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Levels of soluble delta-like ligand 1 in the serum and cerebrospinal fluid of tuberculous meningitis patients[★]

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Abstract

In this study, the levels of soluble delta-like ligand 1 in cerebrospinal fluid and serum of 50 patients with tuberculous meningitis, 30 patients with viral meningitis, 20 patients with purulent meningitis and 40 subjects without central nervous system disease were determined using an enzyme-linked immunosorbent assay. The mean levels of soluble delta-like ligand 1 in both cerebrospinal fluid and serum from patients with tuberculous meningitis were significantly higher compared with those from patients with viral meningitis or purulent meningitis or from subjects without central nervous system disease. Meanwhile, the level of soluble delta-like ligand 1 gradually decreased as tuberculous meningitis patients recovered. If patients deteriorated after treatment, the level of soluble delta-like ligand 1 in cerebrospinal fluid gradually increased. There was no correlation between the level of soluble delta-like ligand 1 and the protein level/cell number in cerebrospinal fluid. Our findings indicate that the levels of soluble delta-like ligand 1 in cerebrospinal fluid and serum are reliable markers for the diagnosis of tuberculous meningitis and for monitoring treatment progress. At the same time, this index is not influenced by protein levels or cell numbers in cerebrospinal fluid.

Key Words: delta-like ligand 1; cerebrospinal fluid; enzyme linked immunosorbent assay; tuberculous meningitis

Abbreviations: DLL1, soluble delta-like ligand 1; TM, tuberculous meningitis; CSF, cerebrospinal fluid

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INTRODUCTION

With the rising morbidity of tuberculosis, early diagnosis and treatment are becoming more and more vital to the prognosis of tuberculous meningitis (TM). Therefore, fast and accurate diagnosis of TM is very important in clinical practice.

Currently, the presence of mycobacterium tuberculosis in cultures of cerebrospinal fluid (CSF) is the gold standard of TM diagnosis, but it is a slow procedure and exhibits poor sensitivity. It is difficult to distinguish between typical TM and other forms of pathogenic meningitis, especially with incomplete treatment of purulent meningitis. High levels of white blood cells and protein in CSF may affect diagnostic results; in particular, high levels of some proteins can lead to false positive results in enzyme linked immunosorbent assay analysis. A number of studies have demonstrated that fatty acid metabolism is closely related to mycobacterium tuberculosis survival, toxicity and the ability to escape immune system attack^[1]. Tuberculosis may result in a disorder of fatty acid metabolism, and delta-like ligand 1 (DLL1) serves as a

regulator in fat metabolism^[2].

In this study, we aimed to measure the levels of soluble DLL1 in both serum and CSF samples from patients with TM, viral meningitis, purulent meningitis or in subjects without central nervous system disease using an enzyme-linked immunosorbent assay, and to discuss the value of soluble DLL1 in TM diagnosis.

RESULTS

Quantitative analysis of involved subjects

A total of 100 patients with central nervous system infectious disease were initially included in this study. A total of 50 patients were assigned to the TM group^[3], 30 patients to the viral meningitis group and 20 patients to the purulent meningitis group. And 40 subjects without central nervous system disease, tumors, immunological disease or inflammation were enrolled in a control group. All 140 cases were involved in the final analysis.

Baseline information of involved subjects

The baseline data of all subjects from the four groups are shown in Table 1. There was

no significant difference among subjects in gender, age, and course of disease ($P > 0.05$).

Table 1 Baseline information of subjects

Item	Tuberculous meningitis group (n = 50)	Viral meningitis group (n = 30)
Gender (male/female, n)	27/23	19/11
Age (mean ± SD, year)	38±21	36±13
Disease duration (mean ± SD, day)	23±19	20±14
Increased number of white blood cells in CSF ($\geq 5 \times 10^6/L$) [n(%)]	41(82)	5(17)
Glucose decrease in CSF (< 2.22 mM) [n(%)]	31(62)	0(0)
Increase protein quantity in CSF (> 0.45 g/L) [n(%)]	37(74)	6(20)
Chloride decrease in CSF (< 120 mM) [n(%)]	21(42)	0(0)
Typical cytological abnormality [n(%)]	40(80)	11(37)

