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Role of cytokines in the assessment of clinical outcome and neuroimaging findings in patients with tuberculous meningitis

[1]Mei-Ling Sharon Tai *MRCP*, [2]Hong-Yien Tan *PhD*, [2]Yean-Kong Yong *PhD*, [2,4]Esaki M. Shankar *PhD*, [5]Shanthi Viswanathan *MRCP*, [3]Hazman Mohd Nor *MMed*, [3]Kartini Rahmat *FRCR*, [1]Jun-Fai Yap *MBBS*, [6]Boon-Seng Ng *MMed*, [1]Chong Tin Tan *FRCP*

[1]Division of Neurology, Department of Medicine; [2]Centre of Excellence for Research in AIDS; & [3]Department of Biomedical Imaging, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [4]Division of Infection Biology & Microbiology, Department of Life Sciences, School of Basic and Applied Sciences, Central University of Tamil Nadu, Thiruvarur, India; [5]Department of Neurology, Hospital Kuala Lumpur; [6]Department of Psychological Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Abstract

Background: Tuberculous meningitis is a life-threatening manifestation resulting from infection by *Mycobacterium tuberculosis*, especially in the developing countries. The molecular aspects of pathogenesis of tuberculous meningitis remain poorly understood. We evaluated the correlation of cerebrospinal fluid (CSF) and serum cytokine levels with the clinical outcome of 15 HIV-negative patients with tuberculous meningitis. We also assessed the association of CSF and serum cytokines with neuroimaging of brain findings in the patients. *Methods:* The prospective longitudinal study was conducted at the University Malaya Medical Centre between 2012 and 2014. Neuroimaging of the brain was performed and the findings of leptomeningeal enhancement, hydrocephalus, tuberculoma, infarcts and vasculopathy were recorded. The CSF and serum specimens were analyzed for IL-1ß, IL-8, IL-10, IL-18, IP-10, IFN-γ, MCP-1, TGF-ß, VEGF, TNF- α, IL-18BPa and MMP-9. The clinical outcome was graded at 3 months based on Modified Rankin scale (mRS). *Results:* On admission and at one month of anti-tuberculosis treatment, the CSF levels of IL-8, IL-1β, IP-10, IFN-γ and VEGF were elevated in all of the patients. Serum IP-10, MCP-1, IL-1β and IL-8 levels were increased on admission and at one month of anti-tuberculosis treatment. There were statistically significant differences between good and poor outcome (mRS at 3 months) for CSF IFN-γ (p=0.033), CSF IL-10 (p=0.033) and serum VEGF (p=0.033) at one month of treatment. None of the patients showed any association between CSF and serum cytokines on admission and at one month of anti-tuberculosis treatment with neuro-radiological findings.
Conclusion: The CSF cytokine levels were not related to TBM disease severity on admission, and changes on MRI/CT scans. CSF levels of IFN-γ and IL-10 at one month of anti-tuberculosis treatment were associated with clinical outcome at 3 months. CSF cytokine levels on admission were not associated with the clinical outcome.

Keywords: Chemokines; cytokines; clinical outcome; tuberculous meningitis; neuroimaging

INTRODUCTION

Tuberculous meningitis (TBM) represents one of the most common and devastating forms of tuberculosis (TB) that involves the central nervous system (CNS).[1] TBM is common in the developing world.[2] TBM accounts for significant rates of morbidity and mortality in the afflicted population worldwide.[1] The molecular aspects underlying the pathogenesis of TBM remain poorly understood. Recent evidence suggests that cytokine and chemokine deregulation could contribute to the onset of chronic inflammation in TBM pathogenesis.[3] Furthermore, it also appears that certain cytokines could traverse into the blood-brain barrier (BBB), potentially to recruit leukocytes across the CNS.[4]

Address for correspondence: Dr. Mei-Ling Sharon Tai, Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. E-mail address: sharont1990@gmail.com

Cytokines that regulate inflammatory responses include interleukins (ILs), chemokines, and type I interferons (IFNs).[3] Interleukins (ILs) are responsible for communication between white blood cells, whereas type I interferons (IFNs) have antiviral effects.[3] Chemokines are members of the cytokine family of regulatory proteins and promote chemotaxis.[3,5] Chemokines are stimulated by primary pro-inflammatory mediators, namely tumor necrosis factor-α (TNF-α) and IL-1β.[5] Cytokines and chemokines have been used as immunological markers for diagnosing active TB disease.[6] Hence, exploring the role of pro- and anti-inflammatory cytokines is key to improving understanding of the pathogenesis of infectious diseases involving the CNS.[7]

One study reported that the concentrations of transforming growth factor-β (TGF-β) were significantly higher in patients with tuberculous pleural effusion.[8] Nonetheless, investigations aimed at evaluating brain neuroimaging combined with CSF and serum levels of cytokines in patients with TBM, after one month of treatment, have scarcely been conducted. Longitudinal studies examining the levels of IL-18 and TGF-ß in TBM have seldom been undertaken till to-date.

The objective of the present study was to evaluate the concentrations of serum and CSF cytokines and chemokines, and correlate their levels with the clinical outcome of HIV-negative patients with TBM. We also determined the association of CSF and serum levels of various cytokines with neuroimaging of brain findings in patients with TBM.

