Original Article

Cerebrospinal fluid cytokines and matrix metalloproteinases in human immunodeficiency seropositive and seronegative patients of tuberculous meningitis

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Abstract

Background: Some important clinical differences exist between human immunodeficiency virus (HIV)-seropositive and HIV-seronegative patients. Alterations in the cerebrospinal fluid (CSF) cytokines and matrix metalloproteinase have been noted in tuberculous meningitis. In HIV-infected patients, the immunopathogenesis is expected to be different. **Materials and Methods:** In this study, 64 patients of tuberculous meningitis (28 HIV seropositive and 36 seronegative) were included. The patients were followed up for six months. Cerebrospinal fluid (CSF) samples of tuberculous meningitis patients and 20 controls were subjected to tissue necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ, IL-10, matrix metalloproteinase (MMP)-2, and MMP-9 estimations. The levels were correlated with the patients' baseline clinical characteristics, CSF parameters, neuroimaging findings, and the outcome. The outcome was assessed and modified with the Barthel index. **Results:** The CSF cytokines and MMP levels were significantly elevated in tuberculous meningitis when compared with the controls. There was no significant difference seen between HIV seropositive and seronegative tuberculous meningitis, except for the IL-1β level, which was significantly lower in the HIV-infected patients. The cytokine and MMP levels did not correlate with the baseline clinical characteristics, disease severity, cerebrospinal fluid characteristics, neuroimaging findings, and outcome. **Conclusion:** In conclusion, HIV infection did not affect a majority of the CSF cytokines and MMP levels in tuberculous meningitis except for IL-1β level. None of the estimated inflammatory parameters correlated with the outcome.

Key Words

Cytokines, human immunodeficiency virus, matrix metalloproteinases, tuberculous meningitis

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Introduction

Tuberculous meningitis is a serious form of *Mycobacterium tuberculosis* infection; it is often associated with high mortality and morbidity.[1,2] Human Immunodeficiency Virus (HIV) infection is associated with an increased risk of disseminated forms of tuberculosis, including tuberculous meningitis.[3] In HIV-associated tuberculous meningitis, the clinical course and outcome are influenced by profound immunosuppression. The CSF parameters in HIV-infected patients may be different when compared with that of HIV-uninfected patients.[4,5]

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Tuberculous meningitis results from the rupture of subpial or subependymal tuberculous foci into the subarachnoid space. *Mycobacteria tuberculosis* interacts with the microglial cells (resident macrophages of the central nervous system) and produces a robust secretion of proinflammatory cytokines and chemokines.[6] Th-1 cells produce interferon (IFN)-γ, an activator of macrophages and monocytes, tissue necrosis factor (TNF)- α and interleukin (IL)-2. Th-2 cells produce IL-4, IL-5, IL-10, and IL-13. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, which are important in degrading the extracellular matrix macromolecules. All these inflammatory mediators play a role either directly or

indirectly (via recruitment of T-lymphocytes into the brain) in the host's defense against *Mycobacterium tuberculosis;* they could also contribute to the damage of the brain.[6-8] MMP-2 and MMP-9 degrade basement membrane proteins like type IV collagen and laminin and play a major role in the breakdown of the blood–brain barrier, as well as brain tissue damage.[9] MMPs have also been shown to play a prominent role in the pathogenesis of tuberculous meningitis.

Several studies, in the past, have demonstrated a rise in the levels of serum and CSF TNF-α and IFN-γ in HIV-negative patients with tuberculous meningitis.[10] The MMP levels were significantly higher in the CSF obtained from the tuberculous meningitis patients.[11] The raised CSF concentrations of protein, interferon γ, interleukin 10, and interleukin 6, have been linked to the pathogenesis of hydrocephalus in patients with tuberculous meningitis.[12] Dexamethasone decreased CSF MMP-9 concentrations early in treatment, and was thought to be responsible for the better outcome in tuberculous meningitis.[13] In this study, we evaluated the CSF cytokines and MMP levels of HIV-seropositive and seronegative patients. We also correlated the CSF cytokine and MMP levels with the clinical manifestations, severity of disease, and outcomes.

Materials and Methods

This prospective follow-up study was conducted from December 2010 to October 2012, in the King George Medical University, Lucknow, Uttar Pradesh, India. This university is a large Tertiary Care Medical Centre, which caters to more than 100 million people. Prior ethical approval for the study was taken from the Institutional Ethics Committee. An informed consent was taken from all the patients or their legal guardians.

We included newly diagnosed patients of tuberculous meningitis. The diagnosis of tuberculous meningitis was based on the consensus of the international criteria for tuberculous meningitis. These patients presented with subacute or chronic meningitis (headache, fever, and/or neck rigidity). The patients had characteristic CSF abnormalities (mononuclear cell pleocytosis, low glucose levels, elevated protein levels). The tuberculous meningitis was categorized into definite, probable, and possible cases based on clinical features, CSF findings, neuroimaging characteristics, evidence of tuberculosis elsewhere, and exclusion of alternative diagnosis.[14] Patients, who were detected positive for HIV 1 or 2, by two different kits, were defined as HIV-infected.[15] Patients with cryptococcal meningitis and those who were already taking antituberculosis treatment, were excluded from the study. Cryptococcal meningitis was diagnosed when CSF India ink staining, antigen testing or culture was positive. CSF for the control group was obtained from the patients who were subjected to spinal anesthesia. These patients of the control group did not have any neurological or systemic diseases. Prior informed consent was obtained from the control subjects as well from patients of tuberculous meningitis.

