Inflammation, Vol. 31, No. 4, August 2008 (© 2008)
DOI: 10.1007/s10753-008-9071-5

Dynamic Changes of Matrix Metalloproteinase-9 in Patients with *Klebsiella pneumoniae* Meningitis

Hung-Chin Tsai,[1,2,3] Sheng-Fu Liu,[4] Kuan-Sheng Wu,[1,2] Yung-Ching Liu,[1,2] Min-Hon Shi,[1,2] Eng-Rin Chen,[3] Chuan-Min Yen,[3] Susan Shin-Jung Lee,[1,2] Yen-Lin Huang,[1,2] and Yao-Shen Chen[1,2,5,6]

Abstract—To quantitate cerebrospinal fluid (CSF) concentrations of matrix metalloproteinase 9 (MMP-9) in adult patients with *Klebsiella pneumoniae* meningitis and to correlate levels of MMP-9 with parameters of intrathecal inflammation and analyze the kinetic changes of MMP-9. In a prospective cohort study, levels of MMP-9 and tissue inhibitor of matrix metalloproteinase (TIMP-1) concentrations were measured in the CSF of six adult patients with meningitis and 11 controls. MMP-9 and TIMP-1 were detected in all of the six patients at presentation and follow up lumbar puncture. CSF levels of MMP-9 (6.71±7.29 ng/ml) and TIMP-1(454.3±242.9 ng/ml) were higher in patients than in the control group (0.07±0.11 ng/ml and 27.14±39.34 ng/ml, respectively). Levels of MMP-9 correlated with CSF concentrations of protein, cell count and lactate. Repeated lumbar punctures showed that levels of MMP-9 decrease during clinical recovery, although the levels of MMP-9 in the CSF are variable because of the small number of cases. The relative change in gelatin zymography is comparable to the changes of MMP-9 levels found in ELISA. MMP-9 levels in CSF may be a useful tool in follow-up in patients with *K. pneumoniae* meningitis.

KEY WORDS: gelatin zymography; *Klebsiella pneumoniae*; matrix metalloproteinase 9; meningitis.

INTRODUCTION

Bacterial meningitis (BM) is still an important clinical problem. Despite the use of potent antibiotics with excellent *in vitro* activity, such as third-generation cephalosporins, the case fatality rate remains high [1–3]. Survivors may suffer from long-term severe neurological sequelae [4, 5]. In BM, acute breakdown of the blood–brain barrier (BBB), and accumulation of blood-derived leukocytes in the cerebrospinal fluid(CSF) lead to brain edema, cerebral vasculitis, and ultimately neuronal injury [6, 7]. In Taiwan, *Klebsiellae pneumoniae* is one of the most common causative pathogens of community-acquired bacterial meningitis, which is different from other parts of the world [3, 8]. One of the prototypical destructive events in the human brain, initiated by the release of inflammatory cytokines and ending with tissue destruction, is production of matrix metalloproteinases (MMPs) [9]. The MMPs are a family of endopeptidases produced by a variety of inflammatory cells [10, 11]. All of the cell types that exist in the central nervous system (CNS) are potential sources of MMPs. *In vitro*, neurons, astrocytes, microglia [12, 13] and oligodendrocytes [14] express various MMP family members. With respect to MMP-9, it was recently shown that CSF levels of this

[1] Section of Infectious Diseases, Department of Medicine, Kaohsiung Veterans General Hospital, 386, Ta-Chung 1st Road, Kaohsiung, 813, Taiwan
[2] National Yang-Ming University, Taipei, Taiwan, Republic of China
[3] Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[4] College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[5] Institute of Environmental Education, National Kaohsiung Normal University, Kaohsiung, Taiwan
[6] To whom correspondence should be addressed at Section of Infectious Diseases, Department of Medicine, Kaohsiung Veterans General Hospital, 386, Ta-Chung 1st Road, Kaohsiung, 813, Taiwan. E-mail: hctsai1011@yahoo.com.tw

enzyme increase during bacterial meningitis and that it is associated with brain damage [9, 15, 16]. However, to the best of our knowledge, no reports have investigated *K. pneumoniae* meningitis and how it is mediated by kinetic regulation after standard corticosteroid therapy for bacterial meningitis.

