that the NIHSS and HAMA scores of children have an obvious upward trend with the increase of serum TNF-α expression, while the NIHSS and HAMA scores of children have an obvious upward trend with the increase of serum NPY expression.

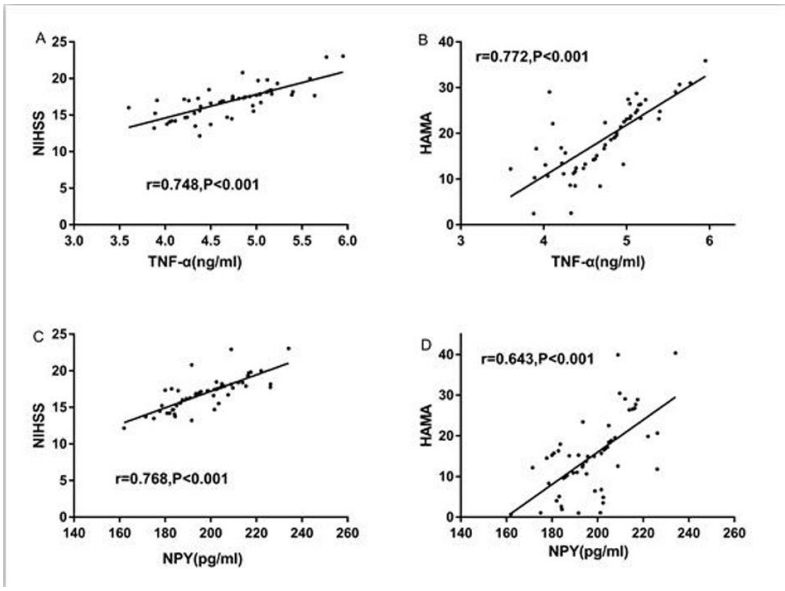

Fig. 3: Correlation between TNF-α, NPY expression and NIHSS, HAMA score in the serum of children
A. TNF-α expression was positively correlated with NIHSS score (r=0.748, *P*<0.001) B. TNF-α expression was positively correlated with HAMA score (r=0.772, *P*<0.001) C. NPY expression was positively correlated with NIHSS score (r=0.768, *P*<0.001)
D. NPY expression was positively correlated with HAMA score (r=0.643, *P*<0.001)

Discussion

Epilepsy represents the clinical manifestation of abnormal over-synchronous discharge of neurons mainly located in cerebral cortex. Epilepsy has greater impact on infants and children than any other age group (21). Epilepsy in children has a wide range of clinical manifestations, many other conditions may be similar to epilepsy, which usually makes the diagnosis process challenging and has a considerable risk of misdiagnosis (22). When we nurse epileptic children, attention should not only be paid to the seizure of epilepsy, but also to the evaluation of various aspects of health, such as mental state and sleep (23). Therefore, this study will explore the expression of TNF-α and NPY in children's serum and relationship between TNF-α and NPY and neurological function score, providing reference for clinical prevention and treatment of epilepsy in children.

Studies have proved the antiepileptic effect of NPY (24), and it can prevent TNF-α overexpression and reduce inflammation. In this study, the expression of TNF-α and NPY in the serum of children in the two groups before treatment was first detected. The results revealed that the expression level of TNF-α and NPY in the serum of the RG was significantly higher than that of the CG, which was consistent with the research results of others (25, 26) in the epileptic rat model. Then we detected the expression of TNF-α and NPY in the serum of children before and after treatment; the results manifested that the expression level of TNF-α and NPY after treatment was significantly lower than that before treatment, and there was no significant difference between the two groups. In the study of epileptic

seizure model, Oztas and others (27) verified that NPY expression could cause the level of pro-inflammatory factor TNF-α to decrease, which might have anti-inflammatory effect. We further analyzed the correlation between TNF-α and NPY expression in the serum of children before treatment, and found that there was a positive correlation between TNF-α and NPY expression, and the serum NPY level had a significant upward trend with the increase of TNF-α. Studies have shown that (28), neurodegeneration and oxidative stress can cause a large number of inflammations, the level of pro-inflammatory cytokines in epileptic brain increases and microglial cells are activated, and when glial cells sense injury signals, they will be activated and release pro-inflammatory cytokines. Therefore, we can reasonably guess the high expression of TNF-α as a proinflammatory factor in epileptic patients, and promote the high expression of NPY to reduce the damage (such as inflammatory reaction) that neurons may cause during epileptic seizures, thus showing a positive correlation between the expression of TNF-α and NPY in patients.

Epilepsy has a great impact on children's quality of life, so studies on their neurological function can provide reference for better efficacy. For this reason, we made a statistical analysis on the NIHSS and HAMA scores of the children before and after treatment; the results displayed that the NIHSS and HAMA scores of the children before treatment were higher, and those after treatment were significantly lower than those before treatment; the results demonstrated that the neurological deficits of epileptic children before treatment were serious and some children even had anxiety. After receiving treatment, the neurological function recovered significantly and the disease condition was well controlled. Epileptic children may have executive power and attention dysfunction, abnormal behavior, loss of consciousness during seizure, etc. (29). After treatment, the disease condition was improved and the neurological function was significantly improved. There are significant differences in TNF-α, NPY, NIHSS and HAMA scores before and after treatment in children. We suspected that there is a close relationship among them. Therefore, Pearson test was used to analyze the correlation between the expression of TNF-α and NPY in the serum as well as the NIHSS and HAMA scores before treatment; the results showed that the expression of TNF-α and NPY was positively correlated with the NIHSS and HAMA scores before treatment. The NIHSS and HAMA scores of children showed a significant upward trend with the increase of the expression of TNF-α and NPY in the serum; the results suggested that the expression levels of TNF-α and NPY were closely tied to the neurological function of children before treatment, and these two factors might be used as predictors of neurological function in epileptic children.

At present, there are more researches on laboratory models of epilepsy and less researches on clinical studies. This clinical experiment has made great progress, but it is still limited. As most of the clinical mechanisms of NPY participating in epilepsy are model tests, and this study has not conducted any research on its molecular mechanism, we still do not know how NPY participates in the pathophysiological process of epilepsy clinically. Therefore, we hope to increase the research on clinical molecular mechanisms in the future to provide more valuable reference for clinical treatment and prognosis.

Conclusion

TNF-α and NPY are involved in the occurrence and development of epilepsy in this study and are strongly linked to the neurological function of patients.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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