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Do cytokines correlate with disease activity in tuberculous meningitis?

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Abstract

Background: Tuberculous meningitis (TBM) remains to be one of the most fatal central nervous system infections. The exact pathogenesis of TBM at cellular level remains unclear. In this study, we assessed the cytokine levels in the serum and cerebrospinal fluid (CSF) of TBM patients and determined their correlation with the disease activity. *Methods:* The levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured by enzyme linked immunosorbant assay (ELISA) in both serum and CSF of 38 patients at baseline, and in 17 of these patients at 1 and 6 month of follow-up. Clinical examination and imaging was performed at baseline and on follow-ups. *Results:* There was a remarkable rise in the levels of serum and CSF TNF-α and IL-1β in TBM patients as compared to age and sex matched controls ($p<0.05$). A significant correlation was found between cytokine levels and stages of TBM ($p< 0.05$). TNF-α levels in both serum and CSF and IL-1β levels in serum were found to be significantly higher in those patients who died than those who survived and had better outcome. TNF-α was higher in patients who developed tuberculoma on follow-up than those who did not ($p<0.05$). The cytokine levels progressively declined over time but remained detectable till 6 months in most patients.
Conclusions: The higher levels of TNF-α and IL-1β were associated with poor outcome in TBM. The higher cytokine levels in patients developing tuberculoma on antituberculous therapy and steroids suggests that these patients may benefit from immunomodulation agents like anti-TNF-α antibody.

INTRODUCTION

Tuberculosis affects one third of world's population and is a leading cause of human mortality and morbidity.[1]The pathogenesis of tuberculous meningitis (TBM) at cellular level is poorly understood, however, there is increasing evidence for the significant role of cytokines, in particular, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the pathogenesis of TBM.[2-12] The role of immunomodulatory therapy has been documented in management of tuberculoma in few case reports[13-15], but later studies could not substantiate those findings.[16] Moreover, there are very limited studies on the kinetics of cytokines on antituberculous therapy during follow-up. In this prospective study, levels of TNF-α and IL1-β were measured and they were correlated with the disease activity. The aim of the study was to find out whether the cytokine levels correlate with the severity of the disease and predict outcomes.

METHODS

The patient population consisted of 38 consecutive patients with TBM, admitted in the Department of Neurology of Postgraduate Institute of Medical Education and Research, Chandigarh, India. All patients had a history and clinical findings compatible with the diagnosis of TBM. The diagnosis was made on the basis of the criteria given by Ahuja *et al*.[17] which includes clinical findings plus one or more of the following criteria: Cerebrospinal fluid (CSF) parameters (pleocytosis, elevated protein levels, low glucose levels, and raised adenosine deaminase (ADA) activity), cranial imaging showing hydrocephalus and basilar enhancement, or evidence of concomitant active extraneurological tuberculosis. All presumptive TBM patients had negative culture for bacterial and fungal agents and negative India ink staining. In one patient, the diagnosis was confirmed by isolation of *M. tuberculosis* from CSF. An informed consent was obtained from each patient/legally authorized representative before enrolment in the study. The study was approved by institutional ethics committee.

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A baseline CSF and serum sample was collected from all 38 patients. In 17 of them, samples were also collected after one and six month of antituberculous therapy. As controls, CSF and serum samples were collected from age and sex matched patients undergoing spinal anesthesia for noninfectious urological disorders. It was ensured that these patients do not have disorders that may alter cytokine levels.

