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

Li Qiu, Dongli Zhang, Yan Sang, Nuo Zheng, Jiao Chen, Xuan Qiu, *Xiaoming Liu

Department of Neurology (II), Xuzhou Children’s Hospital, Xuzhou Medical University, Xuzhou, 221006, P.R.China

*Corresponding Author: Email: minglx@yeah.net

(Received 15 Apr 2020; accepted 11 Jul 2020)

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 suffered 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 includ-
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 \( \chi^2 \), and the measurement data were expressed by \((\text{Meas}\pm\text{SD})\). 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/\( \chi^2 \) 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 |
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.

### Table

|                         | RG (mmol/L)   | CG (mmol/L)   | P      | P      |
|-------------------------|---------------|---------------|--------|--------|
| Fasting blood glucose   | 5.91±1.33     | 5.60±1.74     | 1.157  | 0.250  |
| Low density lipoprotein cholesterol | 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)      |        |        |

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](image)

**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.
Acknowledgements

No funding was received in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Fisher RS, Acevedo C, Arzimanoglou A, et al (2014). ILAE official report: a practical clinical definition of epilepsy. *Epilepsia*, 55(4): 475-482.
2. Newton CR, Garcia HH (2012). Epilepsy in poor regions of the world. *Lancet*, 380(9848): 1193-1201.
3. Ngugi AK, Bottomley C, Kleinschmidt I, et al (2010). Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia*, 51(5): 883-890.
4. Ngugi AK, Kariuki SM, Bottomley C, et al (2011). Incidence of epilepsy: a systematic review and meta-analysis. *Neurology*, 77(10): 1005-1012.
5. Mbuba CK, Ngugi AK, Newton CR, et al (2008). The epilepsy treatment gap in developing countries: a systematic review of the magnitude, causes, and intervention strategies. *Epilepsia*, 49(9): 1491-1503.
6. Banerjee TK, Ray BK, Das SK, et al (2010). A longitudinal study of epilepsy in Kolkata, India. *Epilepsia*, 51(12): 2384-2391.
7. Meyer AC, Dua T, Ma J, et al (2010). Global disparities in the epilepsy treatment gap: a systematic review. *Build World Health Organ*, 88(4): 260-266.
8. Camfield P, Camfield C (2015). Incidence, prevalence and etiology of seizures and epilepsy in children. *Epileptic Disord*, 17(2): 117-123.
9. Aaberg KM, Gunnes N, Bakken IJ, et al (2017). Incidence and prevalence of childhood epilepsy: a nationwide cohort study. *Pediatrics*, 139(5): e20163908.
10. Thuman DJ, Logrosino G, Beghi E, et al (2017). The burden of premature mortality of epilepsy in high-income countries: a systematic review from the Mortality Task Force of the International League against Epilepsy. *Epilepsia*, 58(1): 17-26.
11. Koh HK, Kobau R, Whittemore VH, et al (2014). Toward an integrated public health approach for epilepsy in the 21st century. *Prev Chronic Dis*, 11: E146.
12. Institute of Medicine (2012). Epilepsy across the spectrum: promoting health and understanding. Washington (DC): National Academies Press (US).
13. Kokaić M (2016). Combinatorial gene therapy for epilepsy: simultaneous NPY and Y2 overexpression. *Neuropeptides*, 55: 4-5.
14. Li C, Wu X, Liu S, et al (2019). Roles of neuropeptide Y in neurodegenerative and neuro-immune diseases. *Front Neurosci*, 13: 869.
15. Ferreira R, Xapelli S, Santos T, et al (2010). Neuropeptide Y modulation of interleukin-1[beta] (IL-1[beta])-induced nitric oxide production in microglia. *J Biol Chem*, 285(53): 41921-41934.
16. Li Q, Dong C, Li W, et al (2014). Neuropeptide Y protects cerebral cortical neurons by regulating microglial immune function. *Neural Regen Res*, 9(9): 959-967.
17. Han K, Wang QY, Wang CX, et al (2018). Ghrelin improves pilocarpine-induced cerebral cortex inflammation in epileptic rats by inhibiting NF-κB and TNF-α. *Mol Med Rep*, 18(4): 3563-3568.
18. Berg AT, Berkovic SF, Brodie MJ, et al (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia*, 51(4): 676-685.
19. Ortiz GA, Ralph LS (2014). National institutes of health stroke scale (nihss). *Wiley Stats Ref: Statistics Reference Online*.
20. Thompson E (2015). Hamilton rating scale for anxiety (HAM-A). *Oczep Med (Lond)*, 65(7): 601.
21. Hamiwka LD, Singh N, Niosi J, et al (2007). Diagnostic inaccuracy in children referred with “first seizure”: role for a first seizure clinic. *Epilepsia*, 48(6): 1062-1066.
22. Chowdhury FA, Nashef L, Elwes RDC (2008). Misdiagnosis in epilepsy: a review and recognition of diagnostic uncertainty. *Eur J Neurol*, 15(10): 1034-1042.
23. Aaberg KM, Bakken IJ, Lossius MI, et al (2016).
Comorbidity and childhood epilepsy: a nationwide registry study. *Pediatrics*, 138(3): e20160921.

24. Patrício MI, Barnard AR, Green AL, et al (2018). A clinical-grade gene therapy vector for pharmacoresistant epilepsy successfully over-expresses NPY in a human neuronal cell line. *Seizure*, 55: 25-29.

25. Li TR, Jia YJ, Wang Q, et al (2018). Correlation between tumor necrosis factor alpha mRNA and microRNA-155 expression in rat models and patients with temporal lobe epilepsy. *Brain Res*, 1700: 56-65.

26. Botterill JJ, Guskjolen AJ, Marks WN, et al (2015). Limbic but not non-limbic kindling impairs conditioned fear and promotes plasticity of NPY and its Y2 receptor. *Brain Struct Funct*, 220(6): 3641-3655.

27. Oztas B, Sahin D, Kir H, et al (2017). The effect of leptin, ghrelin, and neuropeptide-Y on serum Tnf-α, Il-1β, Il-6, Fgf-2, galanin levels and oxidative stress in an experimental generalized convulsive seizure model. *Neuropeptides*, 61: 31-37.

28. Janigro D, Iffland PH 2nd, Marchi N, et al (2013). A role for inflammation in status epilepticus is revealed by a review of current therapeutic approaches. *Epilepsia*, 54 Suppl 6 (06): 30-32.

29. Li Q, Cao W, Liao X, et al (2015). Altered resting state functional network connectivity in children absence epilepsy. *J Neurol Sci*, 354(1-2): 79-85.