In a prospective cohort study, we quantitated CSF concentrations of MMP-9 by enzyme-linked immunosorbent assay (ELISA) and gelatin zymography in adult patients with *K. pneumoniae* meningitis. We correlated levels of MMP-9 with parameters of intrathecal inflammation and analyzed the kinetic changes of MMP-9.

MATERIALS AND METHODS

Patients

All of the CSF samples from adult patients with suspected BM were evaluated in a prospective cohort study. CSF samples from patients served as controls in cases where lumbar punctures were performed as routine diagnostic procedure for suspected CNS infection and all of the CSF parameters were normal. The patients with confirmed BM who were enrolled in this study were admitted 1–5 days after onset of clinical symptoms. The diagnosis of BM was based on detection of the pathogen in the CSF by gram staining, bacterial culture, or antigen testing, and the presence of CSF pleocytosis [8]. Repeated lumbar punctures were performed when clinically indicated at the physicians' discretion. All patients with BM were treated with third generation cephalosporin and dexamethasone at a dose of 10mg intravenously every six hours for two days. This study protocol was reviewed and approved by the Committees on Medical Ethics of the Kaohsiung Veterans General Hospital.

CSF Samples

CSF samples from patients with BM were obtained by lumbar puncture at the time of admission (first lumbar punctures) but before initiation of antibiotic and steroid therapy. Eight CSF specimens were collected from lumbar punctures performed seven days (range 3–14 days) after the initiation of antibiotic and steroid therapy. Total cell count, differential leukocyte count, and total protein concentration were determined by standard methods. For measurement of MMP-9, CSF samples were centrifuged at 1,500 *g* for 15 min and supernatant were then frozen at −80°C until assayed.

Enzyme-linked Immunosorbent Assay (ELISA) for MMP-9 and TIMP-1

The concentrations of MMP-9 and TIMP-1 were measured by ELISA Kits (R & D System, Inc. USA) and an assay was performed according to the manufacturer's instructions. In brief, the wells of the ELISA plate were first coated with antibodies which captured MMP-9 and TIMP-1 in the CSF specimens after they were added to the wells. Then, biotinylated detection antibodies and streptavidin labeled horseradish peroxidase (HRP) were added step by step to allow the marker enzyme HRP to bind to the solid phase. TMB-Substrate solution was used to visualize and optical density (O.D.) values were read after 2 N sulfuric acid was added to stop the reaction.

Gelatin Zymography

MMP-9 activity was analyzed on a modified SDS-PAGE. The stacking gels contained 4% polyacryamide and the separating gels contained 12.5% polyacrylamide and 0.1% gelatin. The CSF was centrifuged at 10,000 g for 15 min at 4°C in order to remove debris. The protein contents of the supernatants were then mixed with an equal volume of 2× non-reducing sample buffer and 25 μl was loaded per well. The gel was electrophoresed at 90 V at 4°C in running buffer (25 mM Tris, 250 mM glycine and 0.1% SDS) until the bromophenol blue marker dye reached the bottom of the gel. After electrophoresis the gel was washed two times with gentle agitation for 30 min each in 2.5% Triton X-100 at room temperature. After decanting the washing solution, the gel was equilibrated with developing buffer (50 mM Tris–HCl, pH 7.5, containing 200 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35 and 0.01% NaN_3) for 30 min at room temperature with gentle agitation then replaced with fresh developing buffer and incubated at 37°C for 18 h. The gel was stained with 0.25% Coomassie Brilliant Blue R-250 (Sigma) for 1 h and was destained in 15% methanol/7.5% acetic acid. Gelatinase activity was detected as unstained bands on a blue background.

String Test and Polymerase Chain Reaction (PCR) for *K. pneumoniae* Isolates

A modified string test was used to test the hypermucoviscosity phenotype. When the colonies in the 5% sheep blood agar plate had viscous strings of more than 10 mm in length, the test was defined as positive [17]. 16S

rRNA, *magA,* and *rmpA* genes were amplified using the following primers: 5′-GCGGTAATACGGAGGGTGC and 5′-CACATCCGACTTGACAGACC for 16S rRNA gene, 5′-GGTGCTCTTTACATCATTGC and 5′-GCAAT GGCCATTTGCGTTAG for *magA,* 5′-ACTGGGCTAC CTCTGCTTCA and 5′-CTTGCATGAGCCATCTTTCA for *rmpA* [18–20]. Amplification was performed using an initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s, extension at 72°C for 60 s, and final extension at 72°C for 5 min.

