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ORIGINAL ARTICLE

New insights into the role of neuron-specific enolase in tic disorders

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Abstract

Objective Neuron-specific enolase (NSE) has been suggested for demonstrating brain metabolism in neuropsychiatric disorders. This study assessed serum NSE levels in patients with tic disorders (TD).

Methods In this retrospective case-control study, we investigated whether NSE levels were increased in TD patients. Then, the influencing factors and correlations between NSE levels and clinical features were analyzed. Finally, we tested its diagnostic value for identifying tic severity.

Results NSE levels were increased in TD patients, although no statistically significant difference was present between transient TD, chronic TD, and Tourette syndrome. Factors influencing NSE levels assessed by multiple linear regression were the Yale Global Tic Severity Scale (YGTSS) global severity scores and gender. There were significant correlations between NSE levels and tic severity. The optimal cut-off value to distinguish mild tics from moderate-severe tics estimated by receiver operating characteristics curve was 24.95 ng/ml (AUC = 0.683).

Conclusion Our findings suggested that NSE may be a significant biomarker in TD but should be confirmed in further investigation.

Keywords Neuron-specific enolase · Tic disorders · Biomarker · Neuropsychiatric disorders

Introduction

Tic disorders (TD) are characterized by the presence of involuntary contractions of muscle groups, resulting either in motor movements or in verbal utterances and sounds [1]. Generally, tics occur in childhood, vary their severity over time, and gradually decrease or even completely disappear after adulthood [2, 3]. Tics can affect patients' health-related quality of life [4, 5]. TD are hierarchically classified into three types: transient TD, chronic TD, and Tourette syndrome (TS) [1]. While the pathogenesis of TD is complex, current research has indicated it is likely linked to the abnormality of the central nervous system (CNS) [6]. Until now, the relationship between clinical features of TD and CNS abnormalities has remained inconclusive. In recent years, the neuro-biochemical markers have brought new insights to identify neurological abnormalities and provide new tools for scientific research.

Neuron-specific enolase (NSE) is a well-known biomarker for demonstrating brain metabolic abnormalities in neurological and psychiatric diseases. Brain tissue contains a high number of specific proteins that cannot be found in other tissues, offering the opportunity to investigate the structural and functional abnormalities of nerves in brain tissue noninvasively [7]. NSE is the γγ-isoenzyme of the glycolytic enzyme enolase found mainly in the cytoplasm of neurons and cells of neuroendocrine origin [8, 9]. When the plasma membrane is impaired functionally or structurally, NSE is released from neurons. The literature has indicated alterations of NSE in neuropsychiatric disorders [10, 11]. As NSE is selectively expressed in neurons and has high stability in biological fluids, it shows important reference value.

To date, few research assessed serum NSE levels in TD children. Our study was aimed to investigate whether NSE levels were increased. If present, we would explore the influencing factors and observe its levels in different subgroups and determine the correlations between NSE levels and clinical features. Finally, we tested its diagnostic value. Here we present our findings.

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Materials and methods

The project is a retrospective case-control study with drug-naive children (aged 3–18 years) with TD. The study group comprised three subgroups (including100 transient TD, 100 chronic TD, and 100 TS), totally 300 patients who were enrolled from January 2016 to June 2018 in Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. All patients were diagnosed based on DSM-5 criteria. Subjects with psychiatric comorbidities or under treatment, or with hemolyzed blood samples, or with incomplete clinical data were excluded. All related clinical information was collected. The control group included 100 age- and gender-matched children without a history of CNS disease or other significant medical disorders. They were recruited in the same hospital. The Yale Global Tic Severity Scale (YGTSS) was used to evaluate tic severity. There were three important scores from the YGTSS: (a) global severity score; (b) total tic score; (c) overall impairment rating.

NSE measurement (laboratory test) is as follows: 10 ml of blood was collected by venipuncture in the cubital fossa, in a vacutainer tube without anti-coagulant, between 8:00 am and 11:00 am. After collection, the total blood was immediately centrifuged at 4000*g* for 10 min and the resulting serum was stored at − 80 °C for analysis. To measure the serum levels of NSE, the commercial kit was used according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany), using the electrochemiluminescence technique with the Cobas® (Roche®). NSE levels are expressed in ng/ml.