METHODS

Patient enrolment and selection

The patients were selected from a cohort of TBM patients with follow-up and prospective inclusion of new cases. The study was conducted between 2012 and 2014 at the University Malaya Medical Centre (UMMC), and specimens (CSF and blood) were collected. The patients were diagnosed with TBM on the basis of clinical, CSF and radiological criteria. The clinical criteria of TBM included illness lasting for >5 days, change in the level of consciousness, focal neurological symptoms and signs or response to anti-tuberculosis treatment (ATT) drugs in the absence of cryptococcal meningitis. All of the recruited TBM cases were negative for infection with human immunodeficiency virus (HIV).

A detailed medical history and clinical examination was performed. Consciousness was assessed by Glasgow Coma Scale (GCS). Focal neurological deficit was assessed. Severity of meningitis was graded as stage 1 meningitis only, stage 2 meningitis with focal neurological signs or altered mental state and stage 3 meningitis with involvement of neurological signs and stupor. CSF was examined for opening pressure, white blood cells, protein, glucose, bacteria, cryptococci and was also stained for acid-fast bacilli (AFB). CSF specimens were also examined for the presence of *Mycobacterium tuberculosis* by culture and polymerase chain reaction (PCR).

Chest radiography was done for all the patients. Computed tomography (CT) scan of the brain was performed on admission and the information was recorded. Magnetic resonance imaging (MRI) of the brain and magnetic resonance angiography (MRA) was performed using 3.0-Tesla Signa HDx (GE Healthcare, USA). The findings of leptomeningeal enhancement, hydrocephalus, tuberculoma, infarcts and vasculopathy were recorded.

Diagnosis of tuberculous meningitis (TBM)

The CSF criteria for the diagnosis of TBM were positive mycobacterial culture, PCR, CSF AFB smear or histopathological findings of *M. tuberculosis*. The accompanying radiological and microbiological results were chest X-ray or CT thorax features of pulmonary TB (PTB), positive sputum AFB direct smear and abnormal sputum mycobacterial TB culture results. Concomitant presence of other extra-pulmonary TB (EPTB), such as TB of the spine was also noted. The patients with TBM were categorized into definite, probable and possible, based on the various clinical, CSF and radiological features.[9]

Management and clinical progress

The patients were treated with an intensive phase of four ATT drugs (ethambutol, isoniazid, rifampicin and pyrazinamide) followed by a maintenance phase of two drugs (isoniazid and rifampicin). The total duration of ATT was 12-18 months based on the British Infection Society guidelines.[10] Corticosteroid treatment was administered to patients who had severe TBM for a duration of one month followed by tapering off of the dose in the following month. Extra-ventricular drainage (EVD) and/or ventriculo-peritoneal (VP) shunt was performed, if indicated.

The patients underwent regular daily clinical examination. CT or MRI of the brain was repeated 1-2 months after admission and when patients showed clinical deterioration. Their clinical outcome was graded at 3 months on the basis of Modified Rankin scale (mRS). The mRS scale ranges between 0 and 6[11] as follows: 0: No symptoms; 1: No significant disability, able to carry out all usual activities, despite some symptoms; 2: Slight disability, able to look after own affairs without assistance, but unable to carry out all previous activities; 3: Moderate disability, requires some help, but able to walk unassisted; 4: Moderately severe disability, unable to attend to own bodily needs without assistance, and unable to walk unassisted; 5: Severe disability, requires constant nursing care and attention, bedridden, incontinent; 6: Death. In the current study, mRS was categorized into poor (mRS 3-6) or good (mRS 0-2) clinical outcome.[11]

Specimens and cytokine ELISA

Lumbar CSF on admission and one month after onset of treatment was collected in a sterile vial and was immediately stored at -80 °C. Blood samples were also collected at the same time points. Subsequently, the CSF and blood samples were later analyzed for the levels of IL-1ß, IL-8/CXCL-8, IL-10, IL-18, IFN gamma-induced protein 10 (IP-10/CXCL10), IFN-γ, monocyte chemoattractant protein (MCP-1/CCL2), IL-18 binding protein (IL-18BPa), matrix metallopeptidase-9 (MMP-9), TGF-ß, vascular endothelial growth factor (VEGF) and TNF-α, using ELISA kits procured from R&D System (Minneapolis, USA). Independent ELISAs were performed. The samples were performed in duplicate. The coefficient of variance (CV) cutoff was <20%.

Statistical analysis

All statistics were performed using Statistical Package for Social Sciences, SPSS version 20 software (SPSS Inc., Chicago, USA). Changes in serum and CSF cytokine levels after one month treatment was evaluated by Wilcoxon Signed-Rank Test. A comparison of the serum and CSF cytokine levels between good and poor outcome at 3 months (mRS) was analysed using Mann–Whitney U-test. An assessment of comparison between early stage (stage 1) and advanced stages (stages 2 and 3) of the serum and CSF cytokine levels was analysed using Mann–Whitney U-test. *p* value of <0.05 was taken as statistically significant.

A comparison of all the serum and CSF cytokine levels was made for the presence or absence of MRI brain abnormalities (hydrocephalus, leptomeningeal enhancement, tuberculoma, infarcts and vasculopathy). This was expressed as mean ±standard deviation (SD).

