European Review for Medical and Pharmacological Sciences 2016; 20: 1474-1478

Expression level of NSE, S100B and NPY in children with acute miliary phthisis and secondary tubercular meningitis

C.-X. ZHANG[1], D.-J. ZHANG[2], Y.-L. WANG[1], W. HAN[3], G.-C. SHI[2], H.-Q. ZHANG[2]

[1]The First Department of Tuberculosis, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan, China
[2]The Third Department of Tuberculosis,The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan, China
[3]The Second Department of Tuberculosis, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan, China

Abstract. – OBJECTIVE: To explore the expression level of neuron-specific enolase (NSE), S100B and neuropeptide Y (NPY) in children with acute miliary tuberculosis and secondary tubercular meningitis.

PATIENTS AND METHODS: 28 children diagnosed with acute miliary tuberculosis and secondary tubercular meningitis were included into group A, 25 children diagnosed with pure acute miliary tuberculosis were included into group B and 23 children diagnosed with suspected meningitis were included into group C. The levels of NSE, S100B and NPY in cerebrospinal fluid and serum were detected.

RESULTS: The levels of NSE, S100B and NPY in cerebrospinal fluid and serum of group A were significantly higher than the levels in the other two groups, differences were statistically significant ($p<0.05$). A multifactor retrospective analysis suggested that secondary tubercular meningitis was significantly correlated with the high expression of S100B, NPY and NSE in cerebrospinal fluid and serum.

CONCLUSIONS: Early detection of the expression levels of NSE, S100B and NPY in cerebrospinal fluid and serum was of great value for the diagnosis of tubercular meningitis secondary to acute miliary tuberculosis.

Key Words:
Acute miliary tuberculosis, Tubercular meningitis, NSE, S100B, NPY.

Introduction

About 20% of children with acute miliary tuberculosis develop secondary tubercular meningitis and the fatality rate reaches 50%-75%[1,2]. Large amounts of tuberculotoxin released in a short period of time can rapidly invade the blood stream and even penetrate the blood-brain barrier[3,4]. Neuron-specific enolase (NSE) is a special type of acid-soluble protein in neurons and neuroendocrine cells[5]. S100B is a marker protein of glial cells[6]. Neuropeptide Y (NPY) is a kind of nerve polypeptide that is widely distributed in the nervous system[7]. A previous study has confirmed that these three specific biochemical markers are of great value in the diagnosis and prognosis of neurological diseases[8]. This study analyzes the sensitivity and specificity of the above three proteins in the diagnosis of acute miliary tuberculosis and secondary tubercular meningitis and provides a new reference for clinical diagnosis and treatment.

Patients and Methods

Patients

Patients diagnosed in our hospital from January 2014 to October 2015 were enrolled in the study: 28 children diagnosed with acute miliary tuberculosis and secondary tubercular meningitis were included into group A, 25 children diagnosed with pure acute miliary tuberculosis were included into group B and 23 children diagnosed with suspected meningitis were included into group C. All cases were free of birth defects, autoimmune or genetic metabolic diseases. All children were examined by tuberculin test, sputum exam, bac-

Corresponding Author: Chunxiao Zhang, MD; e-mail: lafhjg@163.com

terial culture, X-ray and CT. Group A consisted of 16 males and 12 females; ranging in age from 3-8.3 years (5.3±1.2 years on average). The clinical course for children in group A ranged from 0.5 h to 2.5 days (mean: 1.0 ± 0.5 days). The main clinical manifestations were fever (n=15), cough (n=3), fever with cough (n=4), hemoptysis (n=3), and a positive meningeal irritation sign (n=20). Group B had 13 males and 12 females, aged from 2.7-8.6 years (5.6±1.4 years on average); the clinical course ranged from 1 h to 3 days (mean: 1.2 ± 0.3 days). The main clinical manifestations were fever (n=10), cough (n=7), fever with cough (n=5), and hemoptysis (n=3). Group C had 12 males and 11 females; aged from 3.2-8.5 years, (5.5±1.6 on average); the clinical course ranged from 6 h to 6 days (mean: 1.8 ± 0.7 days). The main clinical manifestation was a positive meningeal irritation sign (n=15). The differences on gender and age of the two groups were not statistically significant (p>0.05).