The SPSS 25.0 program was used for the statistical analysis. The Mann-Whitney and Kruskal-Wallis tests were used to compare NSE levels in subgroups, and the Spearman correlations were used to assess the relationship between the NSE levels and clinical features. A multiple linear regression model was used to estimate influencing factors. The optimal cut-off value was to distinguish tic severity using receiver operating characteristics (ROC). Statistical significance was determined when p value < 0.05.

Results

Patient characteristics

Patient characteristics are shown in Table 1. In the transient TD group (25 female/75 male), the mean age was 7.42 ± 2.58 years. In the chronic TD group (22 female/78 male), the mean age was 8.72 ± 2.59 years. In the TS group (25 female/75 male), the mean age was 8.41 ± 2.72 years. In the control group (26 female/74 male), the mean age was 7.97 ± 3.25 years. There was no statistical difference in gender ratio or age between transient TD, chronic TD, TS, and the control group respectively.

Serum NSE levels in the TD groups

The serum NSE levels are shown in Table 1. Compared with the control group, NSE levels were increased in three TD subgroups (transient TD, chronic TD, TS) and the differences were statistically significant (Mann-Whitney $p = 0.000$, $p = 0.000$, $p = 0.000$, respectively). However, we found no statistically significant difference in NSE levels between the transient TD, chronic TD, and TS groups (Kruskal-Wallis $p = 0.556$).

Factors influencing serum NSE levels by multiple linear regression

There was no difference in NSE levels between the transient TD, chronic TD, and TS groups; thus, we combined the data of 300 patients for statistical analysis. To predict influencing factors, we used the multiple regression model (Table 2). In this model, NSE levels were the dependent variable. A range of predictor variables covered three domains: Firstly, non-tic-related factors were included. They were gender, age, age of onset, and disease duration. Secondly, factors reflecting tic severity were included: the YGTSS global severity scores and total tic scores. Lastly, the electroencephalogram (EEG) classifications were included. We found the YGTSS global severity scores and gender were the influencing factors, and other factors did not affect the NSE levels observed in this study.

Serum NSE levels in subgroups

Based on the results of multiple linear regression, 300 TD patients were classified according to tic severity firstly. Severe TD patients contain less; thus, we combined moderate and severe TD patients together. NSE levels were increased in moderate-severe TD group compared with mild TD group (median 23.01 vs. 21.62; $p = 0.001$). Then, stratifying by gender, patients were subdivided and classified into four subgroups. NSE levels were increased in mild male TD group than mild female group (median 21.74 vs. 19.58; $p = 0.016$), and in moderate-severe female TD group than mild female group (median 22.46 vs. 19.58; $p = 0.012$), and in moderate-severe male TD group than mild male group (median 23.11 vs. 21.74; $p = 0.004$). No difference between moderate-severe female TD group and male TD group was found ($p = 0.142$).

Table 1 Patient characteristics and serum NSE levels

	Overall	Transient TD	Chronic TD	TS	Control group
n	300	100	100	100	100
Gender: female/male	72/228	25/75	22/78	25/75	26/74
Age (mean ± SD), y	8.18 ± 2.68	7.42 ± 2.58	8.72 ± 2.59	8.41 ± 2.72	7.97 ± 3.25
Age of onset (mean ± SD), y	6.50 ± 2.31	6.97 ± 2.49	6.53 ± 2.30	5.99 ± 2.06	
Disease duration (median, IQR), m	12 (8–24)	5 (2–8)	24 (12–24.0)	24 (12–36)	
YGTSS global severity score (mean ± SD)	25.86 ± 8.36	23.76 ± 7.23	24.52 ± 7.45	29.30 ± 9.21	
YGTSS total tic score (mean ± SD)	15.86 ± 6.54	14.26 ± 5.18	14.62 ± 5.99	18.70 ± 7.36	
YGTSS overall impairment rating (median, IQR)	10 (10–10)	10 (10–10)	10 (10–10)	10 (10–10)	
Serum NSE levels (median, IQR), ng/ml	22.07 (19.34–24)	22.60 (19.37–24.74)	21.74 (18.68–24.69)	22.38 (19.57–24.97)	17.12 (14.80–19.79)

IQR interquartile range, *SD* standard deviation, *y* year, *m* month, *YGTSS* Yale Global Tic Severity Scale.

