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BRIEF REPORT

Elevated Matrix Metalloproteinase Concentrations Offer Novel Insight Into Their Role in Pediatric Tuberculous Meningitis

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We collected lumbar and ventricular cerebrospinal fluid and serum from 40 children treated for tuberculous meningitis and measured the concentrations of gelatinases and their inhibitors. The concentrations of matrix metalloproteinase 9 (MMP-9), MMP-2, tissue inhibitor of metalloproteinase 1 (TIMP-1), and TIMP-2 were significantly elevated in the lumbar CSF samples, and we found interesting dynamics for MMP-9 that offer novel insight into its role in pediatric patients with tuberculous meningitis.

Keywords. cerebrospinal fluid; matrix metalloproteinase; MMP-2; MMP-9; pediatric; tissue inhibitors of metalloproteinase; TIMP-1; TIMP-2; tuberculous meningitis.

Tuberculous meningitis (TBM), the most severe form of tuberculosis, results in high morbidity and mortality rates in children. Central to its pathology is a profuse inflammatory response and the associated breakdown in the blood–brain barrier, in which the matrix metalloproteinases (MMPs) play a role. MMPs are an enzyme family of the zinc-dependent endopeptidases responsible for the degradation of the extracellular matrix, which is involved in normal physiological functions and in various pathologies [1]. Previous studies in adults have implicated MMP-9 and MMP-2 (known as the gelatinases) in the pathophysiology of TBM; higher levels of them are found in the cerebrospinal fluid (CSF) of patients with TBM than are found in controls and patients with other meningitides, and they are associated with poorer neurological outcomes [2–4]. MMP-9 was also proposed as a target on which steroids act to suppress to improve patient outcome in TBM [5]. Gelatinases are innately inhibited by tissue inhibitors of metalloproteinases (TIMPs); TIMP-1 targets MMP-9, and TIMP-2 targets MMP-2. To our knowledge, MMPs have not been studied in pediatric patients with TBM. In this study, we aimed to assess the acute temporal profile of gelatinases and their inhibitors in 2 CSF compartments (lumbar and ventricular) and serum.

METHODS

Patients with TBM who were enrolled as part of a prospective study on biomarkers conducted from October 2010 to August 2013 served as the study cohort. Inclusion criteria were a definite or probable TBM diagnosis (per consensus research criteria [6]) and having had fewer than 2 doses of drug treatment for TBM before CSF and serum samples were obtained. Severity of the patients' TBM was determined using the following "refined" British Medical Research Council criteria[7] on admission: stage I, a Glasgow Coma Score (GCS) of 15 without focal neurological signs; stage IIa, a GCS of 15 with neurological deficit or a GCS of 13 or 14 with or without neurological deficit; stage IIb, a GCS of 10 to 12 with or without focal neurological deficit; and stage III, a GCS of <10 with or without neurological deficits. Eight children who underwent elective neurosurgery to section their filum terminale, which was thickened by fat and tethered the spinal cord (a condition in which the CSF is typically normal and not associated with inflammation) were included as control patients. Patients with TBM were treated with the standard 4-drug regimen of rifampicin (20 mg/kg), isoniazid (20 mg/kg), pyrazinamide (40 mg/kg), and ethionamide (20 mg/kg) for 2 months, followed by 4 to 6 months of rifampicin and isoniazid. Prednisone (2 mg/kg per day) was used for the first 3 weeks. Demographic, laboratory, and radiological data were collected. Patient outcome 6 months after admission was assessed by using the Pediatric Cerebral Performance Category Scale [8]; scores were dichotomized as 1 to 3 for good outcome (normal function to moderate disability) and 4 to 6 for poor outcome (severe disability to death).