The study was approved by the Medical Ethics Committee (MEC) of University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. All the patients, or their legally acceptable representatives, provided informed consent before enrolment.

RESULTS

Clinical demographic characteristics

The study consisted of 15 patients with TBM, and the baseline characteristics of the participants are listed in Table 1. The mean age was 34.20±10.28 years (range 19-59). Nine (60%) patients were female patients. Five (33%) were Malay, six (40%) were Indians and four (27%) were non-Malaysian patients. The three most common symptoms were fever (87%), headache (73%) and vomiting (73%). Two (13%) patients presented with stage 1, nine (60%) patients with stage 2 and four (27%) with stage 3 meningitis. The patients had GCS with range of 8-15 (median 14). Pulmonary TB was present in five (33%) patients. The diagnosis was definite in 11 (73%) patients and was possible in four (27%) patients. None of the patients had probable TBM.

The CSF cells ranged between 0 and 795 (mean 35.10±19.93)/mm^3 and were predominantly lymphocytic. The mean CSF protein was 1.63±1.39 g/dl, and glucose 2.06±1.43 mmol/L.

Neuroimaging findings

The CT and MRI of the brain revealed leptomeningeal enhancement in thirteen (87%), hydrocephalus in twelve (80%), infarct in thirteen (87%) and tuberculoma in eight (53%) patients. Vasculopathy was observed in 11 of the 14 patients (79%). One patient did not have MRA of the brain performed.

Cytokine levels at baseline and one month after the start of the treatment

The serum and CSF cytokine levels on admission and at one month are presented in Table 2.

a) CSF cytokine levels: On admission (baseline), the CSF levels of IL-8, IL-1β, IP-10, IFN-γ and

Table 1: **Baseline characteristics, cerebrospinal (CSF) and sputum results of TB meningitis patients**

Characteristics	Patients, n=15
Age, median (Range)	33 (19-59)
Gender (n, %)	
Male	6 (40%)
Female	9 (60%)
Ethnic group (n, %)	
Malay	5 (33%)
Indian	6 (40%)
Non-Malaysians	4 (27%)
Clinical features (n, %)	
Fever	13 (87%)
Headache	11 (73%)
Vomiting	11 (73%)
Altered sensorium	9 (60%)
Loss of appetite	8 (53%)
Loss of weight	6 (40%)
Limb weakness (hemiparesis/paraparesis)	6 (40%)
Neck stiffness	4 (27%)
Stage of illness on admission (n, %)	
Stage 1	2 (13%)
Stage 2	9 (60%)
Stage 3	4 (27%)
Other TB sites (n, %)	
Pulmonary tuberculosis (PTB)	5 (33%)
TB spine	1 (7%)
Other medical illnesses (n, %)	
Diabetes mellitus (DM)	1 (7%)
Systemic lupus erythematosus (SLE)	1 (7%)
Cerebrospinal fluid results on admission	
Opening pressure, cm H2O (mean ± SD)	35.10±19.93 (range 14-75)
White blood cells, cells/ml (mean ± SD)	153.79 (range 0-795)
Lymphocyte differential in percentage (mean ± SD)	68.60 ± 37.26 (range 0-100)
Neutrophil differential in percentage (mean ± SD)	28.55 ± 36.59 (range 0-100)
Glucose, mmol/L (mean ± SD)	2.06 ± 1.43 (range 0.5-5.3)
Protein, g/L (mean ± SD)	1.63 ± 1.39 (range 0.6-5.4)
Acid-fast-bacilli direct smear (n, %)	1(7%)
Mycobacterium tuberculosis culture positive (n, %)	10 (67%)
Tuberculous PCR (n, %) positive (out of 14 samples)	4 (29%)
Sputum (n, %)	
Mycobacterial culture positive	1 (7%)
Diagnosis (n, %)	
Definite	11 (73%)
Probable	0
Possible	4 (27%)
Outcome (mRS) at 3 months (n, %)	
0	0
1	1 (7%)
2	3(20%)
3	0
4	2 (13%)
5	6 (40%)
6	3 (20%)

Table 2: Serum and CSF cytokine and IL-18BPa levels in TBM patients on admission and at 1 month