Correlation analyses between serum NSE levels and clinical features

Firstly, we performed a correlation analysis with 300 TD patients. The results are shown in Table 3. There was a significant positive correlation between NSE levels and total tic scores ($r = 0.281$, $p = 0.000$), and overall impairment rating scores ($r = 0.279$, $p = 0.000$), and the YGTSS global severity scores ($r = 0.318$, $p = 0.000$). In this part, there were no significant correlations between serum NSE levels and age ($p = 0.321$), age of TD onset ($p = 0.304$), and disease duration ($p = 0.708$). Then, we performed the correlation analysis in three subgroups (Table 3). We found a moderate and significant correlation between NSE levels and the YGTSS global severity scores (Fig. 1); no other significant correlations were found.

ROC curve for serum NSE levels

The optimal cut-off value was to distinguish tic severity using ROC curve. The optimal cut-off value to distinguish mild tics from moderate-severe tics was 24.95 ng/ml, with sensitivity of 31.6% and specificity of 88.3%. The result is shown in Fig. 2.

Discussion

Here, we show for the first time. Serum NSE levels were increased in TD patients and significantly related to tic severity as measured with the YGTSS. The multiple linear regression model showed the YGTSS global severity scores and gender were factors influencing NSE levels. And NSE levels were increased in mild male TD group than mild female group, and in moderate-severe female group than mild female group, and in moderate-severe male group than mild male group. Our findings highlighted the importance of tic severity and gender in future studies on NSE levels in TD. Using the ROC curve, the optimal cut-off value to distinguish mild tics from moderate-severe tics was 24.95 ng/ml. Until now, there is still a lack of laboratory indicators related to tic severity. Our study indicated that NSE levels, as a significant biomarker, could provide reference value for TD.

NSE is a glycolitic enzyme involved in the metabolism of neuronal cells primarily localized in the cytoplasm of the neurons. As it is not secreted, increments in NSE levels in serum might indicate metabolic dysfunction and structural damage to the neuronal tissue [12]. NSE is released soon after cellular injury of the nervous tissue and then shows detectable increases

Table 2 Summary of the multiple linear regression model. The YGTSS global severity scores and gender were factors influencing NSE levels

Variables	Unstandardized regression coefficients (95% CI)	Standard error	*p* value
YGTSS global severity score	0.234 (0.114 to 0.353)	0.061	0.000
Gender			
Female (reference)	(Reference)	(Reference)	0.039
Male	1.067 (0.052 to 2.082)	0.516	
Age	− 1.048 (− 2.522 to 0.427)	0.749	0.163
Age of onset	0.959 (−0.516 to 2.434)	0.749	0.202
Disease duration	0.072 (−0.052 to 0.197)	0.063	0.254
Total tic score	− 0.076 (− 0.228 to 0.077)	0.078	0.329
EEG classification			
Normal (reference)	(Reference)	(Reference)	0.447
Abnormal	− 1.043 (− 3.742to1.656)	1.371	

EEG electroencephalogram, *CI* confidence interval

Table 3 Significant correlation analyses between NSE levels and other clinical features

Variables	Serum NSE levels[a]	(*p* value)
Entire TD sample	22.07 (19.34–24)	–
Total tic score	$r = 0.281$	0.000
Overall impairment rating	$r = 0.279$	0.000
Transient TD	22.60 (19.37–24.74)	–
Total tic score	$r = 0.288$	0.004
Overall impairment rating	$r = 0.252$	0.011
Chronic TD	21.74 (18.68–24.69)	–
Age	$r = -0.218$	0.029
Age of onset	$r = -0.222$	0.026
Total tic score	$r = 0.248$	0.013
Overall impairment rating	$r = 0.316$	0.001
TS	22.38 (19.57–24.97)	–
Total tic score	$r = 0.299$	0.002
Overall impairment rating	$r = 0.274$	0.006