Lumbar and ventricular CSF and serum were obtained during clinically indicated procedures. These fluids were multiplexed on the BioPlex platform (Bio-Rad Laboratories, Hercules, California) using customized 2-plex MILLIPLEX MAP (multianalyte profiling) plates (Merck Millipore, Burlington, Massachusetts)

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according to manufacturer instructions. Access to ventricular CSF was not clinically indicated for the control patients. To avoid the effect of treatment on gelatinase concentrations, we excluded samples taken after the patient had more than 2 doses of treatment and hemolyzed and xanthochromic samples. Statistical analyses were performed using SPSS 22.0 (IBM Corp, Armonk, New York) and GraphPad Prism 6.07 (GraphPad Software, La Jolla, California). Medians and interquartile ranges are reported where applicable. Statistical significance was set at a 2-tailed *P* value of .05. Univariate analyses were performed using the Mann–Whitney U, Kruskal–Wallis, or Friedman test (for related variables), the χ^2 or Fisher exact test, and the Spearman correlation test. We corrected for multiple comparisons with the Dunn multiple-comparisons post hoc test, and adjusted *P* values were calculated.

This study was approved by the Human Research Ethics Council of the University of Cape Town, and informed written consent was obtained for all participants from their parents or legal guardians (approval HREC 318/2010).

RESULTS

The patient's clinical characteristics are shown in Table 1. In total, 108 lumbar, 38 ventricular, and 84 serum samples from 40 patients with TBM were collected (up to 4 weeks after admission) and analyzed. Eight lumbar CSF and 8 serum samples from control patients were analyzed.

The median concentrations of lumbar CSF MMP-9 were significantly higher in the patients with TBM than in the control patients at all time points (Figure 1A). The same was true also for median lumbar CSF TIMP-1 concentrations except at week 4 (Figure 1D). Median ventricular CSF MMP-9 and TIMP-1 concentrations were significantly higher in the patients with TBM than in the control patients during admission and week 1 (Figure 1B and E). The median serum MMP-9 concentrations at admission were significantly higher in the patients than in the control patients (Figure 1C), and median serum TIMP-1 concentrations showed no significant differences (Figure 1F). Overall, the median lumbar CSF MMP-9 concentrations were significantly decreased between admission and week 1 (average decrease, 1.1 ng/mL; $P = .04$) (Supplementary Data B), and an overall increasing trend was seen in subsequent weeks.

Median concentrations of lumbar CSF MMP-2 and TIMP-2 were significantly higher in the patients with TBM than in the control patients for all time points except for MMP-2 on admission and TIMP-2 in week 4 (Figure 1G and J). Unlike lumbar MMP-9 concentrations, no significant differences were seen between the time points. The median ventricular CSF MMP-2 concentration on admission was lower in patients with TBM than in control patients, whereas those for the remaining time points for MMP-2 and all ventricular TIMP-2 concentrations showed no significant differences (Figure 1H and K). The median serum MMP-2 concentrations at admission and week 1 were significantly lower in patients with TBM than in the controls, whereas TIMP-2 concentrations showed no significant differences (Figure 1I and L).

Analytes were found to be highest in the serum (compared to the 2 CSF compartments) for all except TIMP-1.

The admission MMP/TIMP ratios (calculated using their median values) were 0.003 (95% confidence interval [CI], 0.0009–0.005) for MMP-9/TIMP-1 and 0.729 (95% CI, 0.336–1.422) for MMP-2/TIMP-2; these ratios were less than 1, which suggests a greater abundance of TIMP relative to MMP.

Positive correlations were found for MMP-2 and TIMP-2, and negative correlations were found for MMP-9 and TIMP-1 (Supplementary Data C1). The same positive correlation for MMP-2/TIMP-2 was seen in the control samples but not for control MMP-9/TIMP-1 concentrations (Supplementary Data C2). No correlation was found between the admission analyte concentrations with the CSF–serum albumin quotient (Q-alb) or with patient clinical, radiological, or CSF chemistry data. An increase in lumbar MMP-9 concentrations between admission and week 4 was associated with good outcome 6 months after admission (risk ratio, 2.1 [95% CI, 1.23–3.53]; $P = .01$).