All patients were subjected to a detailed clinical evaluation. The severity of tuberculous meningitis was assessed by the modified British Medical Research Council (BMRC) staging system (stage I: Alert and oriented without focal neurological deficits; stage II: Glasgow coma score of 14–11 or 15 with focal neurological deficits; stage III: Glasgow coma score of 10 or less, with or without focal neurological deficits).[16,17] Disability assessment was done as per the modified Barthel index (MBI), which included the degree of dependence for bowel and bladder, grooming, toilet use, transfer, mobility, dressing, feeding, use of stairs, and bathing. For each activity, a score of 0 indicated complete dependence and a score of 2 or 3 indicated that the patient could do that particular activity independently.[18]

Patients were also subjected to a complete hemogram, routine biochemistry, renal and liver function tests, and a chest radiograph. The CSF examination included routine microscopy for acid-fast bacilli staining, tuberculosis polymerase chain reaction, and mycobacterial culture. Drug sensitivity testing was also done. CD4 + T cell count was performed in all HIV-seropositive patients. Gadolinium-enhanced brain magnetic resonance imaging (MRI) was performed with a Signa Excite 1.5 Tesla instrument (General Electric Medical Systems, Milwaukee, WI, USA). The neuroimaging features recorded were the abnormal enhancement of leptomeninges, tuberculomas, hydrocephalus (communicating or non-communicating), and infarcts.

For estimation of cytokines and MMPs, 3 ml of the CSF sample, from both cases and controls, was collected in a sterile container and was centrifuged at 1000 rpm for 10 minutes and the supernatant was stored at -80°C. Later on these samples were analyzed for quantitative expressions of IL-1β, IL-10, TNF-α, IFN-γ (Legend Max Human ELISA kits, California, US) and MMP-2 and MMP-9 (Abcam Human ELISA kit, United Kingdom) by sandwich enzyme linked immunosorbent assay (ELISA) pre-coated with a capture antibody. The assays were performed according to the manufacturer's instructions. Absorbance reading at 450 nm was obtained by a Bio-Rad Microplate Reader. Optic density reading was analyzed by a computer-based curve fitting software using a 5- or 4-parameter logistic curve fitting algorithm. All estimations were done in duplicate.

The patients received standard antituberculosis treatment, in accordance with the World Health Organization treatment guidelines.[19] The patients received two months of daily oral isoniazid (5 mg/kg of body weight; maximum, 300 mg), rifampicin (10 mg/kg; maximum, 600 mg), pyrazinamide (25 mg/kg; maximum, 2 g/day), and intramuscular streptomycin (20 mg/kg; maximum 1gm/day) followed by seven to nine months of isoniazid and rifampicin. Supplemental pyridoxine was given (25 – 50 mg/day). The patients also received dexamethasone for eight weeks. In addition, the patients received intravenous dexamethasone for four weeks (0.4 mg/kg body weight per day, which was then tapered off decreasing 0.1 mg/kg every week) and then oral treatment for four weeks, starting at a total of 4 mg per day and decreasing by 1 mg each week. All patients also received symptomatic treatment for fever, raised intracranial pressure, and seizures. For the HIV-seropositive group, antiretroviral therapy was started as early as antituberculosis drugs were tolerated (not later than eight weeks).[20]

The patients were followed up at one, three, and six months. Reconstitution inflammatory syndrome was considered if, new, recurrent or worsening clinical features of tuberculous meningitis were noted within three months of antiretroviral therapy. Cytokine and matrix metalloproteinase levels were correlated with the clinical features, radiological findings, laboratory parameters, and the outcome. The outcome was assessed at six months on the basis of the modified Barthel index score as, improved (MBI = 20), partially improved (MBI = 12-19), disabled (MBI < 12), and death.

Statistical analysis

The data was analyzed using the statistical software package, SPSS 16 and Microsoft Excel. A difference between two values was considered to be significant only if the *p* value was found to be <0.05. The two-sample t-test was used to see the difference between the mean of two different groups, if the data was normally distributed. If data was not found to be normally distributed, a non-parametric equivalent of the two-sample t-test, the two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to test the level of significance between two values in the quantitative data. However, the chi- square test was used to look at the same in qualitative data. The Kruskal-Wallis test and *post hoc* analysis was performed to see the differences in cytokine and matrix metalloproteinase expressions in HIV-seropositive, HIV-seronegative, and control groups, and in other places where three or more groups had to be analyzed (stage of tuberculous meningitis and outcome). Binary logistic regression analysis was performed to assess the predictors for mortality and disability.