Item	Purulent meningitis group (n = 20)	Control group (n = 40)
Gender (male/female, n)	7/13	22/18
Age (mean ± SD, year)	42±14	42±17
Disease duration (mean ± SD, day)	19±15	20±14
Increased number of white blood cells in CSF ($\geq 5 \times 10^6/L$) [n(%)]	19(95)	1(2)
Glucose decrease in CSF (< 2.22 mM) [n(%)]	10(50)	0(0)
Increase protein quantity in CSF (> 0.45 g/L) [n(%)]	19(95)	1(2)
Chloride decrease in CSF (< 120 mM) [n(%)]	7(35)	0(0)
Typical cytological abnormality [n(%)]	19(95)	0(0)

Typical cytological abnormality refers to tuberculous meningitis showing a mixed cellular response, viral meningitis shows a lymphocytic response and purulent meningitis shows a response to neutrophils. CSF: Cerebrospinal fluid.

Level of soluble DLL1 in CSF

Enzyme-linked immunosorbent assays showed that the CSF level of soluble DLL1 was above 1.0 ng/mL in 43 cases of the TM group (86%), whereas the level in subjects of the viral meningitis, purulent meningitis and control groups was always below 1.0 ng/mL. Statistical analysis showed that there were highly significant differences in the level of soluble DLL1 between the TM group and viral meningitis, purulent meningitis and control groups ($P < 0.01$), while there were no significant differences among viral meningitis, purulent meningitis and control groups ($P > 0.05$; Table 2).

Soluble DLL1 content in serum

Enzyme-linked immunosorbent assays showed that serum levels of soluble DLL1 were higher than 6.0 ng/mL in 42 cases (84%) of the TM group, whereas only 2, 1 and 1 cases from viral meningitis, purulent meningitis and control groups, respectively, were higher than

6.0 ng/mL. Statistical analysis showed that there were highly significant differences in the serum levels of soluble DLL1 between the TM group and viral meningitis, purulent meningitis and control groups ($P < 0.01$), while there were no significant differences among viral meningitis, purulent meningitis and control groups ($P > 0.05$; Table 2).

Table 2 Content of soluble delta-like ligand 1 (ng/mL) in cerebrospinal fluid and serum of subjects

Group	n	Content in cerebrospinal fluid	Content in serum
Tuberculous meningitis	50	2.97±1.86	10.32±4.86
Viral meningitis	30	0.23±0.22 ^a	0.79±1.59 ^a
Purulent meningitis	20	0.27±0.25 ^a	0.69±1.30 ^a
Control	40	0.17±0.15 ^a	0.88±2.34 ^a

Data are expressed as mean ± SD. ^a $P < 0.01$, vs. tuberculous meningitis group (*F*-test followed by least significant difference *t*-test).

Soluble DLL1 levels in the CSF of TM patients before and after treatment

In the TM group, 18 cases received lumbar puncture treatment for 1–2 weeks, and their soluble DLL1 CSF levels were monitored. The CSF levels of soluble DLL1 gradually decreased in 14 TM patients who showed improved symptoms, while in the other four TM patients, who presented aggravated symptoms, CSF levels of soluble DLL1 gradually increased. The difference between the two groups was significant ($P < 0.05$ or $P < 0.01$; Table 3).

Table 3 Level of soluble delta-like ligand 1 in cerebrospinal fluid (ng/mL) of tuberculous meningitis patients after treatment

Treatment time (week)	Better post-treatment (n = 14)	Worse post-treatment (n = 4)
1	2.88±0.60	3.07±0.32 ^c
2	2.89±0.18	3.44±0.11 ^c
4	2.01±0.31 ^b	3.38±0.16 ^{ad}
6	0.89±0.10 ^b	6.56±1.68 ^{ad}

Data are expressed as mean ± SD. ^a $P < 0.05$, ^b $P < 0.01$, vs. pre-treated time point in the same group (*t*-test); ^c $P < 0.05$, ^d $P < 0.01$, vs. better post-treatment group (*t*-test).