DISCUSSION

Similar to the authors of studies in adults, we found that gelatinase concentrations (and those of their inhibitors) were elevated in pediatric patients with TBM; however, we found no association with admission clinical features or unfavorable outcomes. Unlike in adults [9], admission MMP-9 levels in these pediatric patients did not differ significantly with severity of the presenting clinical condition. Instead, patients whose MMP-9 levels increased during the sampling period were twice as likely to have a better outcome than those whose levels had decreased. Despite increased Q-alb values for all patients (which suggests a compromised blood–brain barrier), no correlation between Q-alb and MMP levels was found in our study.

Like that in adults, the MMP-9 concentration in a normal child's central nervous system (CNS) is minimal/undetectable, as seen in our control patients. In contrast, MMP-2 has an ongoing physiological presence responsible for the remodeling of the extracellular matrix of the CNS [10]. In patients with TBM, infiltrating monocytes secrete MMP-9 and astrocytes secrete both MMP-9 and MMP-2 [10, 11]. Activation of MMP-2 requires TIMP-2 (as part of a trimolecular complex), which might explain the positive correlation that we found. Furthermore, the presence of a negative correlation between MMP-9 and TIMP-1 in patients with TBM but not in control patients might suggest that MMP-9 indicates pathology more than MMP-2. In the adult brain, TIMP-1 is localized to an area of persistent neuronal plasticity [12], and the high levels in our study might reflect the higher neuroplasticity of the pediatric brain.

Table 1. Demographic, Clinical, Radiological and CSF Data[a]

Characteristic	Value	Lumbar Samples (n = 34)	Ventricular Samples (n = 6)
Patients with TBM			
Total no. of patients	40		
Age on admission (median [range]) (years)	*3.7 (0.4–13.1)*		
Sex, male (n [%])	25 (62.5)		
Clinical characteristics on admission			
MRC stage (n [%])			
I	3 (7.5)		
IIa	14 (35)		
IIb	14 (35)		
III	9 (22.5)		
BCG vaccination (n = 29) (n [%])[b]	25 (86.2)		
Recent TB contact (n = 36) (n [%])	16 (44.4)		
Tuberculin skin test (n = 26) (n [%])	18 (69.2)		
HIV infection (n = 38) (n [%])	2 (5.3)		
Duration of symptoms (median [range]) (days)	7 (1–90)		
Weight loss/failure to thrive (n [%])	20 (50)		
Night sweats (n [%])	3 (7.5)		
Persistent cough (n [%])	7 (17.5)		
GCS (median [range])	11 (4–15)		
Focal neurological signs (n [%])[c]	20 (50)		
Headache (N = 35) (n [%])	15 (42.9)		
Neck stiffness (n [%])	31 (77.5)		
Fever (n [%])	28 (70)		
Vomiting (n [%])	20 (50)		
Seizures (n [%])	12 (30)		
Altered level of consciousness (n [%])	37 (92.5)		
Diagnostic data (n [%])			
Definite TBM[d]	22 (55)		
Probable TBM[e]	18 (45)		
Control patients (N = 8)			
Age (median [range]) (years)	2.7 (0.7–5.5)		
Sex, male (n [%])	4 (50)		
CSF parameters			
Glucose (mmol/L)		1.8 (0.6–4.8)	3.5 (1.8–4.3)
Chloride (mmol/L)[f]		106.5 (95–131)	114 (102–126)
Protein (g/L)		1.9 (0.34–20)	0.4 (0.2–1.2)
Total white cell count		158.5 (6–901)	9.5 (0–85)
Polymorphonuclear cells (per mL)		22.5 (0–280)	1 (0–4)
Lymphocytes (per mL)		129 (6–715)	7 (0–85)
CSF/serum albumin ratio[g]		27.1 (0.4–99.7)	6 (2.6–20.3)
Radiological characteristics (n [%])			
Hydrocephalus[h]	40 (100)		
Communicating hydrocephalus	31 (81.5)		
Noncommunicating hydrocephalus	3 (78.9)		
Meningeal enhancement at admission	38 (95)		
Tuberculoma present (overall)	23 (57.5)		
Infarcts	28 (70)		
Miliary TB	5 (12.5)		
Chest radiograph suggestive of TB	21 (52.5)		

Abbreviations: BCG, bacillus Calmette–Guérin vaccine; CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; HIV, human immunodeficiency virus; MRC, Medical Research Council; TB, tuberculosis; TBM, tuberculous meningitis.