Results

In this study, we enrolled 64 patients of tuberculous meningitis (HIV seronegative 36, and seropositive 28) [Figure 1]. All HIV-positive patients were infected with HIV-1 virus. Details of the comparative baseline and follow-up characteristics of the two groups have been provided in Table 1. The HIV-seropositive group had a significantly higher median age, more number of male patients, and a higher incidence of past tuberculosis (like pulmonary) as compared to the HIV-seronegative group [Table 1].

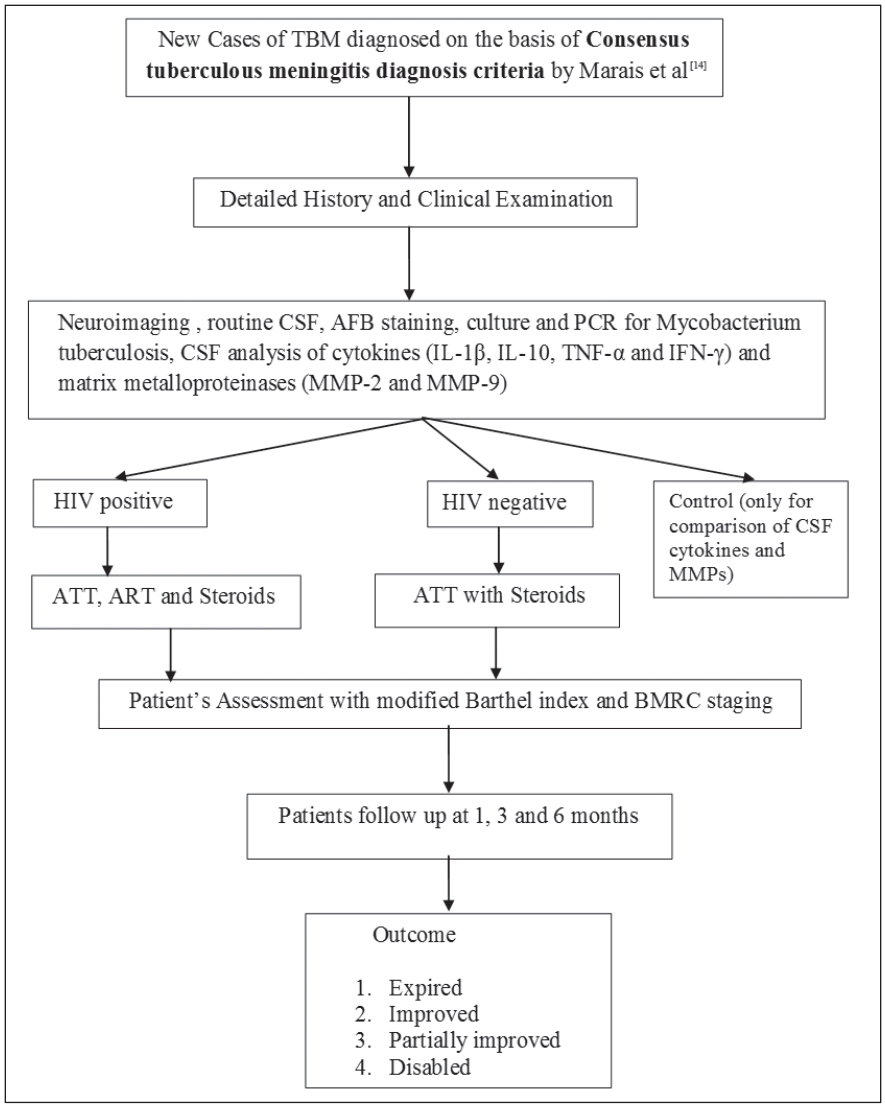

Figure 1: Flow diagram of the study

Table 1: Baseline and follow-up characteristics of the patients of tuberculous meningitis