The laboratory personnel were masked to the clinical diagnosis and group of the subjects, matching each sample by letter coding, and so was the clinician to subsequent levels until the end of the study. The collected blood was kept in a serum separator for 30 minutes. Serum from blood after clotting was separated out and collected in a clean tube and again centrifuged for 10 min at 3000 rpm. The serum thus obtained was stored at -80^{0}C and was used for cytokine (TNF-α, IL-1β) estimation by ELISA. The CSF was collected in the TBM patients as a routine diagnostic procedure by lumbar puncture. Ten ml CSF was collected after discarding the first 1ml. CSF specimens, upon collection, were examined for cell count, levels of glucose and protein, ADA, Gram stain, India ink, VDRL and bacterial and fungal culture. Rest of the samples was stored at -80^{0}C till analyzed for cytokine estimation by ELISA. The cytokines were analyzed using a standard kit provided by Gen Probe Life Sciences Limited, Diaclone, France. First of all 100µl of sample or diluted standard or control was added into wells. Thereafter, 50µl of diluted biotinylated antibodies were added to all wells. After this, it was incubated for 3 hours at room temperature. Then the wells were washed three times. After this 100µl of streptavidin – horse radish peroxidase (HRP) was added to all wells. The plates were then incubated further for 30 minutes at room temperature. The wells were then again washed three times. Subsequently 100µl of ready-to-use tetramethylbenzidine (TMB) was added to all wells and colour was allowed to develop for 12-15 minutes. After this, the reaction was stopped using 100µl H_2SO_4 and absorbance was read at 450nm filter and 620nm reference filter. An average of standard values was calculated and the values from blank wells were subtracted from it. From these values standards curves were then plotted and cytokines levels expressed as pg/ml.

Detailed history, clinical examination and a computed tomography and magnetic resonance imaging of brain was done at admission and at 1 and 6 months of follow up. Further imaging was done as and when required in case of clinical deterioration. All patients received four drugs (rifampicin, isoniazid, pyrazinamide, ethambutol) antituberculous therapy for first 2 months followed by 2 drugs antituberculous therapy (rifampicin, isoniazid) for 10 months along with steroids and patients underwent ventriculoperitoneal shunt,as and when indicated.[18] Outcome was defined as dead, persistent vegetative state, severe disability, moderate disability and good recovery using Glasgow Outcome Scale.[19]

Statistical analysis

The data obtained from the study was compiled and expressed as mean ± standard deviation for data having Gaussian distribution and as median (range) for data having non-Gaussian distribution. For all dichotomous variables Chi-Square test was applied. Mann-Whitney U test was applied to calculate the significance of difference between the mean value of these cytokines in control subjects and patients with TBM as data was skewed. For paired variables having normal distribution, paired t test was used and Kruskal-Wallis test was used for paired variables not normally distributed. A p value of <0.05 was taken as significant. Correlation between the continuous variable with skewed distribution was assessed using Spearman correlation co-efficient. All analyses were performed using SPSS version 10 software (SPSS, Chicago, Illinois).

RESULTS

The mean age of patient with TBM was 31.82 ± 13.19 years (range, 13-70 years). Fever and headache were seen in 37 (97.4%) patients. The triad of fever, headache and vomiting was seen in 28(73.7%) patients. Six patients were classified in Stage 1 (fully conscious without any focal neurological signs); 18 in Stage 2 (depressed level of consciousness and hemiparesis or a single cranial nerve palsy); and 14 in Stage 3 (patients who were comatose or complete hemiplegia or paraplegia).Seizure was observed in 11 (28.9%) patients. Ten patients had generalized seizures and one patient had partial seizure. Cranial nerve palsy was seen in 20 (52.6%) patients. Sixth nerve was the most commonly involved cranial nerve. Other cranial nerves involved were third, seventh, fourth and second cranial nerves in descending order. Steroid therapy was given to all patients. Antituberculous therapy induced hepatitis was observed in 4 (10.5%) patients. Hydrocephalus occurred as a complication in 16 (42.1%) patients. Infarcts were noted in 4 patients (3 in middle

Table 1: Clinical characteristics of tuberculous meningitis patients

Parameters		Frequency (percent)
Age		31.82±13.39*
Sex	Male	26 (66.7%)
	Female	12 (33.3%)
Duration of illness before presentation		58.58±45.29* days
Fever		37 (97.4%)
Headache		37 (97.4%)
Vomiting		28 (73.7%)
All Seizures types		11 (28.94)
Focal seizures		1 (2.63%)
Altered sensorium at presentation		29 (76.3%)
Glasgow coma scale score at presentation		13†
Pulmonary tuberculosis		6 (15.7%)
TB lymphadenitis/TB hip arthritis		3 (7.9%)/1 (2.6%)
Cranial nerve palsy		20 (52.6%)
Infarct		4 (10.5%)
Hemiparesis		7 (18.4%)
Hydrocephalus		16 (42.1%)
Hepatitis		4 (10.5%)
Death		9 (23.7%)

∗Mean±Standard deviation,† Mean

cerebral artery territory and one in the pons).