Correlation analysis between soluble DLL1 CSF levels and protein levels/cell number in CSF

Correlation analysis showed that there was no correlation between the level of CSF soluble DLL1 and protein levels/cell numbers in all involved subjects or in TM patients ($P > 0.05$; Figures 1, 2).

DISCUSSION

Human DLL1 is homologous to the Drosophila Notch ligand, Delta, and is an L-type trans-membrane protein, composed of 723 amino acids. DLL1 has a Notch ligand

region named Delta/Serrate/LAG-2, which is followed by eight tandem epidermal growth factor-like repeating sequences and one short cytoplasmic domain. DLL1 can be amplified through the polymerase chain reaction using a pair of primers whose sequences are from the *Drosophila* Delta gene^[4].

The combination of DLL1 and Notch inhibits the differentiation of muscle, bone marrow or lymphatic progenitor cells^[5-7]. Perturbation of the Notch signaling pathway by gene mutations or abnormal activation of DLL1 may be associated with many diseases, such as stroke, tumor, brain reperfusion injury, Alzheimer's disease and Parkinson's disease, especially the development of brain tumors^[8]. Notch signaling affects the generation of fat cells by regulating transcription factors that can activate fatty acids, thus it plays an important role in fat metabolism^[9]. Genome analysis shows that the mycobacterium tuberculosis genome contains more than 4 000 genes, including 250 genes encoding fatty acid metabolism-related enzymes^[1, 10]. Peptidoglycan, the basic structural component of the mycobacterium tuberculosis cell wall, combines covalently-bound arabinogalactan lactose and branch bacteria acid. Branch bacteria acid is the biggest fatty acid in nature, and is highly involved in virulence and the ability of mycobacterium tuberculosis to escape immune system attack^[1, 10]. All the above evidence indicates the importance of fat metabolism for mycobacterium tuberculosis^[1, 10]. Based on the above theories, we can speculate that tuberculosis, a chronic consumptive disease, can cause a fat metabolism disorder and excessive differentiation of fat cells. The accelerated decrease of DLL1 in TM patients, leads to the down-regulation of Notch signal in neighboring cells and promotes the differentiation of adipose cells.

This study shows that levels of soluble DLL1 in CSF and serum in the TM group were significantly higher than those in viral meningitis, purulent meningitis and control groups. Also, there was no correlation between CSF soluble DLL1 levels and CSF protein levels/cell numbers, which indicated greater diagnostic value in atypical cases.

Biochemical and cytology tests and examinations of CSF are routine for the diagnosis of TM. Analysis of clinical data from 50 TM patients showed the following percentage of cases were abnormal for: white blood cell count (high) 82%, protein levels (high) 74%, glucose levels (low) 62%, chloride (low) 21%, and cytology 80%. Furthermore, 86% of the TM patients had abnormally high CSF levels of soluble DLL1 and in these patients changes in the CSF level of soluble DLL1 did not correlate with changes in CSF levels of protein or cell numbers. All the above evidence suggested that detection of DLL1 levels had a higher sensitivity than other tests and is, therefore, valuable for clinical diagnosis.

The CSF and serum levels of DLL1 in the TM group were increased; the percentage of cases with elevated CSF was 86% (43/50) and that with elevated serum was 84% (42/50). Although the total number of cases was small, the specificity was high; therefore, the study has clinical value. The CSF level of DLL1 in viral meningitis, purulent meningitis and control groups was always below 1.0 ng/mL, whereas there were two cases of viral

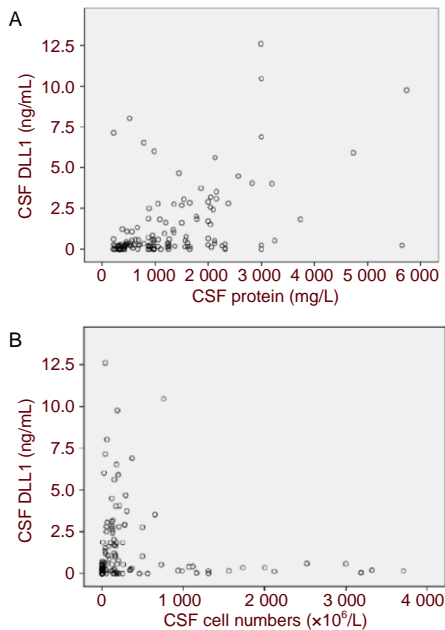


Figure 1 Correlation analysis between soluble delta-like ligand 1 (DLL1) levels and the levels of protein (A), or cell numbers (B) in cerebrospinal fluid (CSF) of all subjects.

