Is serum NSE a useful biomarker in multiple system atrophy?

[1]Qiaoqiao Zhang *MM*, [2]Long Jin *Phar M*

[1]*Department of Neurology,* [2]*Phar M, Department of Pharmacy, Affiliated Dongyang Hospital of Wenzhou Medical University, Dongyang, Zhejiang, China*

Abstract

Objective: Multiple system atrophy (MSA) is a rapidly progressive and devastating neurodegenerative disease with limited lifespan. Biomarkers in diagnosing and evaluating the prognosis of MSA is a rapidly developing field. Neuron-specific enolase (NSE) is a biomarker of neuronal damage applicable in many neurological disorders. This study aimed to determine the usefulness of serum NSE as biomarker of MSA. *Methods:* In this case-control study involving 50 patients with MSA, electrochemiluminescence assay was used to investigate whether serum NSE levels. The disease severity of patients was assessed by Unified System Atrophy Rating Scale (UMSARS part II). The correlation between NSE levels and clinical features in MSA patients was analyzed. *Results:* The NSE levels were higher in MSA patients (11.59±2.52 ng/mL) than the control group (11.30±2.73 ng/mL); however, the difference was not statistically significant (P=0.581). In addition, no statistically significant difference was detected in the NSE levels between the parkinsonian (MSA-P) and cerebellar subtypes (MSA-C). No distinct difference was also detected between males and females of MSA patients. Furthermore, no correlations were established between NSE levels and age, disease duration, and UMSARS II score.
Conclusions: This study suggest that serum NSE is not a useful biomarker of MSA. Future studies should examine whether there is a role for CSF NSE in reflecting neuronal damage in MSA patients.

Keywords: Multiple system atrophy, neuron-specific enolase, biomarker, Unified System Atrophy Rating Scale

INTRODUCTION

Multiple system atrophy (MSA), first described in 1969[1], is a rare, adult-onset, rapidly progressive, and devastating neurodegenerative disorder, leading to irreversible neuronal damage with an aggressive clinical course lasting approximately 9 years from onset to death. People with MSA suffer from severe disabilities, including autonomic failure in combination with levodopa (L-dopa)-unresponsive parkinsonism (MSA-P) or cerebellar ataxia (MSA-C).[2] The incidence of MSA is about 0.6-0.7/100,000 in the population. A geographical discrepancy was detected in the MSA clinical subtypes in worldwide distribution. In the Western countries, MSA-P is the most common subtype, whereas in Japan, MSA-C predominates, implicating the influence of genetic or environmental factors.[3-5] The identified neuropathological hallmarks of MSA is the glial cytoplasmic inclusions (GCIs). Although several studies proposed many factors, such as oxidative stress[6], neuroinflammation[7], mitochondrial dysfunction[8], myelin lipids dysregulation[9,10], genetic polymorphism[11], iron metabolism dysregulation[12], intestinal inflammation[13], and intestinal dysbiosis[14], the exact pathogenesis is not yet clearly elucidated.[15]

Neuron-specific enolase (NSE) is a cytoplasmatic glycolytic enzyme, localized in neurons and neuroendocrine cells. Since NSE is not physiologically secreted, the increase in the NSE level in serum or CSF is considered a biomarker of neuronal damage in neurological disorders.[16-18] NSE is also elevated in neurodegenerative diseases; for instance, serum and CSF of early-stage Creutzfeldt-Jakob disease[19] and CSF of Alzheimer's disease.[20,21] Since MSA is the neurodegenerative disease, we speculated that NSE levels are elevated in MSA and play a role in the pathophysiology of MSA. Hitherto, only a few studies have been reported on the significance of NSE in MSA.[22]

In order to investigate whether there is a role for the NSE-mediated neuronal damage in MSA

pathophysiology, we assessed the serum NSE levels in patients with MSA and analyzed the correlation between serum NSE levels and clinical features in MSA patients.