An extraneurological tuberculous localization was diagnosed in 8 (21%) patients before or during hospitalization. Six patients had pulmonary tuberculosis at initial evaluation. Three patients developed tubercular lymphadenitis and one patient developed right tubercular hip arthritis during follow-up. Ten (26.3%) patients with hydrocephalus underwent ventriculoperitoneal shunting and others were managed conservatively or they expired before any intervention. Nine patients (23.7%) died from progressive neurological deterioration either during first hospital admission or during follow-up (Table 1). Important characteristics of the cerebrospinal fluid are shown in Table 2.

Figure 1 shows the CSF and serum concentrations of TNF-α and IL-1β at presentation.

TNF-α and IL-1β levels were significantly higher in both serum and CSF of TBM patients as compared to controls (Table 3 and 4). No significant difference was observed in cytokine levels between patients with hydrocephalus or hemiparesis, and patients developing tubercular lymphadenitis on follow-up than those who did not. A significant difference was also observed in TNF-α and IL-1β levels in CSF between Stage 1 and 3 TBM ($p<0.001$) and Stage 2 and 3 TBM ($p<0.001$). TNF-α levels in both serum and CSF and IL-1β levels in serum were found significantly higher in those patients who died than those who survived and had better outcome (Table 5). At presentation, 8 patients had tuberculoma whereas, 4 patients developed tuberculoma during follow up. TNF-α levels in CSF and IL-1β levels in serum were significantly

Table 2: Characterics of cerebraspinal fluid

Parameters	Median Values	Range
TLC	130 Cells/cmm	0-1200
Protein	181.5 mg/ml	55-1200
Glucose	27mg/ml	10-63
ADA	12 IU/L	2-33

Table 3: TNF-α levels (pg/ml) at admission in the serum and CSF

	Cases (n=38) (Mean±S.D)	Controls (n=9) (Mean±S.D)	*P* value
CSF	169±96.84	17.11± 12.15	<0.001
Serum	80.95±36.00	12.22± 4.38	<0.001

Table 4: IL-1β levels (in pg/ml) at admission in the serum and CSF

	Cases (n=38)	Controls (n=9)	*P* value
CSF	110.82±54.22	15.22±8.64	<0.001
Serum	71.72±30.42	14.64±18.27	<0.001

Table 5: Relation of CSF and serum cytokine levels (pg/ml) with survival

	Non-survivors (n=9)	Survivors (n=29)	*P* value
CSF TNF-α	233 ±97.02	137.40± 73.26	0.004
CSF IL-1β	112.89 ±22.56	103.12 ±51.51	0.097
Serum TNF-α	107.11 ±35.63	70.24 ±32.99	0.014
Serum IL-1β	81.54 ±21.11	62.62± 13.50	0.007

higher in patients who developed tuberculoma on follow up than those who did not (Table 6). Cytokine levels progressively declined during follow-up both in serum and CSF, however remained elevated in most of the samples (Figure 2).

DISCUSSION

There was a remarkable increase ($P < 0.001$) in the CSF and serum levels of TNF-α and IL-1β in the serum and CSF of TBM patients as compared to the control values, which is similar to the findings of other studies.[3-11] Babu *et al.*, showed positive correlation between severity of the disease and TNF- αlevel in the CSF and serum.[8] However, Mastroianni *et al.* and Donald *et al.* did not demonstrate any correlation.[4,6] In the present study, a significant difference was

Table 6: CSF and serum cytokine levels (pg/ml) in patients developing tuberculoma on follow- up and those who did not develop tuberculoma

	Cytokine levels in patients developing Tuberculoma on follow-up (n=4)	Cytokine levels in patients not developing Tuberculoma on follow-up (n=8)	*P* value
CSF TNF-α at baseline	144±23.76	147.25±93.98	0.308
CSF TNF-α at 1 month	124.75±14.6	81.12±30.02	0.042
CSF TNF-α at 6 month	115.75±19.61	49.5±20.00	0.006
CSF IL-1β at baseline	87.75±8.01	86.0±29.62	0.733
CSF IL-1β at 1 month	88±10.67	66.25±22.72	0.089
CSF IL-1β at 6 month	33±10.98	21.25±9.52	0.103
Serum TNF-α at baseline	80±25.61	72.50±38.99	0.610
Serum TNF-α at 1 month	49±11.31	40.62±17.84	0.494
Serum TNF-α at 6 month	48.25±23.76	32.50±19.44	0.147
Serum IL-1β at baseline	72.32±35.14	64.63±10.15	0.552
Serum IL-1β at 1 month	6.25 ±5.31	9.87±9.87	0.603
Serum IL-1β at 6 month	13.0± 6.05	5.62±4.74	0.031