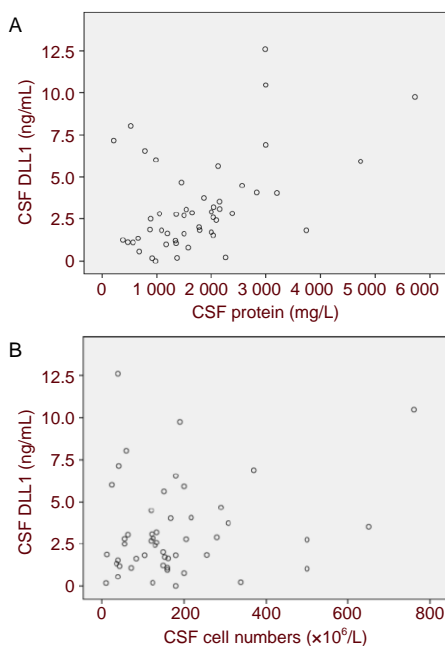


Figure 2 Correlation analysis between soluble delta-like ligand 1 (DLL1) levels and the levels of protein (A), or cell numbers (B) in cerebrospinal fluid (CSF) from tuberculous meningitis patients.

meningitis, one case of purulent meningitis and one healthy control subject with serum levels above 6.0 ng/mL. This index could, therefore, be used to differentiate among TM, viral meningitis and purulent meningitis cases and healthy subjects.

The observation of 18 TM patients over time showed that, 14 patients had reduced levels of soluble DLL1 that correlated with the patients' recovery. These dynamic changes could, therefore, indicate the effects of treatment. In four cases that deteriorated after treatment, the CSF level of soluble DLL1 increased, and the degree of increase reflected the severity of the illness. These results show that testing CSF DLL1 could help to understand TM pathology, determine the effect of treatment over time, and provide reference for clinical treatment.

The exact mechanism underlying the increase in soluble DLL1 in TM patients still needs to be elicited. Cellular immunity, mediated by T lymphocytes, may play a major role in all immune responses in TM^[11]. T lymphocytes include Th1, Th2, Tr, memory T and effective T cells. The balance of the response between Th2 and Th1 plays a vital role in progress, deterioration, control and prevention of tuberculosis^[12]. The Notch pathway is vitally important in the differentiation of T cells from hematopoietic stem cells^[13-14]. It can promote the generation of T $\alpha\beta$, and can collaborate with the Gata3 gene to regulate the differentiation of CD4⁺ cells into Th1/Th2 cells. When mycobacterium tuberculosis invades the central nervous system, T lymphocytes can proliferate significantly and can differentiate because of the local immune reaction. The upregulation of T lymphocytes needs the activation of the Notch signaling pathway. Recently, Kapoor *et al*^[15] demonstrated the activation of Notch signaling in tuberculous granuloma in TM patients. Activation of Notch signaling can make lymphoid progenitor cells differentiate into T cell lineages rather than B cell lineages. The reduction of DLL1 levels in cell subsets, mediated by a distintegrin and metalloprotease, appeared to contribute to the down-regulation of Notch signaling in neighboring cells, and to promote the differentiation of lymphatic progenitor cells. DLL1 is located in the adherens junctions of neural cell processes, which is mediated by the scaffolding protein membrane associated guanylate kinase, WW and PDZ domain containing 1. In TM patients, adherens junctions are destroyed, which leads to the release of DLL1 and to the increase of DLL1 levels^[16]. The clean-up of pathogens in viral meningitis and purulent meningitis patients mainly depends on neutrophils, monocytes and phagocytes and the humoral immune response, which mainly depends on specific antibodies, so the level of soluble DLL1 in viral meningitis and purulent meningitis patients is much lower than that in TM patients. This study demonstrates that the levels of CSF and serum soluble DLL1 were significantly increased in TM patients, but were very low or even undetectable in viral meningitis, purulent meningitis and normal cases. Joint

tests of soluble DLL1 in CSF and serum provide a new approach and reference for early confirmed diagnosis of TM. The potential clinical value is considerable.