METHODS

A total of 50 MSA patients were recruited from the Department of Neurology, Affiliated Dongyang Hospital of Wenzhou Medical University between August 2014 and 2019. The patients were recruited by consecutive sampling. The diagnosis of probable MSA was confirmed by a movement disorders neurologist according to consensus criteria.[23] The MSA-P group consisted of 26 patients, and the MSA-C group consisted of 24 patients. The exclusion criteria were secondary causes of parkinsonism, other neurological diseases, psychiatric symptoms, cancer, known tobacco or ethanol abuse, and severe complications, such as heart disease and renal failure. All MSA patients were evaluated using the Unified System Atrophy Rating Scale (UMSARS part II).[24] Many healthy individuals go to the Physical Check Center of the hospital for physical examinations such as blood test, to evaluate their health status. Fifty age- and gender-matched healthy control subjects without medical illnesses and known tobacco or ethanol abuse were recruited from the Physical Check Center of the same hospital. The demographics of patients and controls are described in Table 1. Written informed consent was obtained from all participants included in the study that was approved by the local ethics committee of this hospital.

Data collection

An equivalent of 3 mL venous blood was collected from participants between 5:00 and 7:00 a.m., following about 8 h of fasting. The sera were collected from the samples by centrifugation at 3000 rpm for 10 min, and stored at −80 °C for subsequent use. The NSE serum levels were detected by electrochemiluminescence assay (ECLIA; Roche Diagnostics, USA).

NSE was routinely tested in the Clinical Laboratory of this hospital. The blood samples withdrawn from the participants were sent to the Clinical Laboratory within 1 h after drawing on the same day for the detection of NSE by ECLIA. We also stored the serum samples at −80 °C for later use. The kit and laboratory method were same as those used above to test the stored serum samples, and no significant differences

were observed when comparing the current and previous detection results.

Statistical analysis

Data analysis was performed using SPSS version 21 (SPSS Inc., IL, USA). Numerical data were summarized using means and standard deviation (SD). A parametric t-test was used to compare the NSE levels between groups. Mann–Whitney U test was used to compare the age between the groups. The associations between NSE levels and demographic and clinical parameters (gender, disease duration, UMSARS II score) were analyzed by Pearson's correlation test. The association between NSE levels and age was analyzed by Spearman's correlation test. $P<0.05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the study population

The demographics and clinical characteristics of patients with MSA and healthy controls are shown in Table 1. A total of 50 MSA patients (male/female (M/F)=29/21, mean age (62.94±9.26) years) and 50 controls (M/F=21/29, mean age (59.06±11.84) years) were recruited in this study. The results did not find gender- (P=0.110) and age-related difference (P=0.083) between the two groups. The mean disease duration of MSA patients was 3.32±1.82 years. The mean UMSARS II score was 22.84±13.03.

Serum NSE levels in patients and controls

The serum levels of NSE were measured using ECLIA. The levels of NSE were higher in MSA patients (11.59±2.52 ng/mL) compared to the control group (11.30±2.73 ng/mL), but the difference was not statistically significant (P=0.581, Table 1). Moreover, no statistically significant difference was detected in the NSE levels between MSA-P (11.6±2.85 ng/mL) and MSA-C (11.59±2.1 ng/mL, P=0.992). Similarly, no difference was observed between the genders of MSA patients (Male: 11.25±2.20 *vs*. Female: 12.06±2.89, P=0.271). Also, no correlation was established between NSE levels and age, disease duration, and UMSARS II score (Figure 1).