Baseline characteristics	Total (*n* = 64)	HIV negative (*n* = 36)	HIV positive (*n* = 28)	*P* value HIV-negative versus HIV-positive
Age [median(min–max)] (in years)	31 (17–60)	25 (17–60)	35.5 (21–52)	< 0.001
Gender 1. Male	45 (70.3%)	19 (52.8%)	26 (92.9%)	< 0.001
2. Female	19 (29.7%)	17 (47.2%)	2 (7.1%)	
Duration of illness (in days) [median (min–max)]	90 (10–730)	90 (10–365)	90 (10–730)	0.90
Altered sensorium	36 (56.2%)	17 (47.2%)	19(67.8%)	0.09
Focal neurological deficit	11 (17.2%)	4 (11.1%)	7 (25.0%)	0.14
Seizures	16 (25.0%)	9 (25.0%)	7 (25.0%)	1.0
Vision impairment	5 (7.8%)	5 (13.9%)	0	< 0.001
Past history of TB	15 (23.4%)	5 (13.9%)	10 (35.7%)	0.04
Meningeal signs	52 (81.2%)	28 (77.8%)	24 (85.7%)	0.42
GCS [median, (min–max)]	14 (3–15)	14 (3–15)	13 (4–15)	0.16
Stage of TBM				
Stage 1	14 (21.9%)	9 (25%)	5 (17.9%)	0.46
Stage 2	30 (46.9%)	18 (50%)	12 (42.9%)	
Stage 3	20 (31.2%)	9 (25%)	11 (39.3%)	
Papilledema	20 (31.2%)	12 (33.3%)	8 (28.6%)	0.68
Optic atrophy	1 (1.6%)	1 (2.8%)	0 (0%)	0.37
Oculomotor palsy	3 (4.7%)	2 (5.6%)	1 (3.6%)	0.71
Abducens palsy	13 (20.3%)	11 (30.6%)	2 (7.1%)	0.02
Facial palsy	6 (9.4%)	3 (8.3%)	3 (10.7%)	0.74
Overall cranial nerve involvement	19 (29.7%)	14 (38.9%)	5 (17.9%)	0.04
Hemiparesis	9 (14.1%)	4 (11.1%)	5 (17.9%)	0.44
Paraparesis	2 (3.1%)	1 (2.8%)	1 (3.6%)	0.85
Quadriparesis	2 (3.1%)	1 (2.8%)	1 (3.6%)	0.85
Hemoglobin g/dl (mean±SD)	11.0±2.2	11.5±1.8	10.5±2.5	0.06
Total leukocyte count [median (min–max)] (/μl)	6975 (1700–19200)	7400 (3730–19200)	5900 (1700–16100)	0.05
Blood sugar [median (min–max)] (mg/dl)	106 (78–188)	111.5 (78–188)	102.5 (80–174)	0.13
Serum sodium [median (min–max)] (meq/L)	135 (119–145)	135 (119–145)	134 (120–144)	0.35
Blood urea [median (min–max)] (mg/dl)	27.5 (18–166)	26 (18–47)	28.8 (18–166)	0.07
Serum creatinine [median (min–max)] (mg/dl)	0.7 (0.4–3.5)	0.6 (0.4–1.3)	0.9 (0.4–3.5)	0.004
Abnormal LFTs	7 (10.9%)	3 (8.3%)	4 (14.3%)	0.45
Abnormal chest X-ray	22 (34.4%)	12 (33.3%)	10 (35.7%)	0.84
CSF total cells [median (min–max)]	135 (5–1380)	140 (30–1020)	126.5 (5–1380)	0.82
CSF Cell groups (0–50, 51–100, >100)	12 (18.8%)	5 (13.9%)	7 (25%)	0.48
	14 (21.9%)	9 (25%)	5 (17.9%)	
	38 (59.4%)	22 (61.1%)	16 (57.1%)	
CSF Protein [median (min–max)]	223 (50–4846)	265 (73–4846)	196 (50–1460)	0.13
CSF Protein groups (0–100,101–250, >250)	10 (15.6%)	4 (11.1%)	6 (21.4%)	0.32
	25 (39.1%)	13 (36.1%)	12 (42.9%)	
	29 (45.3%)	19 (52.8%)	10 (35.7%)	
CSF Sugar [median (min–max)]	35.3 (3.4–96)	35.3 (5–96)	34 (3.4–94)	0.32
CSF/blood sugar ratio	0.29 (0.03–0.94)	0.32(0.03–0.94)	0.29(0.03–0.79)	0.51
CSF/blood sugar ratio groups (<0.5 and ≥0.5)	53 (82.8%)	30 (83.3%)	23 (82.1%)	0.90
	11 (17.2%)	6 (16.7%)	5 (17.9%)	
Positive AFB stain	15 (23.4%)	9 (25.0%)	6 (21.4%)	0.74
Positive PCR	41 (64.1%)	24 (66.7%)	17 (60.7%)	0.62
Positive Culture*	16 (25.0%)	9 (25.0%)	7 (25.0%)	1.0
Hydrocephalus	39 (60.9%)	25 (69.4%)	14 (50.0%)	0.11
Exudates	21 (21.1%)	7 (19.4%)	7 (25.0%)	0.59
Meningeal enhancement	30 (46.9%)	14 (38.9%)	16 (57.1%)	0.15
Infarct	12 (18.8%)	7 (19.4%)	5 (17.9%)	0.87
Tuberculoma	7 (10.9%)	2 (5.6%)	5 (17.9%)	0.12
Neuroimaging abnormality	48 (75.0%)	27 (75%)	21 (75.0%)	1.0
Definite	43 (67.2%)	26 (72.2%)	17 (60.7%)	0.33
Probable	8 (12.5%)	4 (11.1%)	4 (14.3%)	0.70

(*Continued*)

Table 1: (Continued)

Baseline characteristics	Total (*n* = 64)	HIV negative (*n* = 36)	HIV positive (*n* = 28)	*P* value HIV-negative versus HIV-positive
Possible	13 (20.3%)	6 (16.7%)	7 (25.0%)	0.41
MBI at enrollment [median (min – max)]	6 (0 – 20)	9 (0 – 20)	2.5 (0 – 20)	0.04
MBI at six months [median (min – max)]	18.7 (8 – 20)	20 (10 – 20)	18.7 (8 – 20)	0.01
Drug-induced hepatitis	4 (6.2%)	3 (8.3%)	1 (3.6%)	0.44
Outcome				
Improved	30 (46.9%)	23 (63.9%)	7 (25.0%)	0.04
Partially improved	7 (10.9%)	2 (5.6%)	5 (17.9%)	
Disabled	4 (6.2%)	2 (5.6%)	2 (7.1%)	
Death	23 (35.9%)	9 (25.0%)	14 (50.0%)	