[a] Spearman's correlation coefficients

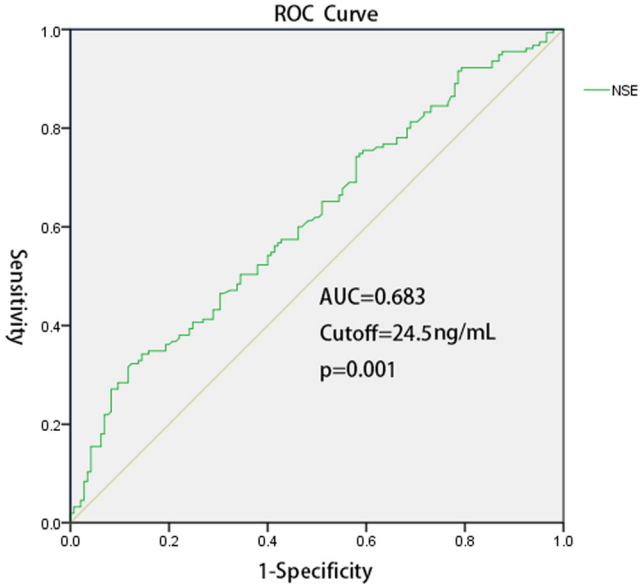

Fig. 2 ROC curve for NSE levels in TD patients

in concentration in blood. Previous studies investigated this protein has been used for traumatic brain injury [13], stroke [14], and hypoxic encephalopathy [15]. On the other hand, NSE can indicate metabolic dysfunction in the neuronal tissue. Wiener et al. reported decreased NSE levels in patients with major depression, which might reflect reduced neuronal metabolic activity [8]. Hawro et al. reported the low concentrations of NSE detected in patients with neuropsychiatric systemic lupus erythematosus may also reflect the reduced neuronal metabolic activity [16]. Besides, NSE is also a highly specific marker for neuroendocrine cells. Using immunostaining techniques, NSE not is only observed in neurons but also been demonstrated in a variety of neuroendocrine cells including