Cytokines	On admission		1 month	
Serum	Median (IQR)	Range	Median (IQR)	Range
IL-8	19.7 (9)	15.4-36.1	24.6 (24.9)	15.4-46.8
Free IL-18	17.7 (24.0)	8.1-59.9	11.9 (10.1)	9.0-22.6
IL-1β	7.4 (0.7)	6.5-8.2	7.5(1.1)	6.5-8.2
IP-10	339.0(630.0)	107.2-2142.1	858.6(1702.7)	187.2-2542.9
TGF-ß	21.7 (7.5)	18.5-37.2	21.7 (6.8)	17.3-31.6
MMP-9	258.5 (236.3)	115.2-1204.6	141.1 (223.9)	67.9-1067.2
IFN-γ	-13.6 (35.2)	-21.9 to 29.2	-17.2 (14.1)	-25 to 22.9
IL-10	2.4 (0.3)	2.1-3.0	2.5 (1.0)	2.2-3.5
VEGF	94.8 (137.6)	53.6-266.6	89.9 (160.6)	47.6-810.0
MCP-1	81.7 (61.9)	49.0-138.3	156.2 (122.5)	57.1-213.0
TNF-α	1.5 (0.3)	1.2-2.1	1.5 (0.3)	1.2-3.0
IL-18BPa	112.5 (48.6)	89.3-200.1	138.9 (132.9)	89.3-256.0
Cytokines	**On admission**		**1 month**	
CSF	Median (IQR)	Range	Median (IQR)	Range
IL-8	853.2 (1637.0)	284.0-2802.4	1374.1 (1729.5)	169.9-3403.9
Free IL-18	3.6 (1.3)	2.6-5.3	2.6 (2.2)	2.1-4.4
IL-1β	10.2 (12.1)	9.3-1104.1	11.0 (17.3)	8.5-32.5
IP-10	7631.3 (821.3)	5858.8-7994.1	7499.0 (3161.9)	856.2-8021.7
TGF-ß	28.9 (12.3)	21.0-42.9	27.6 (5.3)	22.3-34.4
MMP-9	283.1 (214.6)	62.9-460.7	257.8 (533.7)	115.2-909.5
IFN-γ	61.5 (181.2)	6.3-237.5	117.7 (176)	4.2-258.3
IL-10	11.8 (24.3)	2.3-63.2	11.4 (16.6)	2.9-27
VEGF	41.6 (78.8)	32.9-204.8	88.3 (120.2)	32.9-211.9
MCP-1	1114.7 (1670.6)	639.1-3331.6	1113.7 (828.2)	651.0-2488.6
TNF-α	3.5 (3.4)	1.7-8.5	3.0 (2.4)	1.9-6.0
IL-18BPa	4035.1 (7215.2)	1419.4-12486.5	6346.3 (7618.5)	871.2-15019.6

Footnotes: All pg/ml except MMP-9 in ng/ml

VEGF cytokines were raised in all of the samples (100%). CSF IL-10 levels were increased in 57% of the samples. CSF IL-18BPa level was also elevated in all of the samples. The levels of TGF-ß, MMP-9, MCP-1, free IL-18 and TNF-α were normal across all the CSF samples.

After one month of treatment, CSF IL-8, IL-1β, IP-10, IFN-γ and VEGF were still elevated in all the samples (100%). CSF levels of IL-10 were increased in 42% of the samples. Of note, CSF IL-18BPa levels were increased in all of the CSF samples of the patients with TBM. The levels of TGF-ß, MMP-9, MCP-1 and TNF-α were normal in all of the CSF samples. Interestingly, we also

observed that the CSF levels of the free IL-18 were decreased in 57.1% of the CSF samples one month following the initiation of ATT.

b) Serum cytokine levels: At baseline, there was an increase in serum levels of IP-10 in 91%, MCP-1 in 73%, IL-1β in 55% and IL-8 in 18% of the blood samples. The levels of serum TGF-ß, IFN-γ, IL-10, VEGF, TNF-α, free IL-18 and MMP-9 were normal in all of the blood samples. At one month, there was an increase in serum IP-10 in 100%, MCP-1 in 73%, IL-1β in 42%, IL-8 in 42%, MMP-9 in 8% and IFN-γ in 8% of the blood samples. The levels of serum TGF-ß, IL-10, VEGF, TNF-α, MMP-9 and free IL-18 were normal in all of the blood samples. The IL-18BPa levels were normal in all of the blood samples on admission and at one month of treatment.

The median serum IFN-γ at one month of treatment and on admission was -17.2 and -13.6 respectively (p=0.036). The median serum MCP-1 at one month of treatment was 156.2, which was increased compared to 81.7 on admission (p=0.017, Table 3). The median serum IL-8 at stage 1 on admission was 15.9, and the median increased to 23.2 at stages 2 and 3 (p=0.075). The median serum IL-18BPa at stage 1 was 91.6, and the value was elevated to 131.5 at stages 2 and 3. (p=0.075)

Table 3: Changes in cytokine and IL-18BPa levels at baseline (admission) and after 1 month treatment

Serum cytokines	**z**	***p* value**
IL-8 (1 month)--IL-8 (admission)	-1.260	0.21
Free IL-18 (1 month)—free IL-18 (admission)	-0.980	0.33
IL-1β (1 month)--IL-1β (admission)	-0.318	0.75
IP10 (1 month)—IP10 (admission)	-1.680	***0.093***
TGF-ß (1 month)— TGF-ß (admission)	-0.420	0.67
MMP-9 (1 month)— MMP-9 (admission)	-1.540	0.12
IFN-γ (1 month)— IFN-γ (admission)	-2.100	**0.036**
IL-10 (1 month)--IL-10 (admission)	-1.101	0.27
VEGF (1 month)-- VEGF (admission)	-0.420	0.67
MCP-1 (1 month)-- MCP-1 (admission)	-2.380	**0.017**
TNF-α (1 month)-- TNF-α (admission)	-1.289	0.20
IL-18BPa (1 month)— IL-18BPa (admission)	-1.260	0.21
CSF cytokines	**z**	***p* value**
IL-8 (1 month)--IL-8 (admission)	-0.169	0.87
Free IL-18 (1 month)-free IL-18 (admission)	-0.169	0.87
IL-1β (1 month)--IL-1β (admission)	-0.676	0.50
IP10 (1 month)—IP10 (admission)	-0.85	0.40
TGF-ß (1 month)— TGF-ß (admission)	-1.270	0.20
MMP-9 (1 month)— MMP-9 (admission)	-0.676	0.50
IFN-γ (1 month)— IFN-γ (admission)	-0.676	0.50
IL-10 (1 month)--IL-10 (admission)	-0.734	0.46
VEGF (1 month)-- VEGF (admission)	-1.363	0.17
MCP-1 (1 month)-- MCP-1 (admission)	-0.676	0.50
TNF-α (1 month)-- TNF-α (admission)	-0.105	0.92
IL-18BPa (1 month)— IL-18BPa (admission)	0.000	1.00