HIV = Human immunodeficiency virus, TB = Tuberculosis, GCS = Glasgow coma scale, min = Minimum, max = Maximum, TBM = Tuberculous meningitis, LFTs = Liver function tests, CSF = Cerebrospinal fluid, AFB = Acid fast staining, PCR = Polymerase chain reaction, MBI = Modified Barthel index, *Drug-resistant strain was not isolated

Table 2: Cytokines and matrix metalloproteinases levels in the cerebrospinal fluid of HIV-positive and HIV-negative patients of tuberculous meningitis and controls

Parameters	Control (*n* = 20) median (range)	HIV negative (*n* = 34), median (range)	HIV positive (*n* = 28) median (range)	Kruskal Wallis test *P* value
TNF-α (pg/ml)	23.4 (10.6–34.3)	36.38 (.34–62.5)	29.5 (2.0–85.3)	< 0.001
IL-β (pg/ml)	6.6 (4.6–10.4)	12.1 (2.7–58.7)	8.3 (1.5–88.1)	<0.001
IFN-γ (pg/ml)	86.1 (67.9–106.1)	1388.8 (47.1–1843.2)	1386 (41.3–1652.2)	<0.001
IL-10 (pg/ml)	70.3 (55.0–87.3)	345.3 (5.8–599.0)	160.9 (41.4–745.2)	<0.001
MMP-2 (ng/ml)	60.5 (47.1–69.5)	77.3 (38.8–221.9)	83.2 (48.8–286.5)	0.001
MMP-9 (pg/ml)	466.8 (432.1–986.0)	4962.2 (200.0–5966.0)	3476.2 (491.0–5999.4)	<0.001

MMP = Matrix metalloproteinase, HIV = Human immunodeficiency virus, TNF = Tumor necrosis factor, IL = Interleukin, IFN = Interferon

Table 3: Comparison of cerebrospinal fluid cytokine and matrix metalloproteinase expressions within the groups (including controls) by post hoc analysis

Cytokines and MMPs	Control versus HIV-negative *P* value	Control versus HIV-positive *P* value	HIV-negative versus HIV positive, *P* value
TNF-α	<0.001	0.005	0.07
IL-1β	<0.001	0.036	0.04
IFN-γ	<0.001	<0.001	0.70
IL-10	<0.001	<0.001	0.10
MMP-2	0.034	<0.001	0.32
MMP-9	<0.001	<0.001	0.32

MMP = Matrix metalloproteinase, HIV = Human immunodeficiency virus
TNF = Tumor necrosis factor, IL = Interleukin, IFN = Interferon

Baseline cerebrospinal fluid cytokines and matrix metalloproteinases

The CSF cytokine and matrix metalloproteinase levels were significantly elevated in the tuberculous meningitis patients, of both HIV-positive and HIV-negative groups, as compared to the controls. However, the cytokine levels showed no significant difference between the HIV-seropositive and seronegative groups. Only IL-1β was significantly lower in the HIV-seropositive group [Tables 2 and 3].

High levels of IL-1β and MMP-2 were correlated with vision impairment, higher levels of TNF-α and IL-10, with acid-fast bacilli stain positivity. There was also a positive correlation of TNF-α expression with modified Barthel index at enrollment, IL-1β with CSF sugar levels, and IFN-γ and IL-10 with the number of cells in CSF [Table 4]. We did not find any significant correlation of CSF cytokine and matrix metalloproteinase levels with CD4 + T cell counts [Table 5].

Factors affecting mortality and disability of tuberculous meningitis at six months

After six months, 30 (46.9%) patients improved, seven (10.9%) had partially improved, four (6.2%) had disability, and 23 (35.9%) patients died. There was significantly higher mortality in the HIV seropositive group as compared to the HIV seronegative group ($P = 0.04$). None of HIV-infected patients experienced the reconstitution inflammatory syndrome.

On Univariate analysis, we observed that age, Glasgow coma scale, stage of tuberculous meningitis, facial palsy, cerebral infarction, and the modified Barthel index at inclusion, were significantly correlated with poor outcome [Table 6]. On multivariate analysis, the modified Barthel index at inclusion was a factor that was significantly associated with the outcome ($P = 0.008$ and 0.02) and disability ($P = 0.018$ and 0.02). The comparison of survival between the two groups by log-rank test showed a significant difference between the HIV-seronegative and the HIV-seropositive groups ($P = 0.010$) [Figure 2]. None of the cytokine levels measured by us correlated with the outcome.