Methods

The approval of the Ethics Committee of the hospital and the informed consents of the patients' families were obtained. The levels of NSE, S100B and NPY in cerebrospinal fluid and serum were detected. 5 ml of cerebrospinal fluid were drawn by the conventional method. Each sample was centrifuged at 3000 rpm for 5 min at room temperature, the supernatant was collected and saved. 3 ml of peripheral venous blood were also drawn and centrifuged at 3000 rpm for 10 min at room temperature, the supernatants were collected and the samples preserved at -80°C. An enzyme-linked immunoassay kit (R&D Systems, Inc., Minneapolis, MN, USA) and a ELX800 microplate reader (Biotek, Winooski, VT, USA) were used to detect the content of NSE and S100B, by adhering to the ELISA instructions in the kit.

ELISA Detection

A microporous plate was coated with purified human NSE or S100B insoluble antibodies. The samples were added to the wells of the plate to allow for antigen binding. Then, horseradish peroxidase (HRP)-labeled goat anti-human antibody was added to obtain the antibody-antigen-enzyme-labeled-antibody complexes. Finally, the enzymatic substrate TMB was added, it turned yellow in the positive samples treated with acid. The color intensity was positively correlated with the amount of NSE or S100B in the samples. The optical densities (OD) were determined in a microplate reader at 450 nm and the concentrations of NSE and S100B were calculated using a standard curve.

Determination of NPY (Radioimmunoassay Method)

(1) Appropriate polystyrene tubes were prepared including a non-specific binding tube (NSB), a maximum binding rate tube (S_0), a standard tube S1-S5, and a sample determination tube U1-Un. (2) 300 ml of buffer solution were added into the NSB tube and 200 ml into the S-tube; also, 200 ml of standard solution were added into the S1-S5 tubes separately; next 200 ul of plasma were added to the sample determination tubes Ul-Un. (3) 100 ml of serum were added into all tubes except into the NSB tube; the tubes were left at 4°C for 48h on a shaker platform; then, 100 ml of labeled ^{125}I-NPY were added into all the tubes, and they were again left at 4°C for 24h on a shaker platform. Finally, 500 ml PR reagent were added into all the tubes, and after shaking briefly, they were left to settle at room temperature for 20 min before centrifuging at 3500 rpm for 25 min at 4°C. The supernatants were immediately discarded, and the intensity of radioactivity in each tube was determined using a scintillation counter.

Statistical Analysis

The SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. For continuous variables, data were expressed as mean ± standard deviation. The comparison between groups was made by the one-way ANOVA analysis using S-N-K method. Enumeration data were expressed by percentages (%). The differences between groups were compared using the X^2 test. The one-way ANOVA analysis was represented by the F value. p<0.05 was considered to be statistically significant.

Results

Comparison of the Expression Levels of NSE, S100B and NPY in Cerebrospinal Fluid and Serum

The levels of NSE, S100B and NPY in cerebrospinal fluid and serum in group A individuals were significantly higher than the levels in the other two groups, differences were statistically significant (p<0.05) (Table I).

Table I. Comparison of the expression levels of NSE, S100B and NPY in CSF and serum (ng/ml).

	NSE		S100B		NPY	
Group	CSF	Serum	CSF	Serum	CSF	Serum
A	40.3±5.5	9.3±1.4	3.1±0.4	0.8±0.2	351.1±58.3	258.2±48.7
B	6.5±1.8	4.4±1.7	0.8±0.1	0.4±0.1	73.8±10.2	68.7±19.5
C	7.2±1.6	4.6±1.3	1.1±0.3	0.5±0.2	76.9±15.6	72.3±18.7
F-value	6.728	6.234	6.957	6.365	6.798	6.103
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: group A: acute miliary tuberculosis and secondary tubercular meningitis; group B: pure acute miliary tuberculosis; group C: suspected meningitis.

Correlation of Secondary Tuberculous Meningitis and the Expression Level of NSE, S100B and NPY

A multifactor retrospective analysis suggested that secondary tubercular meningitis significantly correlates with the high expression of S100B, NPY and NSE in the cerebrospinal fluid and serum of affected individuals (Table II).

Discussion

NSE is a specific soluble protein that exists only in brain tissue and has a specific enolase activity. It is composed of sub-units α, β, and γ that have special immune characteristics and exists in the form of a dimer. The γ dimer is exclusive to glial cells; γ-enolase is located in neurons and neuroendocrine tissues and has high tissue specificity[9]. Under normal circumstances, its content in body fluids is quite low. When the brain tissues get damaged and the permeability of the blood-brain barrier increases, nerve cells disintegrate and get destroyed, and then NSE from their cytoplasm is released into the cerebrospinal fluid and enters the blood, leading to the increased amounts found in there[10]. A previous study showed that increased NSE in cerebrospinal fluid and serum directly reflects the degree of damage of brain tissues[8]. Furthermore, since NSE is a very specific marker of neurons and peripheral neuroendocrine cells, its levels in plasma can be reliably used as a diagnostic aid in cases of status epilepticus, Creutzfeldt-Jakob encephalopathy, acute cerebral apoplexy, brain injury and ischemic encephalopathy and reflect the state of injury of the affected cells[9].