Footnotes: All pg/ml except MMP-9 in ng/ml

When a comparison was made between the early and the advanced stages on admission, the median CSF and serum cytokine levels on admission were not statistically significant. A comparison between good and poor outcome for all the CSF and serum cytokine levels on admission and at 1 month is illustrated in Tables 4a-4b.

No patient had any significant association between CSF and serum level of cytokines on admission as well as after one month of treatment with hydrocephalus, tuberculoma, leptomeningeal enhancement, infarct or vasculopathy (Tables 5a and 5b). In particular, there was no association between CSF and serum free IL-18 as well as IL-18BPa with the various neuroimaging findings.

Table 4a: Serum cytokine levels on admission and after 1 month in relation with mRS of TBM patients

Serum cytokines at baseline (on admission)	Range (Median)		z	*p* value
	Good outcome (mRS 0-2), n=3	Poor outcome (mRS 3-6), n=12		
IL-8	15.4-20.6 (18.0)	16.3-36.1 (21.0)	-1.850	***0.064***
Free IL-18	9.7-32.4 (21.1)	8.1-25.2 (8.3)	-0.204	0.84
IL-1β	6.5-7.4 (7.0)	7.1-8.2 (7.5)	-1.138	0.26
IP10	219.0-353.5 (286.3)	107.2-2142.1 (539.7)	-1.429	0.15
TGF-ß	18.5-22.3 (20.4)	18.5-37.2 (21.7)	-0.945	0.35
MMP-9	231.6-351.4 (291.5)	115.2-1204.6 (230.5)	-0.408	0.68
IFN-γ	-21.9 to -16.7 (-19.3)	-20.8 to 29.2	-1.640	0.10
IL-10	2.2-2.6 (2.4)	2.1-3.0 (2.4)	-0.413	0.68
VEGF	53.6-85.0 (69.3)	56.7-266.6 (130.0)	-0.718	0.47
MCP-1	52.8-122.0 (87.4)	49.0-138.3 (81.7)	0.000	1.00
TNF-α	1.2-1.5 (1.4)	1.2-2.1 (1.5)	-1.365	0.17
Serum cytokines after 1 month	**Range (Median)**		**z**	***p* value**
	Good outcome (mRS 0-2), n=3	**Poor outcome (mRS 3-6), n=12**		
IL-8	18.0-40.9 (29.5)	15.4-46.8 (24.6)	-0.185	0.85
Free IL-18	9.0-19.2 (14.1)	10.3-22.6 (11.9)	-1.387	0.17
IL-1β	7.1-8.2 (7.5)	6.5-8.2 (7.9)	-0.470	0.64
IP10	556.2-981.0 (768.6)	187.2-2542.9 (1308.4)	-0.277	0.78
TGF-ß	21.0-22.3 (21.7)	17.3-31.6 (23.0)	-0.187	0.85
MMP-9	95.9-186.3 (141.1)	67.9-1067.2 (148.5)	-0.832	0.68
IFN-γ	-25.0 to -19.8 (-22.4)	-21.9 to 22.9 (-13.6)	-1.757	***0.079***
IL-10	2.2-2.3 (2.3)	2.2-3.5 (2.9)	-0.747	0.46
VEGF	47.6-66.0 (56.8)	62.9-810.0 (119.8)	-2.134	**0.033**
MCP-1	135.0-209.4 (172.2)	57.1-213.0 (156.2)	-0.192	0.93
TNF-α	1.3-1.4 (1.4)	1.2-3.0 (1.5)	-0.653	0.51

Footnotes: All pg/ml except MMP-9 in ng/ml

Table 4b: CSF cytokine levels on admission and after 1 month in relation with mRS of TBM patients