Discussion

In this study we could not observe any significant difference in most of the clinical, neuroimaging, CSF, and follow-up parameters of HIV-infected and HIV-negative patients of

Table 4: Correlation of CSF cytokine and matrix metalloproteinase with patients' clinical features, CSF findings, neuroradiological findings, and the outcome (included both HIV-positive and HIV-negative groups *n* = 62)

Clinical features	TNF-α	IL-1β	IFN-γ	IL-10	MMP-2	MMP-9
	P value					
Altered sensorium	0.73	0.41	0.44	0.49	0.58	0.46
Seizures	0.34	0.47	0.71	0.14	0.43	0.38
Focal neurologic deficit	0.74	0.15	0.82	0.32	0.80	0.69
Glasgow coma scale	0.18	0.61	0.27	0.35	0.61	0.72
Stage of TBM	0.71	0.50	0.46	0.89	0.75	0.27
Vision impairment	0.92	0.003	0.88	0.75	0.03	0.06
Cranial nerve palsy	0.59	0.50	0.26	0.76	0.51	0.39
CSF findings						
CSF total cells	0.86	0.51	0.32	0.50	0.48	0.75
CSF proteins	0.16	0.69	0.003	0.01	0.61	0.59
CSF sugar	0.80	0.01	0.80	047	0.57	0.34
AFB staining	0.002	0.16	0.97	0.001	0.98	0.69
TB PCR	0.18	0.35	0.64	0.48	0.23	0.13
TB culture	0.66	0.57	0.93	0.05	0.34	0.79
Definite TBM	0.61	0.30	0.52	0.71	0.49	0.08
Probable TBM	0.74	0.69	0.31	0.93	0.77	0.41
Possible TBM	0.75	0.37	0.93	0.61	0.58	0.17
Neuroradiological findings						
Hydrocephalus	0.83	0.97	0.72	0.24	0.11	0.86
Exudates	0.05	0.05	0.49	0.39	0.24	0.92
Infarct	0.67	0.42	0.08	0.35	0.51	0.85
Tuberculoma	0.63	0.08	0.64	0.87	0.06	0.80
Disability (disabled/improved)	0.85	0.10	0.16	0.71	0.76	0.50
Outcome(died/alive)	0.77	0.45	0.19	0.93	0.94	0.82

MMP = Matrix metalloproteinase, TNF = Tumor necrosis factor, IL = Interleukin, IFN = Interferon, TBM = Tuberculous meningitis, CSF = Cerebrospinal fluid, AFB = Acid fast staining, TB = Tuberculosis, PCR = Polymerase chain reaction

Table 5: Correlation of cerebrospinal fluid cytokines and matrix metalloproteinases with CD4 + T cell counts in the HIV-seropositive group

Cytokines and MMPs	Nonparametric correlation with CD4 count	
	Kendall's tau b correlation (*P* value)	Spearman's rank correlation (*P* value)
TNF-α	0.846	0.771
IL-1β	0.127	0.124
IFN-γ	0.158	0.145
IL-10	0.417	0.441
MMP-2	0.922	0.973
MMP-9	0.506	0.527

MMP = Matrix metalloproteinase, HIV = Human immunodeficiency virus TNF = Tumor necrosis factor, IL = Interleukin, IFN = Interferon

tuberculous meningitis. Our observations were consistent with the findings of many other studies done in the past.[4,21-24] Some differences in these two groups have also been described from time to time. For example, Dubé and co-workers noted a higher incidence of intracerebral tuberculoma in HIV-infected patients. A South African study noted a more frequent hydrocephalus and infarcts in HIV-positive tuberculous meningitis.[23] In children, a significantly higher number of HIV-infected patients had pulmonary tuberculosis. Obstructive hydrocephalus and basal enhancement were significantly less frequent in the HIV-infected group.[24] Peculiarities in CSF characteristics in HIV-infected patients of tuberculous meningitis included neutrophilic predominance, a higher rate of smear and culture positivity, and drug resistance.[5] Another study observed that HIV-infected patients had a higher frequency of absence of pleocytosis and multidrug-resistant tuberculous meningitis. CSF protein levels were also significantly lower in HIV-infected patients.[25] We did not observe any such differences in the CSF parameters.

In accordance with the observations of the previous studies, in our study, CSF cytokines (IL-1β, TNF-α, IFN-γ, and IL-10) and MMP-2 and MMP-9 levels were found to be elevated in tuberculous meningitis.[13,26-28] We could not observe any

Table 6: Univariate analysis of factors affecting mortality and disability (MBI < 12) of tuberculous meningitis (included both HIV-positive and HIV-negative groups; *n* = 62)

Factors	Mortality		Disability	
	P value	95% Confidence interval	*P* value	95% Confidence interval
Age	0.007	0.000 – 0.046	0.049	0.003 – 0.122
Altered sensorium	<0.001	0.003 – 0.217	<0.001	0.027 – 0.331
GCS	<0.001	0.000 – 0.046	<0.001	0.000 – 0.046
Stage of TBM*	<0.001		<0.001	
Facial palsy	0.008	0.009 – 0.764	0.03	0.013 – 1.116
Focal neurological deficit	0.025	0.058 – 0.884	<0.001	0.006 – 0.399
Infarct	0.046	0.079 – 1.057	0.048	0.076 – 1.084
MBI	<0.001	0.000 – 0.046	<0.001	0.000 – 0.046

*no common odds ratio estimate and confidence interval computed for stage of TBM as it contained more than two distinct categories (stage 1, stage 2, and stage 3), GCS = Glasgow coma scale, TBM = Tuberculous meningitis, MBI = Modified Barthel index