Statistical Analysis

Results in the serum/CSF concentrations of MMP-9 and TIMP-1 between patient and control groups were compared by using the Mann–Whitney U test. The correlation between MMP-9 concentrations in CSF and CSF parameters were quantified by using Spearman's correlation test. A p value of <0.05 was considered statistically significant.

RESULTS

Patients

During January 2007 to July 2007, six sequential patients with BM caused by *K. pneumoniae* were included in this cohort study. Eleven patients were enrolled and served as a control. All of them had normal CSF parameters. All of the six patients were men. The mean age was 64±12 (range 45–76). All of the six patients had fever and four were unconscious at presentation. Three had seizures and two presented with septic shock initially. Two of the patients were diagnosed as primary meningitis and the other four patients as secondary meningitis caused by *K. pneumoniae* liver abscesses with metastatic infections. *K. pneumoniae* bacteremia was found in five patients. CSF and liver abscess cultures were positive in four and three patients, respectively. Eighty three percent of the patients (5/6) had diabetes mellitus. Six patients underwent a total of 14 lumbar punctures. Five of the patients had a follow-up lumbar puncture at a median of 5 days after the first lumbar puncture (range 3–14 days). Three of the patients had a third lumbar puncture 3, 16, and 17 days after the second lumbar puncture, respectively. At presentation, the CSF protein levels were 5.03±3.54 g/l (range 2.07 to 11.55 g/l); the number of leukocytes was $614\pm903\times10^9$/l (range 42 to $2{,}430\times10^9$/l). The granulocyte percentage in the CSF was 85±7 (range 79–95). The lactate level in the CSF was 114±39 mg/dl (range 70–154 mg/dl). Five of the six patients survived the disease, but one had permanent neurological sequelae (Table 1).

MMP-9 and TIMP-1 Values were Elevated in the CSF of Patients with BM

MMP-9 and TIMP-1 were detected in all six (100%) of the CSF specimens from patients with BM at presentation and follow-up lumbar puncture. The MMP-9 levels in the CSF of the six patients was 6.71±7.29 ng/ml which is several fold higher than the 11 control patients (0.07± 0.11 ng/ml; p=0.001, Mann–Whitney U test). The TIMP-

Table 1. Clinical Manifestations of Six Patients with *Klebsiella pneumoniae* Meningitis

Parameters/patients	A	B	C	D	E	F
Age/sex	73, M	56, M	74, M	76, M	45, M	64, M
Diagnosis	Primary meningitis	Secondary meningitis	Primary meningitis	Secondary meningitis	Secondary meningitis	Secondary meningitis
APACHE II score	28	Not available	24	16	Not available	33
Fever	Yes	Yes	Yes	Yes	Yes	Yes
Unconsciousness	Yes	No	Yes	Yes	Yes	No
Seizure	Yes	No	Yes	Yes	No	No
Septic shock	Yes	Yes	No	No	No	No
Bacteremia	No	Yes	Yes	Yes	Yes	Yes
CSF culture	Positive	Negative	Positive	Positive	Positive	Negative
CSF white cell counts (10^9/l)	180	183	500	42	2,430	350
CSF MMP-9 (pg/ml)	17,971	432	1,121	10,311	10,253	171
Outcome	Death	Recovery	Recovery	Neurological sequelae	Recovery	Recovery

APACHE Acute physiology and chronic health evaluation, *CSF* cerebrospinal fluid, *MMP-9* matrix metalloproteinase 9

1 levels in the CSF of the six patients was 454.3±242.9 ng/ml which is also higher than the 11 control patients (27.14±39.34 ng/ml) (p=0.03, Mann–Whitney U test). The MMP-9 and TIMP-1 levels in serum were not different between the six patients (1.01±0.87 ng/ml and 360±358 ng/ml) and 11 controls (8.79±8.17 ng/ml and 104.8±64.1 ng/ml; Fig. 1).