DISCUSSION

The involvement of NSE in MSA has been rarely investigated.[22] In this study, we aimed to

Table 1: Demographic and clinical characteristics of the study population

Characteristics	MSA (n=50)	Control (n=50)	*P*-value
Gender (M/F)	29/21	21/29	0.110[a]
Age (years) *	62.94±9.26	59.06±11.84	0.083[b]
Disease duration (years) *	3.32±1.82	NA	
UMSARS II score	22.84±13.03	NA	
NSE (ng/mL)	11.59±2.52	11.30±2.73	0.581

Abbreviations: F, female; M, male; NA, not applicable; UMSARS, Unified Multiple System Atrophy Rating Scale.
[a] χ^2 test.
[b] Mann–Whitney *U* test.
*mean±SD

investigate whether NSE play a role in neuronal damage in MSA pathophysiology based on the serum NSE levels. We did not find a significant difference between 50 MSA patients and 50 age- and gender-matched controls. Moreover, we did not find any correlations between NSE levels and age, disease duration, and UMSARS II score associated with the severity of this disease. In addition, the serum NSE levels were similar in both MSA subtypes (MSA-P and MSA-C).

NSE is a glycolytic enzyme localized in the neurons and neuroendocrine cells. It may play a dual role in promoting neuroinflammation and neurotrophy in neurodegenerative diseases. The elevated NSE levels promote inflammatory cytokines and chemokines, which may promote neurodegeneration.[25] Thus, NSE could potentially have clinically useful as a non-invasive quantitative and specific biomarker of the neuronal injury. NSE may also have protective effects by its role in neuronal survival, differentiation, and neurite regeneration via activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways.[26,27] This neuroprotective function is regulated by cathepsin X (Cat X), which truncates the C-terminal end and modifies the activity of the molecule.[28,29]

Previous studies have shown elevated NSE levels in various neurological disorders: serum of traumatic brain injury[30], serum of cerebral venous thrombosis[31], serum of acute spinal cord injury[32], CSF of seizures[33], serum and CSF of Creutzfeldt-Jakob disease[19], serum of stroke[34], and CSF of Alzheimer's disease.[20,21] However, in a previous study, the serum NSE levels did not differ between Parkinson disease's patients and normal controls, which may be attributed to the lesser neuronal damage in Parkinson disease patients.[35] In another study on 36 patients. the serum NSE levels were not significantly higher in idiopathic intracranial hypertension patients compared to controls. Samanci *et al*. explained that this may be attributed to the short biological half-life in of NSE in body fluids.[36] Another study on by Abdo *et al*. showed that the CSF NSE levels in 45 MSA patients did not differ significantly from normal controls, and no differences were observed between the MSA clinical subtypes.[22] In our study, although the serum NSE level was higher in the MSA group than the control group, the difference was not statically significant. There is no previous study reporting serum level of NSE in MSA. The lack of altered levels of serum NSE may be attributed to the short biological half-life of NSE in body fluids, which is approximately 24 h.[25,36]

Based on the NSE-mediated neuron damaging effects, the correlation between NSE levels and clinical disease progression has been demonstrated in patients of various pathologies with impaired blood-brain barrier and neuronal loss.[37-39] However, we did not find any correlations between serum NSE levels and age, disease duration, and UMSARS II score associated with the severity of this disease. Since the pattern of neuronal death in MSA patients is unclear, quantifying the neuronal loss during disease progression is challenging. We did not establish any correlations between serum NSE levels and the duration of MSA disease progression in this study. Since NSE is considered a biomarker of neuronal damage, we speculated that if the neuronal loss in MSA varies with time, the single estimation of serum levels of NSE could not detect this correlation. The serum NSE levels were also similar in both MSA phenotypes, indicating that the enzyme could not differentiate between the two MSA subtypes. This was consistent with the natural history of the MSA subtypes, i.e., no difference in the mean survival between MSA-P and MSA-C patients.[5] Another new finding in this study was that among the 50 MSA patients, the MSA-P group consisted of 26 patients and the MSA-C group consisted of 24
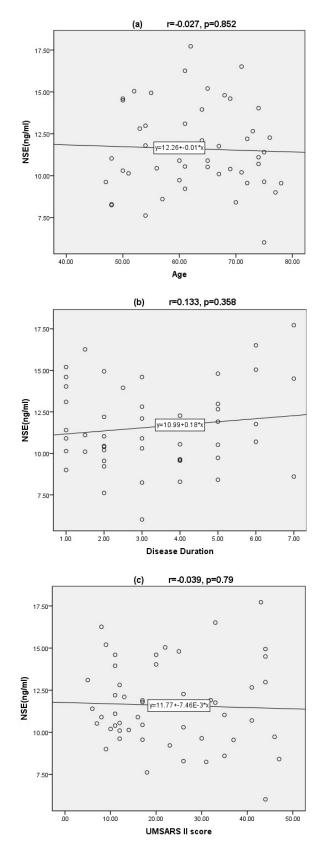
Figure 1. Correlation between NSE levels and age, disease duration, and UMSARS II score.