CSF cytokines on admission	Range (Median)		z	*p* value
	Good outcome (mRS 0-2), n=3	Poor outcome (mRS 3-6), n=12		
IL-8	665.9-2162.5 (2047.1)	284.0-2802.4 (689.4)	-0.707	0.48
Free IL-18	9.7-32.4 (4.0)	8.1-25.2 (8.3)	0.000	1.00
IL-1β	10.2-22.0 (10.7)	9.3-1104.1 (10.1)	-0.892	0.37
IP10	7554.3-7994.1 (7631.3)	5858.8-7984.3 (7569.7)	-0.707	0.48
TGF-ß	24.9-42.9 (37.2)	21.0-37.2 (27.6)	-0.892	0.37
MMP-9	150.8-460.7 (328.2)	62.9-365.4 (278.9)	-0.707	0.48
IFN-γ	22.9-237.5 (200.0)	6.3-175.0 (40.2)	-1.414	0.16
IL-10	3.9-6.2 (200.0)	2.3-15.6 (7.4)	-1.414	0.16
VEGF	35.8-53.6 (41.6)	32.9-204.8 (75.2)	-0.178	0.86
MCP-1	639.1-3331.6 (1114.7)	716.7-2387.3 (926.7)	0.000	1.00
TNF-α	2.7-8.5 (5.8)	1.7-3.9 (3.0)	-1.414	0.16
CSF cytokines after 1 month	**Range (Median)**		**z**	***p* value**
	Good outcome (mRS 0-2), n=3	**Poor outcome (mRS 3-6), n=12**		
IL-8	1374.1-3403.9 (2097.5)	169.9-2233.3 (572.1)	-1.202	0.23
Free IL-18	2.2-4.4 (3.3)	2.1-4.3 (2.6)	-1.202	0.23
IL-1β	11.0-32.5 (21.1)	8.5-26.1 (9.2)	-1.297	0.20
IP10	856.2-7514.8 (7499.0)	4674.5-8021.7 (7442.4)	-0.647	0.52
TGF-ß	27.6-34.4 (28.9)	22.3-27.6 (23.6)	-1.310	0.19
MMP-9	201.2-909.5 (305.0)	115.2-688.8 (206.5)	-1.572	0.17
IFN-γ	117.7-258.3 (197.9)	4.2-139.6 (28.2)	-2.126	**0.033**
IL-10	11.4-27.0 (20.6)	2.9-11.7 (4.3)	-2.126	**0.033**
VEGF	38.7-158.9 (88.3)	32.9-211.9 (80.0)	-0.371	0.71
MCP-1	651-1351.3 (825.5)	712.9-2488.6 (1327.4)	-1.572	0.12
TNF-α	3.0-6.0 (4.5)	1.9-3.6 (2.25)	-1.392	0.16

Footnotes: All pg/ml except MMP-9 in ng/ml

DISCUSSION

M. tuberculosis infection of the brain parenchyma results in the formation of Rich foci. The Rich foci lead to bacterial invasion into the subarachnoid space.[7] This triggers the secretion of TNF-α and IL-1β.[7] TNF-α and IL-1β are responsible for the formation of tuberculoma in the brain.[7] Cytokines and chemokines are necessary for the stimulation of T cells and macrophages.[8] The deregulation of cytokines and chemokines play a key role in chronic inflammation resulting from TBM.[3]

TBM reportedly augments bacterial replication.[6,12] This results in an increase in CSF levels of IL-8, IL-10, IL-1β, IFN-γ and matrix metalloproteinases.[6,12] On admission and at one month of treatment, CSF cytokine for IL-8, IL-1β, IFN-γ and IL-10 were elevated in the patients with

Table 5a: Correlation of serum cytokine levels on admission with MRI/CT scan of brain findings

MRI/CT	IL-8	Free IL-18	IL-1β	IP-10	TGF-ß	MMP-9	IFN-γ	IL-10	VEGF	MCP-1	TNF-α	IL-18-BPa
Hydro (yes/ no)	21.5±4.4/ 23.4±11.1	16.8±11.3/ 12.9±6.8	7.6±0.8/ 7.8±0.4	539.8±303.5/ 820.2±1146	22.1±6.3/ 22.8±4.6	513.9±351.3/ 157.7± 36.9	-10.2±14.5/ -0.7±25.9	2.8±0.6/ 2.3±0.2	114.9±78.8/ 106.6±47.8	121.1±113.5/ 104.4±30.6	1.5±0.2/ 1.6±0.2	122.2±24.0/ 132.4±59.4
Lepto enhan (yes/ no)	22.2±6.9/ 21.5±3.7	15.7±9.8/ 0	7.6±0.7/ 7.9±0.4	607± 634/ 657.9±471.6	23.0±6.0/ 19.2±0.9	405.7±345.0/ 466.5±430.3	-9.3±16.4/ 0.0±26.6	2.6±0.6/ 2.9±0.8	123.4±72.9/ 64.5±11.0	120.8±106.9/ 97.3±19.6	1.5±0.3/ 1.7±0.3	125.6±36.9/ 122.1±20.2
Tuber (yes/no)	22.7±6.7/ 20.3±5.9	16.7±11.4/ 14.3±9.4	7.7±0.8/ 7.6±0.3	685.1±670/ 432.8±284	21.2±3.1/ 25.2±10.4	358.3±246.8/ 572.7±555.3	-7.4±16.6/ -8.0±23.2	2.8±0.6/ 2.3±0.1	107.8±50.3/ 125.6±122.1	133.7±108/ 71±34.8	1.6±0.3/ 1.4±0.2	128.4±35.8/ 116±31.6
Infarct (yes/no)	22.2±6.4/ 21.1±8.1	15.3±10.7/ 11.9	7.5±0.5/ 8.5±0.8	669±637.3/ 379±237.1	22.9±6.1/ 19.8±1.8	412.8±356.3/ 434.4±356.8	-5.8±18.9/ -15.7±1.5	2.5±0.4/ 3.2±1.1	118±76.1/ 88.5±22.8	87.9±30.4/ 245.5±211.3	1.5±0.3/ 1.6±0.3	126.1±34.2/ 120.1±43.5
Vasculo-pathy (yes/no)	22.2±6.4/ 26.8 (1pt)	15.3±10.7 /0	7.5±0.5/ 9	669±637.3/ 546.6	22.9±6.1/ 21	412.8±356.3/ 686.7	-5.8±18.9/ -14.6	2.5±0.4/ 3.9	118±76.1/ 72.3	87.9±30.4/ 394.9	1.5±0.3/ 1.8	126.1±34.2/ 150.8