Correlation of MMP-9 and the CSF Parameters

Only the CSF samples at first (n=6) and second lumbar puncture (n=5) were used for correlation with CSF parameters. There was an association between MMP-9 levels and protein, cell counts and lactate values in the CSF (Table 2).

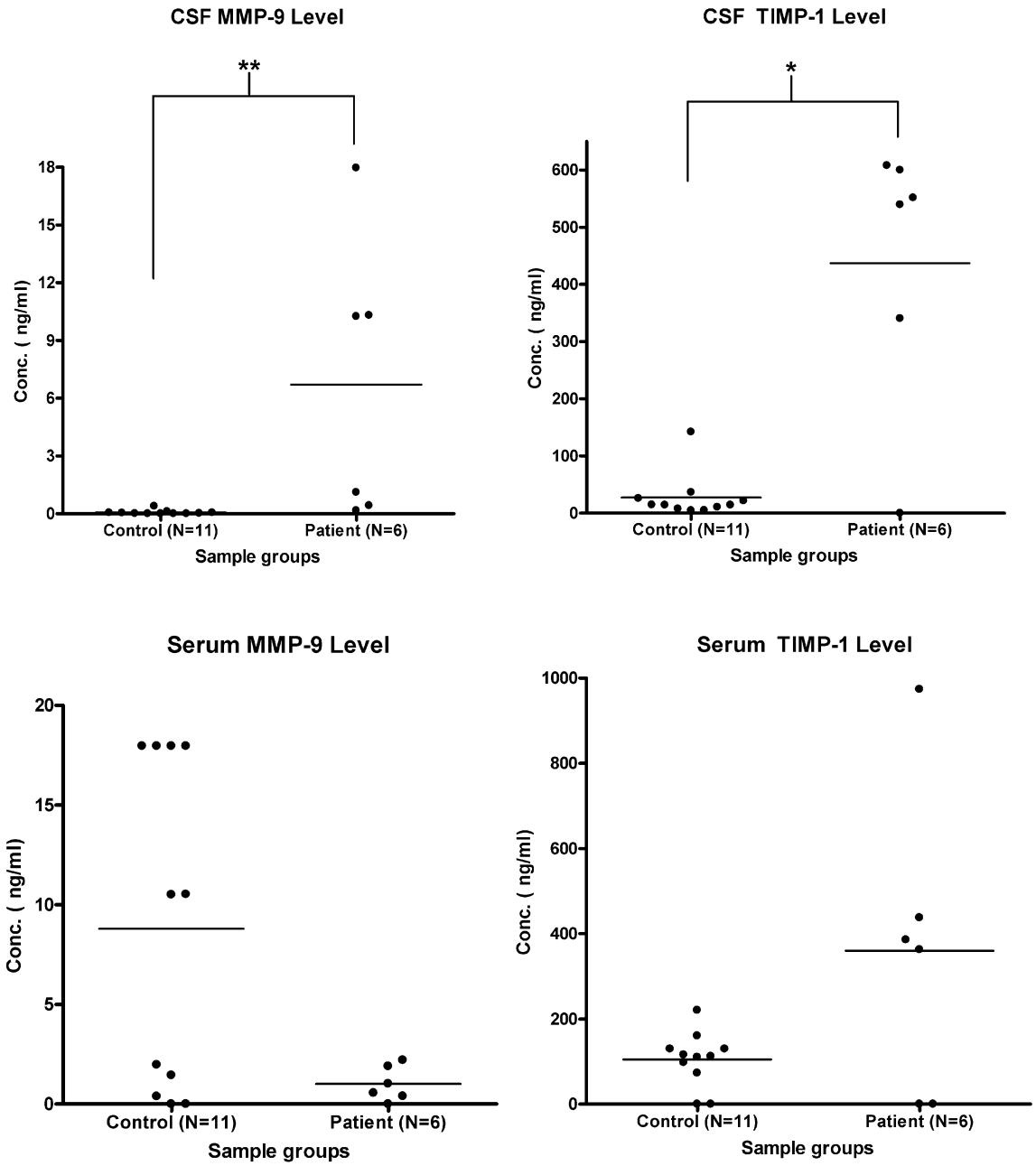

Fig. 1. The MMP-9 and TIMP-1 levels in CSF in six patients were several fold higher than the 11 control patients. ($^{*}p<0.05$, $^{**}p<0.005$, Mann–Whitney U test).