The pathogenesis mechanism of tubercular meningitis is characterized by extensive parenchymal damage to the brain. The bacillus *Mycobacterium tuberculosis* can directly destroy the brain parenchymal cells, leads to neuron damage, inflammatory exudation and caseous necrosis; the blood-brain barrier is also destroyed, and the pia mater shows a diffused haze gray-yellow serous cellulose exudation dominated by monocytes and lymphocytes. Dissemination throughout the pia mater leads to scattered miliary tubercles; the arterioles in pial vessels and brain parenchyma present inflammatory changes, starting from the adventitia, destroying elastic fibers and causing vascular intimal inflammation, finally leading to vascular occlusion, cerebral infarction or hemorrhage[2,3]. The results from our study show the NSE

Table II. Correlation of secondary tuberculous meningitis with the expression level of NSE, S100B and NPY.

Variable/statistics	β	SE	Wald	p-value	OR	95% CI
CSF NSE	0.404	0.471	16.342	0.0001	4.713	3.667-5.897
Serum NSE	1.208	0.459	6.926	0.0085	2.347	1.361-3.229
CSF S100B	0.163	0.47	9.123	0.0133	3.200	1.274-4.038
Serum S100B	1.001	0.481	4.331	0.0374	1.721	1.065-2.985
CSF NSE	1.024	0.485	7.458	0.0347	1.984	1.036-2.204
Serum NSE	0.973	0.516	3.324	0.0376	1.024	0.064-2.039

SPSS statistical terms: β (Correlation Coefficient), SE (standard error), Walds (statistic value), OR (odd ratio) and 95% CI (95% confidence interval).

levels increased in both cerebrospinal fluid and blood reflecting all of those pathological changes. However, the NSE levels were significantly higher in cerebrospinal fluid; and the levels of NSE in cerebrospinal fluid and serum in children with acute miliary tuberculosis and secondary tubercular meningitis were significantly higher than the levels in children with pure acute miliary tuberculosis and suspected meningitis. A relevant study, comparing the levels of NSE in different encephalomeningitis, found that the amount of NSE in tubercular meningitis was the highest, indicating that NSE can be a sensitive index for diagnosis of tubercular meningitis[11]. Nevertheless, the reason for this difference and the nature of the relation between *Mycobacterium tuberculosis* and NSE needs to be more clearly explained.

S100B is an acidic calcium binding protein that mainly exists in Schwann cells. Since most S100B protein exist in brain tissues, it is also considered to be a specific marker of brain tissues. A recent study[12] suggested that the increase of S100B in cerebrospinal fluid and blood is closely related to the degree of brain damage and can signal brain death in organ donors. S100B protein is a brain damage marker with many special features: a biological half-life of only 2h, thermal stability, and serum concentration unaffected by serum content of heparin, protamine or the presence of hemolysis[13]. Therefore, S100B protein is probably a good marker of the acute phase of brain injury[14]. The results of our study show that the S100B level increases in both cerebrospinal fluid and blood (with a more prominent increase in cerebrospinal fluid) and the levels in children with acute miliary tuberculosis and secondary tubercular meningitis are significantly higher than those in children with pure acute miliary tuberculosis or suspected meningitis, indicating that the increase of S100B plays a special role to play in the pathogenesis of tubercular meningitis.

NPY is widely distributed in the central and peripheral nervous system of mammals. Within the sympathetic nervous system, it acts mainly by strongly stimulating blood vessel contraction[15]. In the central nervous system, NPY has been shown to exhibit a multitude of functions including decreasing stress and anxiety, decreasing the frequency of epileptic attacks, inhibiting reproduction, reducing blood pressure, heart rate and metabolism, and also promoting appetite (hence its potential as a target of anti-obesity drugs)[16]. Additionally, in the periphery, NPY can induce vascular contraction and vascular smooth muscle proliferation; result in elevated blood lipids, glucose tolerance and release of fat from cells[17]. In particular, NPY is closely related to the cardiovascular system, it is of great pathophysiological significance in the occurrence and development of essential hypertension, congestive heart failure, coronary heart disease and diabetes mellitus[15]. Of interest to our research, a study suggested that the increased content of NPY in brain damage might be related to the regulation of blood pressure, heart rate and other internal environments[17].