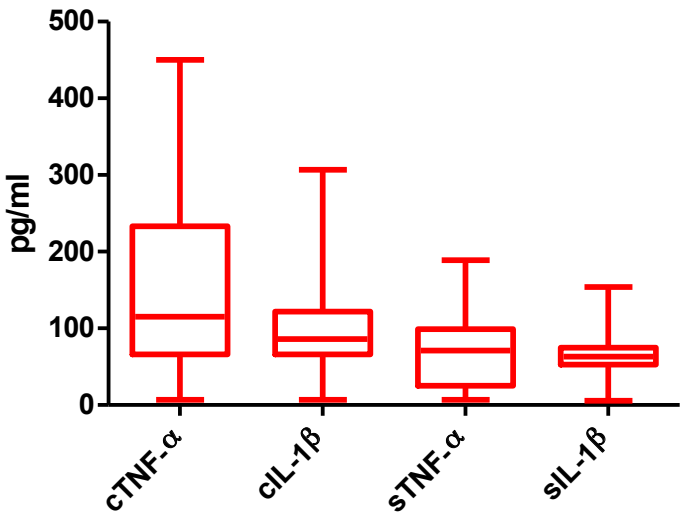

Figure 1: CSF and serum concentrations of TNF-α and IL-1β at presentation (p <0.05). The box plots show the range, 90th percentiles (bars), 75th and 25th percentiles (boxes), and median (bar in boxes).