Hydro=hydrocephalus, lepto enhan-leptomeningeal enhancement, tuber=tuberculoma
Footnotes: All pg/ml except MMP-9 in ng/ml

Table 5b: Correlation of CSF cytokine levels on admission with MRI/CT scan of brain findings

MRI/CT	IL-8	Free IL-18	IL-1β	IP-10	TGF-ß	MMP-9	IFN-γ	IL-10	VEGF	MCP-1	TNF-α	IL-18-BPa
Hydro (yes /no)	1378.8±998.2/ 1223.3±1328.3	3.9±1.0/ 3.0±0.5	231.3±488/ 10±1	7238.3±824.5/ 7985.2±2.6	31±9/ 31.7±7.8	219.9±109.7/ 413.1±67.4	100.6±100.9/ 109.4±128.1	19.4±25.1/ 15.1±17.2	90.1±71.4/ 34.4±2.1	1657.6±1157.7/ 877.4±337	4.1±2.6/ 4.1±2.4	6304.5±4358.1/ 5590.5±5898.8
LeptoEnhan (yes /no)	1334.4±81.9/ 0	3.6±0.9/ 0	168.1±412.8/ 0	7451.7/ 765.6	31.2±8.0/ 0	275.1±132.9/ 0	103.1±7.7/ 0	18.1±21.8/ 0	74.2±64.3/ 0	1434.7±1028.3/ 0	4.1±2.4/ 0	6100.5±4310.8/ 0
Tuber (yes /no)	1336.7±918.1/ 1331.3±1276	3.1±0.6/ 4.4±0.9	10±0.6/ 378.8±628.2	7671.9±395.5/ 7158.3±1139	29.3±5.5/ 33.7±11.4	312.9±132.1/ 224.7±142	75.8±85/ 139.6±119.6	11.5±11.2/ 27±32	39.5±9.5/ 120.3±81.8	1456±1267.2/ 1406.2± 872.6	3.6±1.5/ 4.7±3.5	6120±4066.1/ 6073.8±5562.8
Infarct (yes /no)	1196.4±998.5/ 2162.5 (1 pt)	3.8±0.9/ 2.6	194.3±445.7/ 114.7	7361.3±796.7/ 7994.1	30.2±8.3/ 37.2	244.3±114.7/ 460.7	87±96.3/ 200	16.6±23.4/ 27.2	80.6±68/ 35.8	1567.3±1058.9/ 639.1	3.8±2.4/ 5.8	5490.4±4378.6/ 9761.5
Vasculopathy (yes /no)	22.2±6.4/ 0	3.8±0.9/ 0	7.5±0.5/ 0	669±637.3/ 0	22.9±6.1/ 0	412.8±356.3/ 0	-5.8±18.9/ 0	2.5±0.4/ 0	118±76.1/ 0	87.9±30.4/ 0	1.5±0.3/ 0	126.1±34.2/ 0

Hydro=hydrocephalus, lepto enhan-leptomeningeal enhancement, tuber=tuberculoma
Footnotes: All pg/ml except MMP-9 in ng/ml

TBM, in agreement with the existing literature.[6,12] IL-8 is a chemokine produced by diverse immune cells.[13] IL-8 functions as a chemoattractant for neutrophils and a subset of T lymphocytes.[13] IL-8 is mainly secreted when macrophages phagocytose bacterial pathogens, notably *M. tuberculosis.*[13] In addition, the CSF levels of VEGF both at baseline and at one month were raised, concurring with previous observations.[7,14,15]

To our knowledge, this is the very first study to have examined the levels of IL-18 in the patients with TBM. At one month of ATT, CSF free IL-18 was decreased in 57.1% of the TBM patients. The role of IL-18 in the pathogenesis of TBM is yet to be explored. Previous investigations suggest that IL-18 accelerates lymphocyte apoptosis and down-regulates IFN-γ, while allowing the production of Th2 cytokine IL-10.[17]

Notwithstanding that the levels of IFN-γ and IL-10 were higher, the increase of IL-18BPa is likely contribute to balanced levels of both these cytokines across the CNS. These observations suggest a role of IL-18BPa in the recovery of Th1/Th2 balance in the CNS compartment in TBM.[16] IL-18BPa is a protein inhibitor of IL-18 and a soluble decoy receptor for IL-18.[17] The IL-18BPa binds to IL-18 and prevents the binding of IL-18 to its cognate receptor.[17] Therefore, IL-18BPa negatively regulates the biological activity of IL-18.[17]