Conclusions

The results of our work showed that the levels of NPY were increased in both cerebrospinal fluid and blood (with a higher increase in cerebrospinal fluid), in the children enrolled in our study. Again, the levels in children with acute miliary tuberculosis and secondary tubercular meningitis were significantly higher than those in children with pure acute miliary tuberculosis and suspected meningitis, indicating that the increase of NPY may also play a role in the pathogenesis of tuberculous meningitis. To sum up, early detection of the expression levels of NSE, S100B and NPY in cerebrospinal fluid and serum can be highly valuable for the diagnosis of tubercular meningitis secondary to acute miliary tuberculosis.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Xia P, Jiao Y. Septic cavernous sinus thrombosis caused by tuberculosis infection. BMJ Case Rep 2014; pii: bcr2014206209.
2) Kimura H, Imaizumi K. Progress in management of severe tuberculosis or tuberculosis with severe complication. Kekkaku 2014; 89: 571-582.
3) Chatterjee D, Radotra BD, Vasishta RK, Sharma K. Vascular complications of tuberculous meningitis: an autopsy study. Neurol India 2015; 63: 926-932.
4) Salway RJ, Sangani S, Parekh S, Bhatt S. Tuberculoma-induced seizures. West J Emerg Med 2015; 16: 625-628.
5) Tawk RG, Grewal SS, Heckman MG, Rawal B, Miller DA, Edmonston D, Ferguson JL, Navarro R, Ng L, Brown BL, Meschia JF, Freeman WD. The relationship between serum neuron-specific enolase levels and severity of bleeding and functional outcomes in patients with

nontraumatic subarachnoid hemorrhage. Neurosurgery 2015; 24: 1-3.

6) Hajdukova L, Sobek O, Prchalova D, Bilkova Z, Koudelkova M, Lukaskova J, Matuchova I. Biomarkers of brain damage: S100B and NSE concentrations in cerebrospinal fluid--A normative study. Biomed Res Int 2015; 2015: 379071.

7) Bette M, Roehrenbeck A, Dietzschold B, Weihe E. Neuropeptide Y up-regulation in cerebrocortical neurons after Borna Disease Virus infection is unrelated to brain inflammation in rats. Neurosci Lett 2004; 366: 197-200.

8) Alatas OD, Gurger M, Atescelik M, Yildiz M, Demir CF, Kalayci M, Ilhan N, Acar E, Ekingen E. Neuron-Specific enolase, s100 Calcium-Binding protein b, and heat shock protein 70 levels in patients with intracranial hemorrhage. Medicine (Baltimore) 2015; 94: e2007.

9) Isgro MA, Bottoni P, Scatena R. Neuron-specific enolase as a biomarker: Biochemical and clinical aspects. Adv Exp Med Biol 2015; 867: 125-143.

10) Havnes MB, Kerlefsen Y, Møllerløkken A. S100B and NSE serum concentrations after simulated diving in rats. Physiol Rep 2015; 3: e12546.

11) Song TJ, Choi YC, Lee KY, Kim WJ. Serum and cerebrospinal fluid neuron-specific enolase for diagnosis of tuberculous meningitis. Yonsei Med J 2012; 53: 1068-1072.

12) Krzych LJ, Czempik PF, Saucha W, Kokocinska D, Knapik P. Serum S100B protein concentration in brain-dead organ donors: A pilot study. Anaesthesiol Intensive Ther 2015; 47: 320-323.

13) Chiang LM, Chen WY, Yang YC, Jeng MJ. Elevation of serum S100 protein concentration as a marker of ischemic brain damage in extremely preterm infants. J Chin Med Assoc 2015; 78: 610-616.

14) Peng Q, Yu N. Clinical application of S100B protein in children with central nervous system infections: a review. Zhonghua Er Ke Za Zhi 2015; 53: 393-396.

15) Reichmann F, Holzer P. Neuropeptide Y: a stressful review. Neuropeptides 2015; 30: S0143-4179

16) Paiva SP, Veloso CA, Campos FF, Carneiro MM, Tilan JU, Wang H, Umans JG, Zukowska Z, Kitlinska J. Elevated levels of neuropeptide Y in preeclampsia: A pilot study implicating a role for stress in pathogenesis of the disease. Neuropeptides 2015; 25: S0143-4179.

17) Morl K, Beck-Sickinger AG. Intracellular trafficking of neuropeptide y receptors. Prog Mol Biol Transl Sci 2015; 132: 73-96.