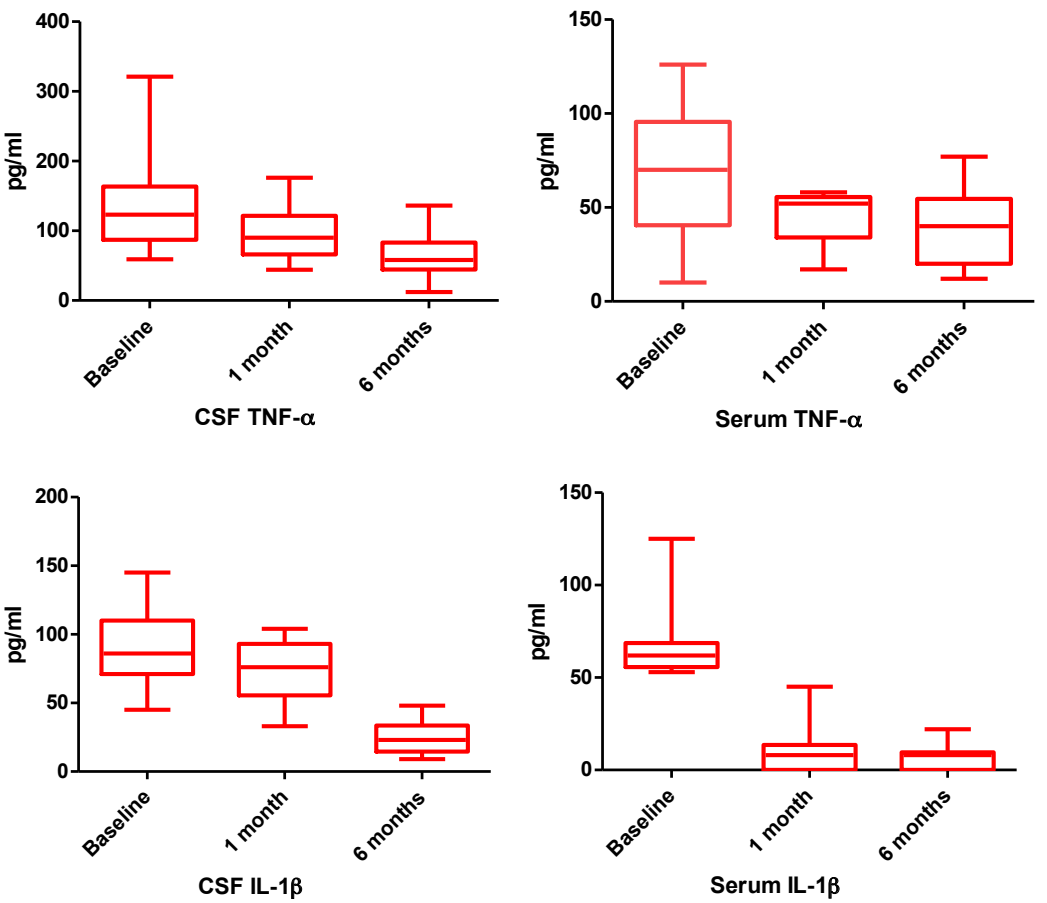

Figure 2: CSF and serum concentrations of TNF-α and IL-1β over time (P <0.05). The box plots show the range, 90th percentiles (bars), 75th and 25th percentiles (boxes), and median (bar in boxes).

observed in TNF-α and IL-1β levels in CSF between Stage 1 and 3 TBM (p<0.001) and Stage 2 and 3 TBM (p<0.001). Result of various studies is summarized in Table 8.

We also tried to find out whether cytokines affect the occurrence of neurological complications of TBM or not. The patients who died during the course of the disease had significantly higher levels of TNF-α in CSF and TNF-α and IL-1β in serum, however, no significant difference was detected between those developing hydrocephalus or infarcts and those not. These apparently contradictory findings may need to be examined in future studies with larger sample size.

In previous investigations, Donald *et al.* documented the presence of TNFα, IL-1β and IFN-γ in the CSF of children with complicated forms of TBM and their persistence throughout the first month of treatment. Similarly, in a study by Mastroianni *et al*., cytokines TNF-α in CSF remained elevated for remarkably long time of 16 months.[6] In our study, the cytokine levels progressively declined in patients having 6-month follow up, although they remained detectable in most of the patients. These results differ from those reported in pyogenic meningitis where the levels of proinflammatory cytokines (TNF-a, IL-6, and IL-8) and anti-inflammatory mediators (IL-10 and sTNFRs) are very high at the onset of the disease and decrease rapidly 6–24 hr after the initiation of treatment.[20]This suggests an entirely distinct immunologic process in acute pyogenic meningitis as compared to that in TBM.

We also evaluated intracytokine correlation in baseline values of cytokines in both CSF and serum (Table 7). A significant positive correlation was found between CSF TNF-α and CSF IL-1β (r =0.512, p = 0.001), serum TNF-α and CSF TNF-α (r = 0.41, p <0.05), CSF TNF-α and serum IL-1β (r = 0.457, p = 0.004) and similarly between serum TNF-α and serum IL-1β (r =0.392, p <0.05). This finding is similar to that obtained by Mastroianni *et al*.[5] and Babu *et al*.[8] This phenomenon indicates that these cytokines go hand in hand in the pathogenesis of TBM. Mastroianni *et al*.[6] also showed a positive correlation between ADA activity in CSF and CSF TNF-α but in our study, no such correlation was observed. We could not evaluate for any difference in cytokine levels between those patients who received steroid therapy and those who did not receive steroids as all of our patients were on steroids. However, in previous studies, no significant difference could be demonstrated between these variables.[4,6]

The presence of tuberculoma in patients with TBM either at presentation or their development de novo or increase in size during treatment is one of the major problems encountered by a treating physician or a neurologist as these are very refractory to the currently available treatment options in TBM. In the development of tuberculoma, immune system plays a crucial role particularly cytokines like TNF-α and IL-β. However, no study in the past has evaluated the effect of cytokines in the development of tuberculoma in patients with TBM during treatment. In the current study, there was no significant difference in the CSF or serum cytokine levels between patients presenting with tuberculoma and those patients presenting without it. However, TNF-α levels in the CSF at one and six months of follow-up andIL-1β in the serum at 6 months of follow-up were significantly higher in patients who developed tuberculoma on follow-up than those who did not. This finding further substantiates the fact that these cytokines are integral in the development and enlargement of CNS tuberculoma.