Table 2. Spearman's Correlation Test Showed an Association between $MMP\text{-}9_{CSF,}$ CSF Parameters in Patients with *Klebsiella pneumoniae* Meningitis

	$MMP\text{-}9_{CSF1}$		$MMP\text{-}9_{CSF2}$	
Variable	*r*	*p*	*r*	*p*
CSF protein	0.886	0.019*	0.829	0.042*
CSF wbc	−0.429	0.397	0.829	0.042*
CSF neut	0.975	0.005*	0.400	0.600
CSF lac	0.900	0.037*	0.900	0.037*

$MMP\text{-}9_{CSF1}$, and $MMP\text{-}9_{CSF2}$, represent the CSF MMP-9 levels at presentation, and follow-up. CSF protein, CSF wbc, CSF neut and CSF lac, are the protein concentration, white blood cell count, neutrophil count and lactate in the CSF

*$p<0.05$

Time Course of MM-9 Expression in CSF by ELISA and Zymography in Patients with Repeated Lumbar Punctures

The heterogeneity of the study population regarding acuity of disease at hospital admission rendered the collective comparison of kinetic regulation of MMP-9 and other parameters inconclusive. We therefore compared concentrations of CSF samples from individual patients who had serial lumbar punctures. MMP-9 underwent rapid kinetic changes (Fig. 2). A 73 year old man (patient A) who died had the highest MMP-9 levels in CSF at presentation (17,971 pg/ml) and 14 days later (17,970 pg/ml). The levels of MMP-9 in the CSF are variable because of the small number of cases. The relative changes in gelatin zymography are compatible to the changes of MMP-9 levels found in ELISA (Fig. 3). No banding at 92 KD (corresponding to MMP-9) was found in the controls (data not shown).

String Test and PCR for magA, and rmpA

Four CSF *K. pneumoniae* isolates were available for string testing and PCR reaction. Two of the four isolates were from liver abscesses with metastatic meningitis and the other two were from primary meningitis. Three isolates tested positive for hypermucoviscosity. We identified the *rmp*A gene in all four isolates. The two isolates from primary meningitis all tested negative for the *mag*A gene and the other two isolates from metastatic meningitis were all positive for the *mag*A gene.

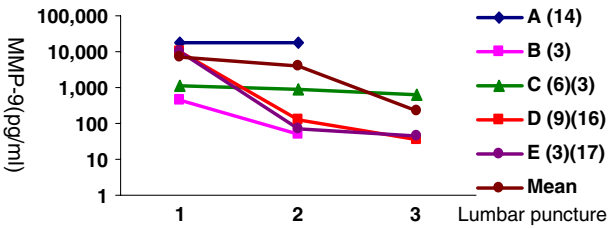

Fig. 2. Dynamic changes of CSF MMP-9 concentrations in five patients who had a second follow-up lumbar puncture. The number in parentheses indicated the time interval (days) between two lumbar punctures. Patient A who died had the highest MMP-9 levels in CSF at presentation (17,971 pg/ml) and 14 days later (17,970 pg/ml).

DISCUSSION

This study demonstrates the upregulation of MMP-9 in CSF specimens from patients with *K. pneumoniae* meningitis. CSF levels of MMP-9 in cases of *K.*

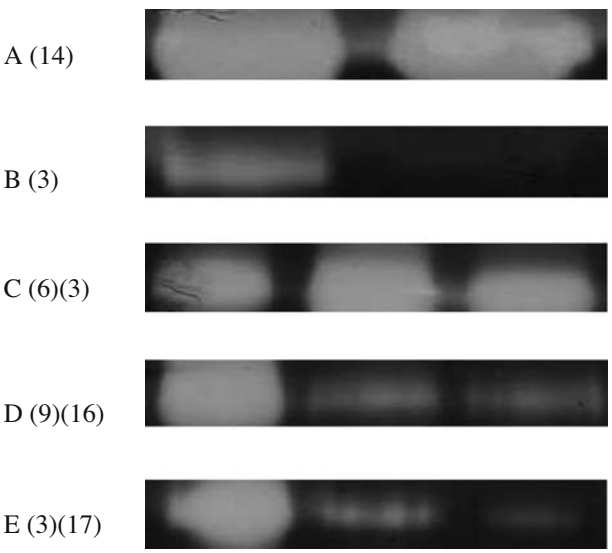

Fig. 3. Dynamic changes of gelatin zymography for MMP-9 concentrations in five patients who had repeated lumbar punctures. The number in parentheses indicated the time interval (days) between two lumbar punctures.