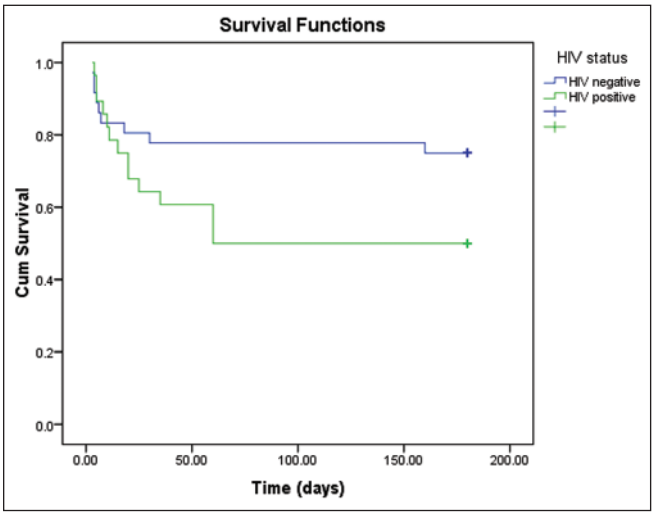

Figure 2: Kaplan Meier survival analysis in HIV-infected and HIV non-infected patients of tuberculous meningitis

difference in the cytokine levels of HIV-negative and HIV-positive patients, except IL-1β. Patel and co-workers too did not find any difference in the levels of these cytokines in CSF of HIV-seropositive and seronegative patients. Our HIV-infected patients had significantly lower levels of IL-1β. The cytokine IL-1ß is a key mediator of inflammation and plays an important role in the host's resistance to *M. tuberculosis* infections. IL-1β participates in a variety of anti-inflammatory activities, like, cell proliferation, differentiation, and apoptosis. Interaction between *M. tuberculosis* and the cells of immune system results in the secretion of several chemokines and cytokines, the most important being TNF-α, IL-1 (IL-1β, IL-18), IL-12, and IFN-γ. Infection of IL-1-deficient mice with *M. tuberculosis* is associated with lower production of other cytokines, defective granuloma formation, and lower survival.[29]

There are conflicting reports about the relation of CSF cytokines and disease severity and the outcome. Although, we noted a low CSF IL-1β level in HIV-infected patients, none of the parameters we measured, was correlated with the outcome. Similar to our findings, Misra and co-workers noted that the release of cytokines in CSF was not related to disease severity, MRI changes or patients' clinical outcome.[27] On the contrary, in some other studies a positive correlation between CSF cytokines and disease outcome had been demonstrated. Mustafa and co-workers observed that TNF-α and IL-1β were associated with disease progression and death in patients with bacterial meningitis.[30] Babu and co-workers found a significant positive correlation between TNF-α and IFN-γ with the severity of tuberculous meningitis.[10] Simmons and colleagues observed that CSF IL-6 levels were independently associated with severity of the disease. Elevated CSF inflammatory cytokines were not associated with death or disability in HIV-negative tuberculous meningitis patients. They also observed that HIV infection attenuated multiple CSF inflammatory parameters and low CSF IFN-γ levels were associated with death in HIV-positive tuberculous meningitis, suggesting that IFN-γ was crucial for conferring immunity and survival of the host.[31]

One weakness of our study is the lack of a control group of HIV + patients (without tuberculous meningitis), which may have helped to dissect out the contribution of HIV infection to the alterations in inflammatory markers. However, this is the first Indian study, which compares two groups of tuberculous meningitis.

In conclusion, HIV infection did not affect a majority of CSF cytokine and MMP levels in tuberculous meningitis, except for the IL-1β level. None of inflammatory parameters was correlated to disease severity or the outcome.