Circulating levels of cytokines present in the sera may not be reflective of the local immune responses at disease sites, although these would provide a milieu for activation of host cells.[8] Raised levels of cytokines and chemokines may be due to an overproduction of local mediators, which are released into the circulation from local sites.[8]

In this study, there was an increase in serum IL-1β and IL-8 in the patients with TBM on admission and at one month post initiation of ATT. The persistence of serum IL-1β may be due to continuous production of this cytokine at the area of inflammation.[18] Infection with *M. tuberculosis* results in the activation of caspase-1 and IL-1β secretion.[19] Potassium efflux and the lysosomal proteases cathepsin B and cathepsin L are required for *M. tuberculosis*-induced caspase-1 activation and IL-1β production.[19]

Furthermore, the level of CSF and serum TGF-ß was normal in all of the patients with TBM, which is suggestive of the TGF-ß being less likely to play a role in the TBM pathogenesis.

Interestingly, the levels of CSF and serum TNF-α on admission and on follow-up were normal. This could likely be attributed to non-expression of TNF-α by non-virulent strains of *M. tuberculosis.*[7] TBM may be associated with insufficient production of TNF-α accompanied by relative increase of soluble TNF-receptors, which may increase the duration of the illness.[6,20]

The median serum MCP-1 at one month of ATT was significantly increased compared to the levels at baseline. MCP-1 has been reported to play an important role in granuloma formation and protection against *M. tuberculosis* infection (8). A previous study has demonstrated an increase in MCP-1 in TBM.[21] Increased MCP-1 in TBM indicates higher gradients for monocyte recruitment present in the host circulation. These may be required to recruit monocytes to the meninges, and therefore could result in an increase in the systemic levels of MCP-1.[8]

IP-10 is a new immunologic marker for TB infection and is used for TB detection in serum and pleural fluid specimens.[22] In this study, there was an increase in serum IP-10 in the TBM patients on admission and at one month of ATT. To our knowledge, this is the first study on CSF IP-10 in TBM patients, and has provided a new finding of elevated IP-10 in the CSF of TBM patients.

None of our patients showed any significant association between CSF level of cytokines and neuro-radiological features of TBM. This could be attributed to the small sample size in our study. In the literature, hydrocephalus is associated with elevated CSF concentrations of IFN-γ and IL-10.[6,23,24] Furthermore, tuberculoma has been linked with raised concentrations of IL-10 and IL-1β.[8,17,18,24] Cerebral infarct is related to increased concentrations of IL-10 and IL-8.[6,7,13,23,24] In addition, there was no association between IL-18BPa and neuro-radiological findings.

VEGF has been associated with vasculopathy in other diseases and has been localized in the microvessels and perivascular cells in TBM.[7,25] In bacterial meningitis, TGF-β has been reported to contribute to cerebral vasculopathy.[7,26] However, our study suggests that VEGF and TGF-β may have a less likely role in TBM vasculopathy.

In our study, CSF IL-10 level at one month of treatment was associated with clinical outcome. However, previous studies showed that CSF IL-10 was not related to prognosis.[6,7,27] CSF IL-1β and TNF-α on admission or at one month in our study was not associated with patients' outcome, similar to previous studies.[6,7,27,28]

Interestingly, the clinical outcome of our TBM patients was not associated with the levels of CSF MMP-9. There have been contrasting

reports whether raised levels of CSF MMP-9 were associated with clinical outcome. High levels of CSF MMP-9 have been correlated with clinical outcome such as focal neurological deficit and mortality in several studies.[7,21,29-31] However, others showed that CSF MMP-9 level was not associated with clinical outcome.[3] Our study also demonstrated that CSF cytokine IL-1ß, IL-8, IL-10, TNF-α, IFN-γ and MMP-9 levels were not associated with the stage of TBM, consistent with previous studies.[6,28,30]

There are limitations in our study; the first and foremost is the small sample size and the absence of a control group. A larger longitudinal study that encompasses different time-points of measurements of the parameters studied herein is warranted to definitively confirm the results of the current study, especially to associate the role of serum TGF- ß and CSF TGF-ß with the pathogenesis of TBM.

Furthermore, the lack of association between neuro-radiological findings and cytokine levels both at baseline and one month post-ATT initiation could be attributed to the short time duration of the study given that TBM is a chronic inflammatory condition. Therefore, we also speculate that the dynamics of cytokine levels and neuro-radiological findings might change over time, potentially leading to significant correlations.

In conclusion, our study showed that the CSF cytokine levels were not related to severity of disease on admission, and brain changes observed on the MRI/CT scans. CSF IFN-γ and IL-10 levels at one month of treatment were associated with clinical outcome at 3 months. CSF cytokine levels on admission were not associated with outcome. Our results also suggest that increase in IL-18BPa is likely a contributing factor for the balanced levels of IFN-γ and IL-10 across the CNS. These observations suggest a role of IL-18BPa in the recovery of Th1/Th2 balance in the CNS compartment in TBM; although, this remains to be investigated with in-depth longitudinal studies.

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DISCLOSURE

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