pneumoniae meningitis were 10- to 100-fold higher than in the controls. Furthermore, MMP-9 underwent rapid kinetic changes in those who recovered. MMP-9 is capable of degrading endothelial basement components, and is of strategic importance in the migratory processes into the CNS [10]. The elevated levels found in both bacterial and viral meningitis [9, 21], support a central role for MMP-9 in meningitis. The finding of a correlation between MMP-9 and CSF parameters in bacterial meningitis is supported by previous research [9] as well as our study. Elevated levels of MMP-9 have been reported to contribute to blood–brain barrier and CSF–brain barrier damage, leading to neuroinflammatory processes and edema of the meninges [9, 15, 16, 15, 16, 22].

In Taiwan, *K. pneumoniae* meningitis is one of the most common causative pathogens of community-acquired bacterial meningitis [3, 8]. It has been known to commonly cause invasive infections, including pyogenic liver abscess, bacteremia, pneumonia and endophthalmitis [17, 19]. The association of the *magA* and *rmpA* gene with the hypermucoviscosity phenotype relevant to the clinical syndrome of *Klebsiella pneumoniae* infection has been reported in Taiwan [23]. We identified the *rmp*A gene in all four isolates. The two isolates from primary meningitis all tested negative for the *magA* gene and the other two isolates from metastatic meningitis all tested positive. This is consistent with a previous report of the strong association between *magA* and metastatic *K. pneumoniae* meningitis secondary to liver abscesses [19]. Furthermore, together with the findings of Ma et al. [24] and Yu *et al.* [23] that most *K. pneumoniae* isolates causing primary meningitis were *magA* negative, we hypothesis that that the *magA* gene is probably responsible for the two distinct entities, metastatic and primary *K. pneumoniae* meningitis.

The source of the elevated MMP-9 in the CSF observed in the present study appears not to be peripheral, as no differences in MMP-9 levels were observed in serum samples from the patients with meningitis and the control group studied. Potential sources of the MMP-9 are CSF-infiltrating immune cells, ependimocytes, microglia [11, 25] and other CNS parenchymal or endothelial cells. Animal models of bacterial meningitis have suggested degranulation of PMN as the primary source of CSF MMP-9 [26]. In our study, the CSF levels of MMP-9 correlated with CSF white blood cells (granulocytes). It is possible that the white blood cells were one of the sources of CSF MMP-9, among others.

In those who recovered, the MMP-9 levels in CSF had a kinetic decline. Patient A (Fig. 2) who died of meningitis, had persistently high CSF MMP-9 levels 12 days after stopping corticosteroid treatment. Although corticosteroids have been shown to suppress the expression of MMP-9 [27] in the CSF during acute CNS inflammation, it is likely that the beneficial effects of corticosteroids on our patients is the result of their downregulatory effect on MMP-9 expression, among other factors.

These results are consistent with findings with multiple sclerosis, where levels of MMP-9 in the CSF and number of gadolinium-enhancing lesions on MRI were reduced significantly following treatment with intravenous methyl prednisolone [28].

We did not measure the serum/CSF albumin for MMP-9 index. Serum MMP-9 levels in the control group was higher compared with the values in the CSF. On the contrary, we found that MMP-9 levels in the CSF of our patients was higher compared to the serum level. Based on our study (n=6), the possibility of MMP-9 accumulation in CSF caused by passive influx instead of intrathecal production still can not be totally excluded.

In our small cases series, we found that patients with *K. pneumoniae* meningitis and the presence of MMP-9 proteins in the CSF, suggest a critical role for MMP-9 as an effector of blood brain barrier damage and of neuronal injury in patients with *K. pneumoniae* meningitis. There was an association between MMP-9_{CSF}, CSF protein, and white cell count. CSF MMP-9 levels might be an adjuvant follow up marker in patients with *K. pneumoniae* meningitis. However, larger case studies are needed to justify this conclusion.