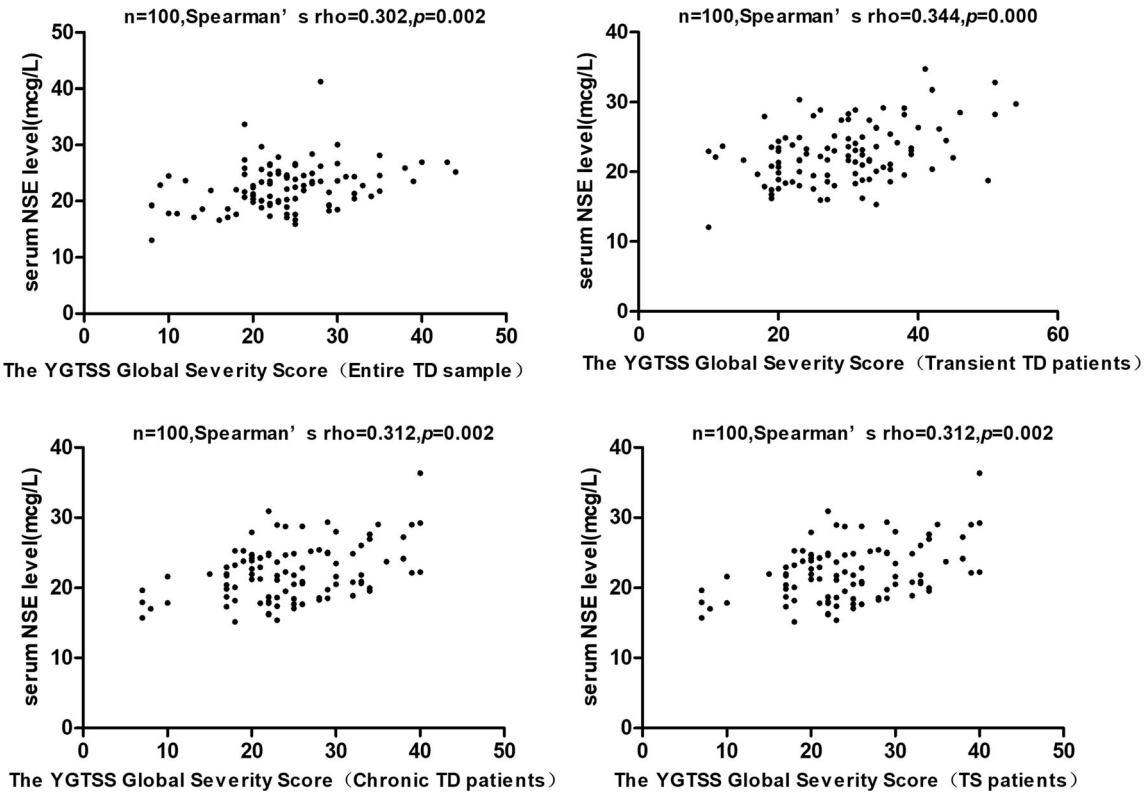

Fig. 1 Correlation analyses showing a moderate and significant correlation between NSE levels and the YGTSS global severity score

pinealocytes, pituitary glandular, and peptide-secreting cells, and so on. Since the original description of NSE in 1965, there have been accounts of its use in clinical practice.

TD is a complex neuropsychiatric disorder with abnormalities mainly in the neuro-transmission of dopamine and γ-aminobutyric acid (γ-GABA) [17, 18]. Subsequently, brain metabolite abnormalities in the GABA-Glu-Gln cycle were reported [19]. As this was the first study reporting an increase in NSE levels in TD, the underlying connections between abnormal neuro-transmission metabolism and the release of NSE were uncertain. It had to be confirmed by using neuroimaging evaluation of brain metabolism including positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). Aside from these neuro-transmission abnormalities above, neuroendocrine factors may be also involved. The heightened responsivity of hypothalamic-pituitary-adrenal (HPA) axis was reported in the neuropathology of TD [20]. Chappell also found TD patients secreted significantly more adrenocorticotropic hormone (ACTH) than control subjects following lumbar puncture [21]. ACTH is released from the adrenocorticotropic cell in anterior pituitary gland which is one kind of neuroendocrine cell that secretes NSE. Thus, we propose that the increase of NSE levels may be also generated by neuroendocrine cells (such as adrenocorticotropic cells). Future cellular experiments to observe the secretion of NSE in these neuroendocrine tissues and cells will be conducted and expound the underlying pathophysiology.

Here, some restrictions have to be considered. Our sample size was too small to confirm the validity of our conclusions. Moreover, the current study did not make use of imaging techniques and cellular experiments. The serum NSE levels are significantly increased in TD and correlate with some clinical features; thus, the utility of NSE as a biomarker of TD is valuable. Further studies are needed to clarify the role of NSE in TD and to evaluate how it may change with successful treatment.

Author contributions Authors Juanjuan Hao and Min Wu designed the study, collected and analyzed the data, and wrote the manuscript. Authors Xin Zhang and Keyu Jiang made a contribution on revising the manuscript for content. All authors approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Xinhua Hospital Ethics Committee affiliated to Shanghai JiaoTong University School of Medicine (Approval No. XHEC-D-2018-047).