SUBJECTS AND METHODS

Design

A clinically comparative observational study.

Time and setting

Experiments were performed from 2008 to 2010 at the Department of Neurology, First Affiliated Hospital of Zhengzhou University, China.

Subjects

A total of 100 patients with central nervous system infectious disease were consecutively enrolled from December 2008 to November 2010 at the Department of Neurology of the First Affiliated Hospital of Zhengzhou University, China.

TM group

Fifty patients, aged 14–68 years, were enrolled according to previously described diagnostic criteria^[3]. Confirmed diagnosis was only made after assessing the following criteria: clinical symptoms, X-ray chest radiograph/CT, tuberculosis polymerase chain reaction and CSF analysis, and after anti-tuberculosis pharmacological treatment. In the TM group, four cases were acid-fast stain positive, 38 cases had abnormally high levels of adenosine deaminase, 16 cases were positive for TB antibody, and five cases were positive for Mycobacterium tuberculosis.

Viral meningitis group

The group consisted of thirty cases, aged 16–60 years. Six cases were positive for herpes simplex virus, five cases were positive for cytomegalovirus, and two cases were positive for EB virus.

Diagnosis criteria: The diagnosis of all enrolled viral meningitis patients conformed to the previously described^[17] criteria. Confirmed diagnosis was only made after assessing the following criteria: clinical symptoms, magnetic resonance imaging and detection of viral antibodies, and after anti-virus pharmacological treatment.

Purulent meningitis group

The group consisted of twenty cases, aged 18–75 years. Diagnosis criteria: The diagnosis of all enrolled purulent meningitis patients conformed to previously described criteria^[18]. Confirmed diagnosis was only made after assessing the following criteria: clinical symptoms, magnetic resonance imaging and CSF inspection, and after antibiotic pharmacological treatment.

Control group

The group consisted of 40 subjects, aged 17–64 years. All subjects were people without central nervous system diseases, tumors, immunological disease or inflammation.

After acquiring the subject's or their families' written informed consent, lumbar puncture was performed in accordance with the *Declaration of Helsinki*.

Methods

CSF and specimen harvest

Serum and CSF samples were collected at enrollment. 2 mL CSF was collected through lumbar puncture (between the L_{4,5} intervertebral space). Serum was extracted from 2 mL of peripheral blood taken from the ulnar vein. All specimens were stored at -80°C until use. To reduce error, CSF and serum were tested simultaneously. For dynamic observation of TM patients, 2 mL of CSF was collected before and after treatment. All samples were stored at -80°C.

Enzyme-linked immunosorbent assay detection of soluble DLL1 levels in CSF and serum

The level of soluble DLL1 in CSF and serum was quantitatively determined in strict accordance with the instructions of the enzyme-linked immunosorbent assay kit (cat#: AG-45A-0027EK-KI0; AdipoGen, Korea). Quality control: samples were detected with recombinant proteins in each experiment. Standard samples were marked with known concentrations (#0 to #7). Blank control and specimens were all tested twice. Mean values were calculated and considered in the analysis to reduce the error.

Main outcome measures

Routine CSF examination, biochemistry, cytology, and bacterial cultivation from TM patients were performed and the level of adenosine deaminase and the presence of tuberculosis antibody were determined.

TM treatment

TM patients were treated as previously described^[3].