ACKNOWLEDGMENTS

This work is supported by the Summer Medical Student Research Program 2007, from the Medical Foundation in Memory of Dr. Deh-Lin Cheng and also supported by grant VGHKS96-027 from Kaohsiung Veterans General Hospital, and grant NSC-96-2320-B-075B-002 from the National Science Council, Republic of China.

REFERENCES

1. Lu, C. H., W. N. Chang, and H. W. Chang. 2000. Adult bacterial meningitis in Southern Taiwan: epidemiologic trend and prognostic factors. *J. Neurol. Sci.* **182**:36–44.

2. Tang, L. M., S. T. Chen, W. C. Hsu, and C. M. Chen. 1997. *Klebsiella* meningitis in Taiwan: an overview. *Epidemiol. Infect.* **119**:135–142.
3. Lee, P. Y., W. N. Chang, C. H. Lu, M. W. Lin, B. C. Cheng, C. C. Chien, C. J. Chang, and H. W. Chang. 2003. Clinical features and *in vitro* antimicrobial susceptibilities of community-acquired *Klebsiella pneumoniae* meningitis in Taiwan. *J. Antimicrob. Chemother.* **51**:957–62.
4. Lu, C. H., W. N. Chang, Y. C. Chuang, and H. W. Chang. 1998. The prognostic factors of adult gram-negative bacillary meningitis. *J. Hosp. Infect.* **40**:27–34.
5. Tang, L. M., and S. T. Chen. 1994. *Klebsiella pneumoniae* meningitis: prognostic factors. *Scand J Infect Dis*. **26**:95–102.
6. Pfister, H. W., and W. M. Scheld. 1997. Brain injury in bacterial meningitis: therapeutic implications. *Curr. Opin. Neurol.* **10**:254–254.
7. Täuber, M. G., Y. S. Kim, and S. L. Leib. 1997. Neuronal injury in meningitis. In: *In defense of the brain*, Peterson, P. K., and J. S. Remington eds. Blackwell, Malden, MA, pp. 124–143.
8. Fang, C. T., Y. C. Chen, S. C. Chang, W. Y. Sau, and K. T. Luh. 2000. *Klebsiella pneumoniae* meningitis: timing of antimicrobial therapy and prognosis. *QJM*. **93**:45–53.
9. Leppert, D., S. L. Leib, C. Grygar, K. M. Miller, U. B. Schaad, and G. A. Holländer. 2000. Matrix metalloproteinase (MMP)-8 and MMP-9 in cerebrospinal fluid during bacterial meningitis: association with blood–brain barrier damage and neurological sequelae. *Clin. Infect. Dis.* **31**:80–84.
10. Yushchenko, M., F. Weber, M. Mäder, U. Schöll, M. Maliszewska, H. Tumani, K. Felgenhauer, and W. Beuche. 2000. Matrix metalloproteinase-9 (MMP-9) in human cerebrospinal fluid (CSF): elevated levels are primarily related to CSF cell count. *J. Neuroimmunol.* **110**:244–251.
11. Yong, V. W., C. A. Krekoski, P. A. Forsyth, R. Bell, and D. R. Edwards. 1998. Matrix metalloproteinases and diseases of the CNS. *Trends Neurosci.* **21**:75–80.
12. Gottschall, P. E., and S. Deb. 1996. Regulation of matrix metalloproteinase expressions in astrocytes, microglia and neurons. *Neuroimmunomodulation*. **3**:69–75.
13. Apodaca, G., J. T. Rutka, K. Bouhana, M. E. Berens, J. R. Giblin, M. L. Rosenblum, J. H. McKerrow, and M. J. Banda. 1990. Expression of metalloproteinases and metalloproteinase inhibitors by fetal astrocytes and glioma cells. *Cancer Res.* **50**:2322–2329.
14. Uhm, J. H., N. P. Dooley, L. Y. Oh, and V. W. Yong. 1998. Oligodendrocytes utilize a matrix metalloproteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. *Glia*. **22**:53–63.