patients, which is different from the demographics in Japan with more MSA-C.[5] Thus, the current data helped to expand the geographical spectrum of MSA clinical subtypes in Asia.

In conclusion, this study analyzed serum NSE levels in MSA patients. There was no significant difference between patients and controls nor between different clinical phenotypes of MSA patients. The main limitation of our study was the small sample size. Due to ethical restriction, CSF samples from normal controls could also not be obtained. Although we could not demonstrate a significantly elevated level of NSE in MSA, we propose that further studies involving larger number of patient and include CSF samples to elucidate any discrepancies between serum and CSF levels should be performed. There should also be repeat estimation of the levels in the same patient during the clinical course to reflect temporal changes of the neuronal damage.

DISCLOSURE

REFERENCES

1. Graham JG, Oppenheimer DR. Orthostatic hypotension and nicotine sensitivity in a case of multiple system atrophy. *J Neurol Neurosurg Psychiatry* 1969;32(1):28-34. DOI:10.1136/jnnp.32.1.28.

2. Krismer F, Wenning GK. Multiple system atrophy: insights into a rare and debilitating movement disorder. *Nat Rev Neurol* 2017;13(4):232-43. DOI:10.1038/nrneurol.2017.26.

3. Gilman S, May SJ, Shults CW et al. The North American Multiple System Atrophy Study Group. *J Neural Transm (Vienna)*. 2005;112(12):1687-94. DOI:10.1007/s00702-005-0381-6.

4. Kollensperger M, Geser F, Ndayisaba JP, *et al.* Presentation, diagnosis, and management of multiple system atrophy in Europe: final analysis of the European multiple system atrophy registry. *Mov Disord* 2010;25(15):2604-12. DOI:10.1002/mds.23192.

5. Watanabe H, Saito Y, Terao S, *et al.* Progression and prognosis in multiple system atrophy: an analysis of 230 Japanese patients. *Brain* 2002;125(Pt 5):1070-83. DOI:10.1093/brain/awf117.

6. Jellinger KA. Neuropathology of multiple system atrophy: new thoughts about pathogenesis. *Mov Disord* 2014;29(14):1720-41. DOI:10.1002/mds.26052.

7. Vieira BD, Radford RA, Chung RS, Guillemin GJ, Pountney DL. Neuroinflammation in multiple system atrophy: Response to and cause of alpha-synuclein aggregation. *Front Cell Neurosci* 2015;9:437. DOI:10.3389/fncel.2015.00437.

8. Ubhi K, Rockenstein E, Kragh C *et al.* Widespread microRNA dysregulation in multiple system atrophy - disease-related alteration in miR-96. *Eur J Neurosci* 2014;39(6):1026-41. DOI:10.1111/ejn.12444.

9. Bleasel JM, Wong JH, Halliday GM, Kim WS. Lipid dysfunction and pathogenesis of multiple system atrophy. *Acta Neuropathol Commun* 2014;2:15. DOI:10.1186/2051-5960-2-15.

10. Don AS, Hsiao JH, Bleasel JM, *et al.* Altered lipid levels provide evidence for myelin dysfunction in multiple system atrophy. *Acta Neuropathol Commun* 2014;2:150. DOI:10.1186/s40478-014-0150-6

11. Chen J, Mills JD, Halliday GM, Janitz M. Role of transcriptional control in multiple system atrophy. *Neurobiol Aging* 2015;36(1):394-400. DOI:10.1016/j.neurobiolaging.2014.08.015.