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References

1. American Psychiatric Association (2013) The Diagnostic and Statistical Manual of Mental Disorders (5th ed.;DSM-5). American Psychiatric Association, Arlington
2. Bloch MH, Peterson BS, Scahill L, Otka J, Katsovich L, Zhang H, Leckman JF (2006) Adulthood outcome of tic and obsessive compulsive symptom severity in children with Tourette syndrome. Arch Pediatr Adolesc Med 160:65–69
3. McNaught KSP, Mink JW (2011) Advances in understanding and treatment of Tourette syndrome. Nat Rev Neurol 7:667–676
4. Silvestri PR, Chiarotti F, Baglioni V, Neri V, Cardona F, Cavanna AE (2016) Health-related quality of life in patients with Gilles de la Tourette syndrome at the transition between adolescence and adulthood. Neurol Sci 37:1857–1860
5. Cavanna AE, David K, Bandera V, Termine C, Balottin U, Schrag A, Selai C (2013) Health-related quality of life in Gilles de la Tourette syndrome: a decade of research. Behav Neurol 27:83–93
6. Felling RJ, Singer HS (2011) Neurobiology of Tourette syndrome: current status and need for further investigation. J Neurosci 31(35): 12387–12395
7. Eyre H, Baune BT (2012) Neuroplastic changes in depression: a role for the immune system. Psychoneuroendocrinology 37:1397–1416
8. Wiener CD, Jansen K, Ghisleni G, Kaster MP, Souza LD, Lara DR, Portela LV, da Silva RA, Oses JP (2013) Reduced serum levels of neuron specific enolase (NSE) in drug-naive subjects with major depression and bipolar disorder. Neurochem Res 38:1394–1398
9. Busnello JV, Leke R, Oses JP, Feier G, Bruch R, Quevedo J, Kapczinski F, Souza DO, Cruz Portela LV (2006) Acute and chronic electroconvulsive shock in rats:effects on peripheral markers of neuronal injury and glial activity. Life Sci 78:3013–3017
10. Machado-Vieira R, Andreazza AC, Viale CI, Zanatto V, Cereser V Jr, daSilva Vargas R et al (2007) Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithiumantioxidant effects. Neurosci Lett 421:33–36
11. Brandel JP, Beaudry P, Delasnerie-Lauprêtre N, Laplanche JL (1999) Creutzfeldt-Jakob disease: diagnostic value of protein14-3-3 and neuronal specific enolase assay in cerebrospinal fluid. Rev Neurol (Paris) 155:148–151
12. Guzel A, Er U, Tatli M, Aluclu U, Ozkan U, Duzenli Y, Satici O, Guzel E, Kemaloglu S, Ceviz A, Kaplan A (2008) Serum neuron-specific enolase as a predictor of short-term outcome and its correlation with Glasgow Coma Scale in traumatic brain injury. Neurosurg Rev 31:439–444
13. Hamed SA, Eltayeb AA, Mohamad HO, Moussa EM (2013) Brain function in children with uncomplicated epilepsy: relationship to biomarkers of brain damage (neuron specific enolase and S100bprotein). J Pediatr Epilepsy 2:115–124
14. Gonzalez-Garcia S, Gonzalez-Quevedo A, Fernandez-Concepcion O, Pena-Sanchez M, Menendez-Sainz C et al (2012) Short-term prognostic value of serum neuron specific enolase and S100B in acute stroke patients. Clin Biochem 45:1302–1307
15. Cronberg T, Rundgren M, Westhall E, Englund E, Siemund R, Rosen I, Widner H, Friberg H (2011) Neuron-specific enolase correlates with other prognostic markers after cardiac arrest. Neurology 77:623–630
16. Hawro T, Bogucki A, Krupińska-Kun M, Maurer M, Woźniacka A (2015) Serum neuron specific enolase-a novel indicator for neuropsychiatric systemic lupus erythematosus? Lupus 24:1492–1497
17. Singer HS, Butler IJ, Tune LE, Seifert WE Jr, Coyle JT (1982) Dopaminergic dysfunction in Tourette syndrome. Ann Neurol 12: 361–366

18. Tinaz S, Belluscio BA, Malone P, van der Veen JW, Hallett M, Horovitz SG (2014) Role of the sensorimotor cortex in Tourette syndrome using multimodal imaging. Hum Brain Mapp 35:5834–5846
19. Kanaan AS, Gerasch S, García-García I, Lampe L, Pampel A, Anwander A, Near J, Harald EM, Mller-Vahl K (2017) Pathological glutamatergic neurotransmission in Gilles de la Tourette syndrome. Brain 140:218–234
20. Corbett BA, Mendoza SP, Baym CL, Bunge SA, Levine S (2008) Examining cortisol rhythmicity and responsivity to stress in children with Tourette syndrome. Psychoneuroendocrinology 33(6): 810–820
21. Chappell P, Riddle M, Anderson G, Scahill L, Hardin M, Walker D, Cohen D, Leckman J (1994) Enhanced stress responsivity of Tourette syndrome patients undergoing lumbar puncture. Biol Psychiatry 36(1):35–43