15. Leib, S. L., J. M. Clements, R. L. Lindberg, C. Heimgartner, J. M. Loeffler, L. A. Pfister, M. G. Tauber, and D. Leppert. 2001. Inhibition of matrix metalloproteinases and tumour necrosis factor alpha converting enzyme as adjuvant therapy in pneumococcal meningitis. *Brain*. **124**:1734–1742.
16. Leib, S. L., Y. S. Kim, L. L. Chow, R. A. Sheldon, and M. G. Tauber. 1996. Reactive oxygen intermediates contribute to necrotic and apoptotic neuronal injury in an infant rat model of bacterial meningitis due to group B streptococci. *J. Clin. Invest.* **98**:2632–2639.
17. Lee, H. C., Y. C. Chuang, W. L. Yu, N. Y. Lee, C. M. Chang, N. Y. Ko, L. R. Wang, and W. C. Ko. 2006. Clinical implications of hypermucoviscosity phenotype in *Klebsiella pneumoniae* isolates: association with invasive syndrome in patients with community-acquired bacteraemia. *J. Intern, Med.* **259**:606–614.
18. Nadasy, K. A., R. Domiati-Saad, and M. A. Tribble. 2007. Invasive *Klebsiella pneumoniae* syndrome in North America. *Clin. Infect. Dis.* **45**:25–28.
19. Fang, C. T., Y. P. Chuang, C. T. Shun, S. C. Chang, and J. T. Wang. 2004. A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. *J. Exp. Med.* **199**:697–705.
20. Nassif, X., N. Honore, T. Vasselon, S. T. Cole, and P. J. Sansonetti. 1989. Positive control of colanic acid synthesis in *Escherichia coli* by *rmpA* and *rmpB,* two virulence-plasmid genes of *Klebsiella pneumoniae*. *Mol. Microbiol.* **3**:1349–1359.
21. Kolb, S. A., F. Lahrtz, R. Paul, D. Leppert, D. Nadal, H. W. Pfister, and A. Fontana. 1998. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in viral meningitis: up-regulation of MMP-9 and TIMP-1 in cerebrospinal fluid. *J. Neuroimmunol.* **84**:143–150.
22. Rosenberg, G. A., E. Y. Estrada, J. E. Dencoff, and W. G. Stetler-Stevenson. 1995. Tumor necrosis factor-α-induced gelatinase B causes delayed opening of the blood–brain barrier: an expanded therapeutic window. *Brain Res.* **703**:151–155.
23. Yu, W. L., W. C. Ko, K. C. Cheng, H. C. Lee, D. S. Ke, C. C. Lee, C. P. Fung, and Y. C. Chuang. 2006. Association between rmpA and magA genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. *Clin. Infect. Dis.* **42**:1351–1358.
24. Ma, L. C., C. T. Fang, C. Z. Lee, C. T. Shun, and J. T. Wang. 2005. Genomic heterogeneity in *Klebsiella pneumoniae* strains is associated with primary pyogenic liver abscess and metastatic infection. *J. Infect. Dis.* **192**:117–128.
25. Miller, A., S. Shapiro, N. Lahat, and Y. Galboiz. 2002. Matrix metalloproteinases and their inhibitors in brain injury and repair. In: *Brain disease: therapeutic strategies and repair*, Abramsky, O., D. A. S. Comspton, A. Miller, and G. Said eds. Martin Dunitz, UK, pp. 63–68.
26. Keiseier, B. C., T. Seifert, G. Giovannoni, and H. P. Hartung. 1999. Matrix metalloproteinases in inflammatory demyelination: targets for treatment. *Neurology.* **53**:20–25.
27. Harkness, K. A., P. Adamson, J. D. Sussman, G. A. Davies-Jones, J. Greenwood, and M. N. Woodroofe. 2000. Dexamethasone regulation of matrix metalloproteinase expression in CNS vascular endothelium. *Brain*. **123**:698–709.
28. Rosenberg, G. A., J. E. Dencoff, N. Correa Jr, M. Reiners, and C. C. Ford. 1996. Effect of steroids on CSF matrix metalloproteinases in multiple sclerosis: relation to blood–brain barrier injury. *Neurology.* **46**:1626–1632.