12. Kaindlstorfer C, Jellinger KA, Eschlböck S, *et al.* The Relevance of Iron in the Pathogenesis of Multiple System Atrophy: A Viewpoint. *J Alzheimers Dis.* 2018;61(4):1253-73. DOI:10.3233/JAD-170601.

13. Hor JW, Lim SY, Khor ES, *et al.* Fecal calprotectin in Parkinson's disease and multiple system atrophy. *J Mov Disord* 2021;15(2):106-14. DOI:10.14802/jmd.21085.

14. Tan AH, Chong CW, Song SL, *et al.* Altered gut microbiome and metabolome in patients with multiple system atrophy. *Mov Disord* 2018;33(1):174-6. DOI:10.1002/mds.27203

15. Jellinger KA. Potential clinical utility of multiple system atrophy biomarkers. *Expert Rev Neurother* 2017;17(12):1189-208. DOI:10.1080/14737175.2017.1392239

16. Park SH, Hwang SK. Prognostic value of serum levels of S100 calcium-binding protein B, neuron-specific enolase, and interleukin-6 in pediatric patients with traumatic brain injury. *World Neurosurg* 2018;118:e534-e542. DOI:10.1016/j.wneu.2018.06.234.

17. Gojska-Grymajlo A, Zielinski M, Wardowska A, *et al.* CXCR7+ and CXCR4+ stem cells and neuron specific enolase in acute ischemic stroke patients. *Neurochem Int.* 2018;120:134-9. DOI:10.1016/j.neuint.2018.08.009.

18. Bharosay A, Bharosay VV, Saxena K, Varma M. Role of brain biomarker in predicting clinical outcome in hypertensive cerebrovascular ischemic stroke. *Indian J Clin Biochem* 2018;33(2):178-83. DOI:10.1007/s12291-017-0664-3.

19. Kohira I, Tsuji T, Ishizu H, *et al.* Elevation of neuron-specific enolase in serum and cerebrospinal fluid of early stage Creutzfeldt-Jakob disease. *Acta Neurol Scand.* 2000;102(6):385-7. DOI: 10.1034/j.1600-0404.2000.102006385.x.

20. Schmidt FM, Mergl R, Stach B, *et al.* Elevated levels of cerebrospinal fluid neuron-specific enolase (NSE) in Alzheimer's disease. *Neurosci Lett* 2014;570:81-5. DOI:10.1016/j.neulet.2014.04.007.

21. Palumbo B, Siepi D, Sabalich I, Tranfaglia C, Parnetti L. Cerebrospinal fluid neuron-specific enolase: a further marker of Alzheimer's disease? *Funct Neurol* 2008;23(2):93-6.

22. Abdo WF, van de Warrenburg BPC, Kremer HPH, Bloem BR, Verbeek MM. CSF biomarker profiles do not differentiate between the cerebellar and parkinsonian phenotypes of multiple system atrophy. *Parkinsonism Relat Disord* 2007;13(8):480-2. DOI:10.1016/j.parkreldis.2007.02.002.

23. Gilman S, Wenning GK, Low PA, *et al.* Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* 2008;71(9):670-6. DOI:10.1212/01.wnl.0000324625.00404.15.

24. Wenning GK, Tison F, Seppi K, *et al.* Development and validation of the Unified Multiple System Atrophy Rating Scale (UMSARS). *Mov Disord.* 2004;19(12):1391-402. DOI:10.1002/mds.20255.

25. Haque A, Polcyn R, Matzelle D, Banik NL. New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. *Brain Sci* 2018;8(2):33. DOI:10.3390/brainsci8020033.