References

1. Garg RK, Sinha MK. Tuberculous meningitis in patients infected with human immunodeficiency virus. J Neurol 2011;258:3-13.
2. Hosoglu S, Geyik MF, Balik I, Aygen B, Erol S, Aygencel TG, *et al*. Predictors of outcome in patients with tuberculous meningitis. Int J Tuberc Lung Dis 2002;6:64-70.
3. Marais S, Pepper DJ, Marais BJ, Török ME. HIV-associated tuberculous meningitis - diagnostic and therapeutic challenges. Tuberculosis (Edinb) 2010;90:367-74.
4. Thwaites GE, Duc Bang N, Huy Dung N, Thi Quy H, Thi Tuong Oanh D, Thi Cam Thoa N, *et al*. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with tuberculous meningitis. J Infect Dis 2005;192:2134-41.
5. Torok ME, Chau TT, Mai PP, Phong ND, Dung NT, Chuong LV, *et al*. Clinical and microbiological features of HIV-associated tuberculous meningitis in Vietnamese adults. PLoS One 2008;3:e1772.
6. Rock RB, Hu S, Gekker G, Sheng WS, May B, Kapur V, *et al*. Mycobacterium tuberculosis-induced cytokine and chemokine expression by human microglia and astrocytes: Effects of dexamethasone. J Infect Dis 2005;192:2054-8.
7. Birkedal-Hansen H. Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol 1995;7:728-35.
8. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodelling. Trends Genet 1990;6:121-5.
9. Baramova E, Foidart JM. Matrix metalloproteinase family. Cell Biol Int 1995;19:239-42.
10. Nagesh Babu G, Kumar A, Kalita J, Misra UK. Proinflammatory cytokine levels in the serum and cerebrospinal fluid of tuberculous meningitis patients. Neurosci Lett 2008;436:48-51.
11. Lee KY, Kim EH, Yang WS, Ryu H, Cho SN, Lee BI, *et al*. Persistent increase of matrix metalloproteinases in cerebrospinal fluid of tuberculous meningitis. J Neurol Sci 2004;220:73-8.
12. Thwaites GE, Macmullen-Price J, Tran TH, Pham PM, Nguyen TD, Simmons CP, *et al*. Serial MRI to determine the effect of dexamethasone on the cerebral pathology of tuberculous meningitis: An observational study. Lancet Neurol 2007;6:230-6.
13. Green JA, Tran CT, Farrar JJ, Nguyen MT, Nguyen PH, Dinh SX, *et al*. Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. PLoS One 2009;4:e7277.
14. Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, *et al*. Tuberculous meningitis: A uniform case definition for use in clinical research. Lancet Infect Dis 2010;10: 803-12.
15. National AIDS Control organisation. Guidelines on HIV testing. March 2007. Ministry of Health and Family Welfare, Government of India. Available from: http:// www.ilo.org/wcmsp5/groups/public/—ed_protect/—protrav/—ilo_aids/documents/legaldocument/wcms_117314.pdf. [Last accessed on 2013 Oct 1].
16. Thwaites GE, Tran TH. Tuberculous meningitis: Many questions, too few answers. Lancet Neurol 2005;4:160-70.
17. Thwaites GE, Nguyen DB, Nguyen HD, Hoang TQ, Do TT, Nguyen TC, *et al*. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. N Engl J Med 2004;351:1741-51.
18. McDowell I, Newell C. Measuring Health - A Guide to Rating Scales and Questionnaires, 2nd ed. Oxford: Oxford University Press; 1996. p. 56-63.
19. World Health Organization. Treatment of tuberculosis: Guidelines, 4th ed. (WHO/HTM/TB/2009.420) World Health Organization. Available from: http:// whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf. [Last accessed on 2012 Nov 30].
20. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. 2010 revision. Available from: http:// whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. [Last accessed on 2013 Oct 1].
21. Berenguer J, Moreno S, Laguna F, Vicente T, Adrados M, Ortega A, *et al*. Tuberculous meningitis in patients infected with the human immunodeficiency virus. N Engl J Med 1992;326:668-72.
22. Yechoor VK, Shandera WX, Rodriguez P, Cate TR. Tuberculous meningitis among adults with and without HIV infection. Experience in an urban public hospital. Arch Intern Med 1996;156:1710-6.
23. Schutte CM. Clinical, cerebrospinal fluid and pathological findings and outcomes in HIV-positive and HIV-negative patients with tuberculous meningitis. Infection 2001;29:213-7.

24. van der Weert EM, Hartgers NM, Schaaf HS, Eley BS, Pitcher RD, Wieselthaler NA, *et al*. Comparison of diagnostic criteria of tuberculous meningitis in human immunodeficiency virus-infected and uninfected children. Pediatr Infect Dis J 2006;25:65-9.
25. Cecchini D, Ambrosioni J, Brezzo C, Corti M, Rybko A, Perez M, *et al*. Tuberculous meningitis in HIV-infected and non-infected patients: Comparison of cerebrospinal fluid findings. Int J Tuberc Lung Dis 2009;13:269-71.
26. Misra UK, Kalita J, Srivastava R, Nair PP, Mishra MK, Basu A. A study of cytokines in tuberculous meningitis: Clinical and MRI correlation. Neurosci Lett 2010;483:6-10.
27. Mastroianni CM, Paoletti F, Lichtner M, D'Agostino C, Vullo V, Delia S. Cerebrospinal fluid cytokines in patients with tuberculous meningitis. Clin Immunol Immunopathol 1997;84:171-6.
28. Patel VB, Bhigjee AI, Bill PL, Connolly CA. Cytokine profiles in HIV seropositive patients with tuberculous meningitis. J Neurol Neurosurg Psychiatry 2002;73:598-9.
29. Juffermans NP, Florquin S, Camoglio L, Verbon A, Kolk AH, Speelman P, *et al*. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. J Infect Dis 2000;182:902-8.
30. Mustafa MM, Lebel MH, Ramilo O, Olsen KD, Reisch JS, Beutler B, *et al*. Correlation of interleukin-1 beta and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. J Pediatr 1989;115:208-13.
31. Simmons CP, Thwaites GE, Quyen NT, Torok E, Hoang DM, Chau TT, *et al*. Pretreatment intracerebral and peripheral blood immune responses in Vietnamese adults with tuberculous meningitis: Diagnostic value and relationship to disease severity and outcome. J Immunol 2006;176:2007-14.

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