[a]Poor outcome was defined as a score of 1 to 3 (normal to mild/moderate disability) or 4 to 6 (severe disability to death) according to the Pediatric Cerebral Performance Category Scale.

[b]A parent or guardian was not present or the clinic card was missing.

[c]Focal neurological signs included abnormal pupillary response, paresis, cranial nerve palsy, and aphasia.

[d]Definite TBM indicates the detection of bacilli in the CSF.

[e]Probable TBM indicates a combination of clinical, radiological, and CSF criteria (see reference 6).

[f]Lumbar chloride values were available for only 31 patients.

[g]CSF/serum albumin ratios were available for only 27 patients with admission lumbar CSF available and 6 patients with ventricular CSF available. The reference CSF protein range is 0.15 to 0.40 g/L.

[h]Of the 40 patients, 2 immediately underwent shunt placement, and 38 patients underwent an air encephalogram or column test to check for hydrocephalus communication; for these 38 tests, 4 patients had inconclusive results.

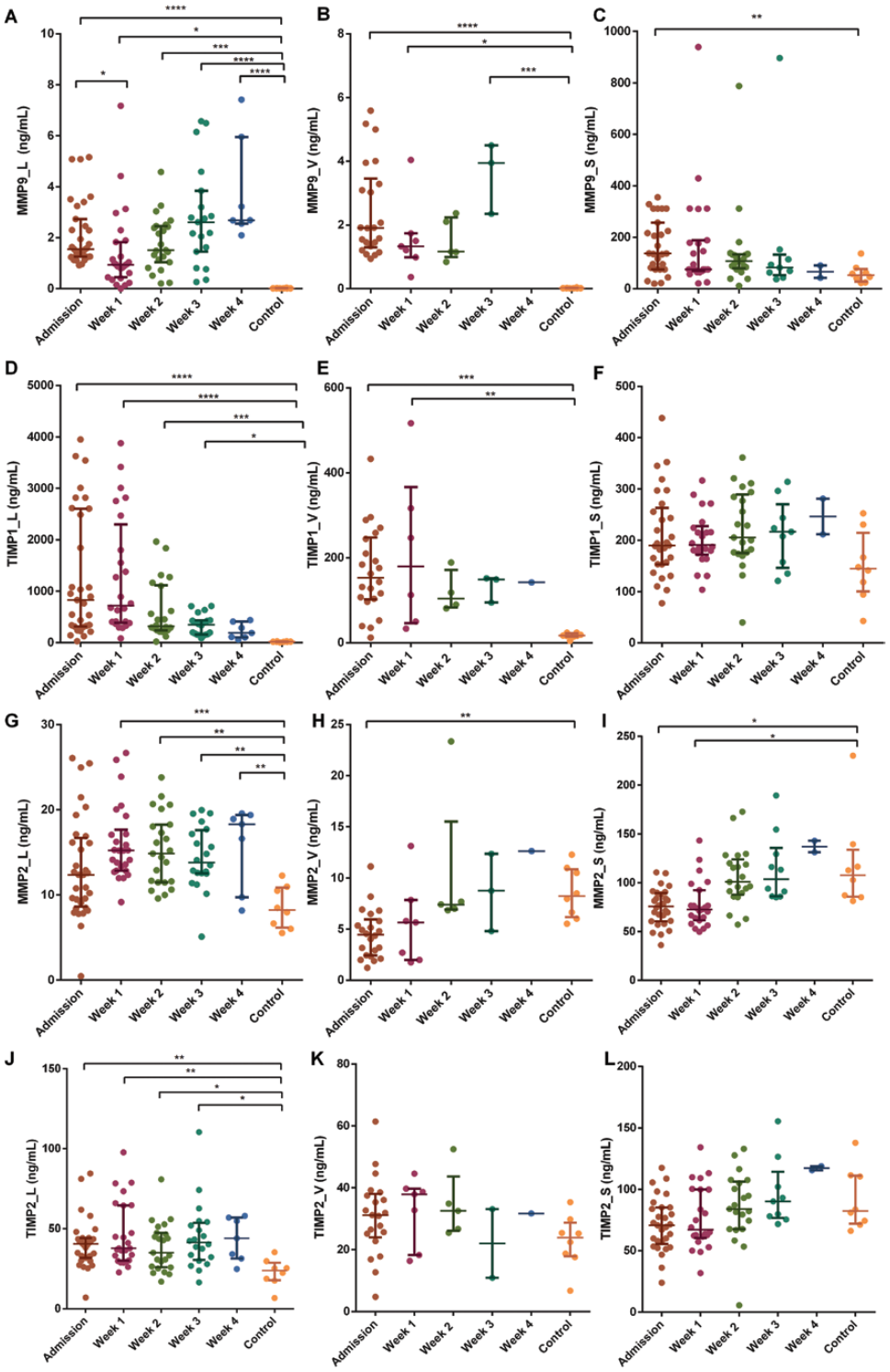

Figure 1. Concentrations of gelatinases and their inhibitors (at all time points) for patients with TBM compared with those of the control patients. **(A)** Lumbar MMP-9. **(B)** Ventricular MMP-9. **(C)** Serum MMP-9. **(D)** Lumbar TIMP-1. **(E)** Ventricular TIMP-1. **(F)** Serum TIMP-1. **(G)** Lumbar MMP-2. **(H)** Ventricular MMP-2. **(I)** Serum MMP-2. **(J)** Lumbar TIMP-2. **(K)** Ventricular TIMP-2. **(L)** Serum TIMP-2. Outliers (defined by >3 standard deviations of the mean) were excluded for all samples. Medians are shown, and whiskers represent the interquartile range. The time points were compared to those of the controls using the Kruskal–Wallis test: *, $P \leq .05$; **, $P \leq .01$; ***, $P \leq .001$. Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

Ventricular CSF sampling is not feasible for routine diagnostic purposes but offers insight into pathophysiology at the site of the disease. Similar to published data [13], our results show a difference in analyte concentrations in the lumbar CSF, ventricular CSF, and serum. The exact mechanisms of these compartmental differences are not clear and require further investigation. Higher serum concentrations of MMP-9, MMP-2, and TIMP-2 might suggest a systemic inflammatory source for these proteins.