26. Zheng J, Liang J, Deng X, *et al.* Mitogen activated protein kinase signaling pathways participate in the active principle region of Buyang Huanwu decoction-induced differentiation of bone marrow mesenchymal stem cells. *Neural Regen Res* 2012;7(18):1370-7. DOI:10.3969/j.issn.1673-5374.2012.18.002.

27. Hafner A, Obermajer N, Kos J. Gamma-enolase C-terminal peptide promotes cell survival and neurite outgrowth by activation of the PI3K/Akt and MAPK/ERK signalling pathways. *Biochem J* 2012;443(2):439-50. DOI:10.1042/BJ20111351.

28. Pislar A, Bozic B, Zidar N, Kos J. Inhibition of cathepsin X reduces the strength of microglial-mediated neuroinflammation. *Neuropharmacology* 2017;114:88-100. DOI:10.1016/j.neuropharm.2016.11.019.

29. Obermajer N, Doljak B, Jamnik P, Fonovic UP, Kos J. Cathepsin X cleaves the C-terminal dipeptide of alpha- and gamma-enolase and impairs survival and neuritogenesis of neuronal cells. *Int J Biochem Cell Biol* 2009;41(8-9):1685-96. DOI:10.1016/j.biocel.2009.02.019.

30. Stefanovic B, Duric O, Stankovic S, *et al.* Elevated serum protein S100B and neuron specific enolase values as predictors of early neurological outcome after traumatic brain injury. *J Med Biochem* 2017;36(4):314-21. DOI:10.1515/jomb-2017-0018.

31. Hu Y, Meng R, Zhang X, *et al.* Serum neuron specific enolase may be a marker to predict the severity and outcome of cerebral venous thrombosis. *J Neurol* 2018;265(1):46-51. DOI:10.1007/s00415-017-8659-9.

32. Du W, Li H, Sun J, *et al.* The prognostic value of serum neuron specific enolase (NSE) and S100B level in patients of acute spinal cord injury. *Med Sci Monitor* 2018;24:4510-5. DOI:10.12659/MSM.907406

33. Shi LM, Chen RJ, Zhang H, Jiang CM, Gong J. Cerebrospinal fluid neuron specific enolase, interleukin-1beta and erythropoietin concentrations in children after seizures. *Childs Nerv Syst* 2017;33(5):805-11. DOI:10.1007/s00381-017-3359-4.

34. Haupt WF, Chopan G, Sobesky J, Liu WC, Dohmen C. Prognostic value of somatosensory evoked potentials, neuron-specific enolase, and S100 for short-term outcome in ischemic stroke. *J Neurophysiol* 2016;115(3):1273-8. DOI:10.1152/jn.01012.2015

35. Schaf DV, Tort ABL, Fricke D, *et al.* S100B and NSE serum levels in patients with Parkinson's disease. *Parkinsonism Relat D.* 2005;11(1):39-43. DOI:10.1016/j.parkreldis.2004.07.002.

36. Samancı Y, Samancı B, Şahin E, *et al.* Neuron-specific enolase levels as a marker for possible neuronal damage in idiopathic intracranial hypertension. *Acta Neurol Belg* 2017;117(3):707-11. DOI:10.1007/s13760-017-0762-2.

37. Beems T, Simons KS, Van Geel WJ, *et al.* Serum- and CSF-concentrations of brain specific proteins in hydrocephalus. *Acta Neurochir (Wien).* 2003;145(1):37-43. DOI:10.1007/s00701-002-1019-1

38. Jauch EC, Lindsell C, Broderick J, *et al.* Association of serial biochemical markers with acute ischemic stroke: the National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator Stroke Study. *Stroke* 2006;37(10):2508-13. DOI:10.1161/01.STR.0000242290.01174.9e

39. Tawk RG, Grewal SS, Heckman MG, *et al.* The relationship between serum neuron-specific enolase levels and severity of bleeding and functional outcomes in patients with nontraumatic subarachnoid hemorrhage. *Neurosurgery* 2016;78(4):487-91. DOI:10.1227/NEU.0000000000001140