In conclusion, the current study confirms that TNF-α and IL-β are involved in the

Table 7: Correlation between CSF and serum levels of various cytokines

	CSF TNF-α	CSF IL-1β	Serum TNF-α	Serum IL-1β
CSF TNF-α	1.00	0.512 (p 0.001)	0.411 (p 0.01)	0.457 (p 0.004)
CSF IL-1β	0.512 (p 0.001)	1.00	0.314 (p 0.055)	0.139 (p 0.404)
Serum TNF-α	0.411 (p 0.01)	0.314 0.055	1.00	0.392 (p 0.015)
Serum IL-1β	0.457 (p 0.004)	0.139 (p 0.404)	0.392 (p 0.015)	1.00

Table 8: Studies showing various types of cytokines measured and their correlation with different parameters

Study	No. of patients	Type of Sample	Cytokines	Follow up of cytokine levels	Comments
Alkin *et al*.[3]	19 adults	CSF	IL-1β, IL-1 receptor antagonist, TNF-α	-	Positive correlation between TNF-α and IL-β levels, and TNF-α levels and conscious state
Babu *et al*.[8]	31 adults	CSF and serum	TNF-α and IFN-γ (also measured NO)	-	Positive correlation between severity of the disease and IFN-γ and TNF-α levels
Donald *et al*.[4]	30 children	CSF	TNF-α, IFN-γ and IL-1β	+	No correlation between cytokine levels and stage of disease; No effect of steroid therapy; Progressive decline in IL-1β and no decline in TNF-α and IFN-γ levels on follow up
Masroianni *et al*.[6]	15 adults	CSF	TNF-α, sTNFR-75, sTNFR-55, IL-10, IL-12	+	TNF-α level declined on follow up, The levels sTNFR-75, sTNFR-55, IL-10 remained elevated for 4-8 months
Thwaites *et al*.[7]	21 adults	CSF and serum	IFN-γ, TNF-α, IL-8, IL-10, TNF-αr1, TNF-αr2, MMP-9, TIMP-1	+	CSF levels of TNF-α was lower and undetectable in 88% patients and 7 days of treatment while TNF-αr1,TNF-αr2 were easily detectable at 60 days of treatment
Patel *et al*.[10]	27adults (17 HIV and seropositive, 10 HIV sero-negative)	CSF	TNF-α, IFN-γ, IL-10	-	-Positive correlation between IL-10 and IFN-γ - positive correlation between disease severity and IFN-γ and TNF-α levels

neuroinflammation caused by *Mycobacterium tuberculosis*. Moreover, the persistence of cytokines even after 6 months suggests that there is continuous production of cytokines at the site of tissue inflammation. This underlines the importance of these cytokines in the local immune response to tuberculous infection of the central nervous system. The finding of higher cytokine levels in all patients, who developed tuberculoma on antituberculous therapy, underlines the role played by these cytokine in the pathogenesis of tuberculoma. Moreover, as all of these patients were on steroid therapy during follow-up, another form of immunomodulatory therapy is probably necessary to prevent development and enlargement of tuberculoma. In the current study, the TNF-α level in CSF and TNF-α in serum and serum IL-1β were associated with poor survival. This may suggest that the patients having higher cytokine level may need other forms of immunomodulatory therapy other than or in addition to steroids. If it is so, Infliximab, which is a chimeric monoclonal antibody against TNF-α, may become a key adjuvant therapeutic choice. Although, in patients presenting with tuberculoma, the cytokines were detectable at 6 months, most of the patients clinically improved and the number and size of tuberculoma reduced over time. This may be due to involvement of other cytokines in pathogenesis which were not examined in the current study or due to pathogen-related factors like species, variable pathogenecity and virulence of *Mycobacterium tuberculosis*. However, a study with larger sample size and probably longer follow up is mandatory to further confirm our findings. Overall, in current study, the cytokine levels correlate well with the disease severity and predict mortality in TBM patients and the continuous release of examined cytokines despite optimum antitubercular therapy suggests that TBM severity

may result mainly from the immune response rather than the organism itself.