Statistical analysis

SPSS 17.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Data are presented as mean ± SD. Comparison among multiple groups was resolved by variance analysis (*F*-test) or Kruskal-Wallis inspection. Binary comparison between groups was performed through the least significant difference *t*-test or the Bonferroni method. Intragroup differences were evaluated using the matching *t* test. Pearson linear correlation analysis showed that the soluble DLL1 level in CSF was closely related with the CSF protein-cell number. A *P* value of less than 0.05 was considered statistically significant.

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Conflicts of interest: None declared.

Ethical approval: This study received permission from the

Ethics Committee of the First Affiliated Hospital of Zhengzhou University, China.

REFERENCES

- [1] Carballeira NM. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. *Proq Lipid Res.* 2008; 47(1):50-61.
- [2] Murata A, Okuyama K, Sakano S, et al. A Notch ligand, Delta-like 1 functions as an adhesion molecule for mast cells. *J Immunol.* 2010;185(7):3905-3912.
- [3] Chinese Medical Association. Guidelines on the Clinical Diagnosis and Treatment (the tuberculosis branch). Beijing: People's Medical Publishing House. 2005:1-55.
- [4] Murata A, Okuyama K, Sakano S, et al. A Notch ligand, delta-like 1 functions as an adhesion molecule for mast cells. *J Immunol.* 2010;185(7):3905-3912.
- [5] Han W, Ye Q, Moore MA. A soluble form of human delta-like-1 inhibits differentiation of hematopoietic progenitor cells. *Blood.* 2000;95(5):1616-1625.
- [6] Hozumi K, Negishi N, Suzuki D, et al. Delta-like 1 is necessary for the generation of marginal zone B cells but not T cells in vivo. *Nature Immunol.* 2004;5(6):638-644.
- [7] Conboy IM, Conboy MJ, Smythe GM, et al. Notch-mediated restoration of regenerative potential to aged muscle. *Science.* 2003;302(5650):1575-1577.
- [8] Nickoloff BJ, Osborne BA, Miele L. Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. *Oncogene.* 2003;22(42):6598-6608.
- [9] Liu RZ, Mita R, Beaulieu M, et al. Fatty acid binding proteins in brain development and disease. *Int J Dev Biol.* 2010;54(8-9): 1229-1239.
- [10] Purohit HJ, Cheema S, Lal S, et al. In search of drug targets for mycobacterium tuberculosis. *Infect Disord Drug Targets.* 2007; 7(3):245-250.
- [11] Tan YJ, Zhang YB, Feng DY, et al. The change and the clinical significance of peripheral blood Th1/Th2 cells in patients with pulmonary tuberculosis. *Zhonghua Jiehe he Huxi Zazhi.* 2004; 27(6):385-389.
- [12] Hartmann P, Plum G. Immunological defense mechanisms in tuberculosis and MAC-infection. *Diagn Microbiol Infect Dis.* 1999;34(2):147-152.
- [13] Wolfer A, Bakker T, Wilson A, et al. Inactivation of Notch 1 in immature thymocytes does not perturb CD4 or CD8T cell development. *Nat Immunol.* 2001;2(3):235-241.
- [14] Wilson A, Ferrero I, MacDonald HR, et al. Cutting edge: an essential role for Notch-1 in the development of both thymus-independent and -dependent T cells in the gut. *J Immunol.* 2000; 165(10):5397-5400.
- [15] Kapoor N, Narayana Y, Patil SA, et al. Nitric oxide is involved in Mycobacterium bovis bacillus Calmette-Guérin-activated Jagged1 and Notch1 signaling. *J Immunol.* 2010;184(6):3117-3126.
- [16] Mizuhara E, Nakatani T, Minaki Y, et al. MAGI1 recruits Dll1 to cadherin-based adherens junctions and stabilizes it on the cell surface. *J Biol Chem.* 2005;280(28):26499-26507.
- [17] Wang WZ. *Neurology.* 5th ed. Beijing: People's Medical Publishing House. 2005:167-168.
- [18] Tan Y, Wang Z, Wang YX, et al. Tuberculous meningitis cerebrospinal fluid in the diagnosis of ammonia enzyme adenosine take off the significance and the dynamic changes observation. *Shiyong Yiji Zazhi.* 2009;25(3):408-409.

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