We found a significant decrease in MMP-9 concentrations between admission and week 1, which reflect a response to prednisone and other drugs for the treatment of tuberculosis. Previous studies found that dexamethasone decreases the CSF MMP-9 concentration early in treatment and proposed this as a mechanism whereby steroids improve the mortality rate in patients with TBM [5, 9]. In contrast, later increases in MMP-9 concentrations in our study overlapped with steroid treatment. Furthermore, because of the intrinsic beneficial roles of MMP-9—including angiogenesis, myelinogenesis, axonal growth, and regulation of synaptic plasticity—MMP-9 might play a role in CNS repair after injury, differing from its pathological role depending on the timing of injury [1, 10]. In pediatric patients with TBM, initially high TIMP-1 levels might alleviate the initial pathological effects of MMP-9, and later increases in the MMP-9 concentration potentially suggest a role in recovery. However, multivariate analysis to control for other factors associated with the outcome are required to test this theory.

Our study was limited by the inability to distinguish between inactive and active forms of measured MMPs. All of our patients had associated hydrocephalus, and our findings might not be generalizable to patients without hydrocephalus. Because of our lack of access to ventricular CSF in the control patients, ventricular analytes from patients with TBM were compared to those from lumbar CSF from control patients.

In conclusion, the key findings of this study can be summarized as follows: (1) Gelatinases and their inhibitors were persistently elevated in pediatric patients with TBM, (2) MMP-9 levels decreased significantly early in treatment, and (3) admission gelatinase concentrations were not associated with neurological complications or poor outcome, but an increase in the MMP-9 concentration during the hospital stay was associated with better outcome.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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