REFERENCES

1. World Health Organization Fact Sheet N104. Tuberculosis (Accessed on 3rd June, 2010 at http://www.who.int/mediacentre/factsheets/fs104/en/index.html)
2. Tsenova L, Bergtold A, Freedman VH, Young RA, Kaplan G. Tumor necrosis factor-alpha is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proc Natl Acad Sci U S A* 1999; 96(10):5657-62.
3. Akalin H, Akdis AC, Mistik R, *et al*. Cerebrospinal fluid interleukin-beta/interleukin-1 receptor antagonist balance and tumor necrosis factor–alpha concentrations in tuberculous, viral and acute bacterial meningitis. *Scand J Infect Dis* 1994;26:667-74.
4. Donald PR, Schoeman JF, Beyers N, *et al*. Concentrations of interferon gamma, tumor necrosis factor alpha, and interleukin-1 beta in the cerebrospinal fluid of children treated for tuberculous meningitis. *Clin Infect Dis* 1995; 21:924-9.
5. Rydberg J, Miorner H, Chandramuki A, Lantz M. Assessment of a possible imbalance between tumor necrosis factor (TNF) and soluble TNF receptor forms in tuberculous infection of the central nervous system. *J Infect Dis* 1995; 172:301-4.
6. 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.
7. Thwaites G E, Simmons C P, Quyen N T H, *et al*. Pathophysiology and Prognosis in Vietnamese Adults with Tuberculous Meningitis. *J Infect Dis* 2003; 188:1105-15
8. Babu G N, Kumar A, Kalita J, Misra UK. Proinflammatory cytokine levels in the serum and cerebrospinal fluid of tuberculous meningitis patients. *Neurosc Lett* 2008; 436:48-51.
9. Chen L, Jiang H, Wei C. Nitric oxide, TNF-alpha and IL-8 in cerebrospinal fluids of tuberculous and cryptococcic meningitis. *Hunan Yi Ke Da Xue Xue Bao* 1997; 22(6): 514-6.
10. Patel VB, Bhigjee, Bill PLA, Connolly CA. Cytokine profile in HIV seropositive patients with tuberculous meningitis. *J Neurol Neurosurg Psychiatry* 2002; 73:598-9.
11. Vankayalapati R, Wizel B, Weis S E, *et al*.Serum cytokine concentrations do not parallel mycobacterium tuberculosis–induced cytokine production in patients with tuberculosis. *Clin Infect Dis* 2003; 36:24-8.
12. Yadav A, Chaudhary C, Keshavan AH, *et al*. Correlation of CSF proinflammatory cytokines with MRI in tuberculous meningitis. *Acad Radiol* 2010; 17:194-200.
13. Robert MT, Mendlson M, Meyer P, Carmicheal A, Lever AM. The use of thalidomide in the treatment of intracranial tuberculomas in adults: two case reports. *J infect* 2003; 47(3):251-5.
14. Schoeman JF, Ravenscroft A, Hartzenberg HB. Possible role of adjunctive thalidomide therapy in the resolution of a massive intracranial tuberculosis abscess. *Childs Nerv Syst* 2001; 17(6):370-2.
15. Schoeman JF, Fleggen G, Seller N, Mendelson M, Hertzenberg HB. Intractable tuberculous infection responsive to thalidomide: report of four cases. *J Child Neurol* 2006; 21(4):301-8.
16. Schoeman JF, Springer P, Van Rensburg AJ, *et al*. Adjunctive thalidomide therapy for childhood tuberculosis meningitis: results of a randomized study. *J Child Neurol* 2004; 19(4):250-7.
17. Ahuja GK, Mohan KK, Behari M, *et al*. Diagnostic criteria for TBM and their validation. *Tuber Lung Dis* 1994; 75(2):149-52.
18. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervoussystem in adults and children. *J Infect* 2009; 59:167-87.
19. Clark WM, Hourihane JM. Clinical stroke scales. In: Herndon RM, ed: Handbook of neurologic rating scales. 2nd ed. Demos medical Publishing, New Delhi, 2006: 276.
20. Kornelisse RF, Savelkoul HFJ, Mulder PHG, *et al*. Interleukin-10 and Soluble tumor necrosis factor receptors in cerebrospinal fluid of children with bacterial meningitis. *J Infect Dis* 1996;173: 1498-502.