The Turkish Journal of Pediatrics 1997; 39: 177-184

TUMOR NECROSIS FACTOR – α AND INTERLEUKIN – 1β LEVELS IN CHILDREN WITH BACTERIAL, TUBERCULOUS AND ASEPTIC MENINGITIS*

*Mehmet Ceyhan MD***, *Güler Kanra MD****, *Zafer Ecevit MD***
*Gülten Seçmeer MD****, *Güliz Erdem MD*****, *Özay Akan MD******
*Osman Müftüoğlu MD*******

SUMMARY: Ceyhan M, Kanra G, Ecevit Z, Seçmeer G, Erdem G, Akan Ö, Müftüoğlu O. (Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Tumor necrosis factor-α and interleukin-1β levels in children with bacterial, tuberculous and aseptic meningitis. Turk J Pediatr 1997; 39: 177-184.

Cerebrospinal fluid levels of tumor necrosis factor-α and interleukin-1β in 78 children with nonbacterial, bacterial and tuberculous meningitis, and in 34 control subjects were analyzed in order to evaluate the involvement of these cytokines in the pathogenesis of acute bacterial meningitis and their discriminative value between different etiologies of meningitis. Tumor necrosis factor-α and interleukin-1β levels were significantly higher in bacterial and tuberculous meningitis than in aseptic meningitis and in control subjects ($p < 0.0001$). There was no difference in the levels of tumor necrosis factor-α and interleukin-1β between nonbacterial meningitis and control groups. The finding that both tumor necrosis factor-α and interleukin-1β are increased in the cerebrospinal fluid of patients with bacterial and tuberculous meningitis whereas normal levels of these two cytokines have been found in patients with nonbacterial meningitis signifies that these cytokines may be used to differentiate between bacterial and nonbacterial meningitis. *Key words: TNF-α, IL-1β, bacterial meningitis, aseptic meningitis.*

One of the most important advances in immunology in recent years is the understanding of the effects of some cytokines on inflammatory responses to infectious agents. Tumor necrosis factor alpha (TNF-α), which is produced mainly by the macrophages as a response to microbial or endotoxic stimuli, has been implicated in the pathogenesis of both local and systemic inflammatory reactions[1].

The importance of TNF-α has been established especially in fatal endotoxemia[2]. In experimental models, common physiological and pathological changes of gram-negative septicemia were observed after TNF-α administration[1,3]. TNF-α

* From the Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Associate Professor of Pediatrics, Hacettepe University Faculty Medicine.

*** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

**** Research Assistant in Pediatrics, Hacettepe University Faculty of Medicine.

***** Instructor in Microbiology, Hacettepe University Faculty of Medicine.

****** Associate Professor of Medicine, Numune Hospital, Ankara.

concentrations are also found to be increased in meningococcemia and meningococcal meningitis[4,5]. The mortality rate and neurologic sequelae have also been found to be higher in cases with increased concentrations of TNF-α[6].

TNF-α has been found to be responsible for at least some of the inflammatory events in bacterial meningitis in controlled studies, and dexamethasone administered before endotoxin was demonstrated to inhibit TNF-α secretion[7]. The important role of TNF-α in the pathogenesis of childhood meningitis has also been shown in clinical studies. TNF-α activity was demonstrated in 93 percent of 42 newborns with gram-negative meningitis in a study done by McCracken et al[3]. In another study, TNF-α levels were found to be increased in 75 percent of infants with bacterial meningitis[8]. Differences in these studies may originate from differences in the sampling time of cerebrospinal fluid (CSF) after the onset of symptoms and differences in the hosts' responses. There was no increase in TNF-α levels in nonbacterial meningitis cases in any of these studies. This finding may help to differentiate between bacterial and nonbacterial cases of meningitis in the presence of high TNF-α levels.

Another important mediator emphasized in the pathophysiology of bacterial meningitis is interleukin-1-beta (IL-1β). Like TNF-α, it is mainly produced by macrophages, but epidermal cells, synovial fibroblasts, lymphocytes, vascular endothelial cells, astrocytes and microglia can all secrete IL-1β[9]. IL-1β secretion can be stimulated by many factors, such as endotoxin in gram-negative bacterial cell walls, the teichoic acid component of the gram-positive bacterial cell wall, immune complexes, C5a and TNF[10].

In clinical studies, IL-1β levels have been detected in the CSF of 95 percent of cases with bacterial meningitis[9]. Although the degree of elevated CSF concentrations of IL-1β was much lower than that of bacterial meningitis, IL-1β concentrations were also found to be elevated in 88 percent of nonbacterial meningitis cases[11]. IL-1β activity was not detected in control subjects without meningeal inflammation. Dexamethasone also inhibits IL-1β secretion, and some of its anti-inflammatory effect is attributed to this inhibition[12,13]. Inexpensive and rapid assays for these two cytokines may help to distinguish between bacterial and nonbacterial causes of meningitis[14].

The etiologies of both bacterial and nonbacterial meningitis are different in Turkey than in developed countries; pneumococcus pneumonia is the major pathogen in bacterial meningitis, and most nonbacterial meningitis cases are due to mumps[15]. Mycobacterium tuberculosis poses a great problem in distinguishing tuberculous meningitis from other causes of bacterial meningitis. TNF-α and IL-1β are thought to be involved in the immune response to mycobacterial infection because they exhibit antimycobacterial effects in vitro; moreover, the mycobacterium tuberculosis cell wall component lipoarabinomannan,

mycobacterial heat shock protein, and M.tuberculosis culture filtrate devoid of contaminating lipopolysaccharide are reported to stimulate the production of TNF-α and IL-1β[16, 17]. TNF-α levels were found to be moderately increased in tuberculous meningitis in only one clinical study[14]. Still, the importance of these cytokines in tuberculous meningitis must be evaluated.

Material and Methods

Patients: Patients between the ages of one month and 15 years were evaluated in four study groups:

Group 1: Bacterial meningitis cases: Symptoms indicative of meningitis and fulfilling at least one of the following two criteria[18]:

1. Bacterial isolation and/or latex agglutination.
2. At least one of the following, if no pathogen were isolated:
   a) White blood cell (WBC) greater than 2,000 or polymorphonuclear leukocyte (PMNL) count greater than 1,180 per cubic milimeter of CSF.
   b) CSF protein greater than 220 mg/dl,
   c) CSF to blood ratio of glucose less than 0.25.

Group 2: Tuberculous meningitis cases: Cases with symptoms indicative of meningitis and routine CSF laboratory tests suggesting bacterial meningitis and detection of acid-resistant bacilli on Ziehl-Neelsen stain and/or culture-positive cases; or, cases with a positive family history for tuberculosis who were non-responsive to conventional antimicrobial therapy who had a clinical cure after anti-tuberculous therapy and who had lymphoycte-predominant CSF pleocytosis, hypoglycorrhachia and blockage of CSF circulation demonstrated by cisternography or other imaging techniques.

Group 3: Nonbacterial meningitis cases: Cytochemical values of CSF suggesting meningitis with normal CSF glucose and negative bacteriological tests (culture, gram stain, conventional antigen testing) and cases that had not received antimicrobial therapy during the previous two weeks.

Group 4: Control subjects: Cases with normal CSF findings. These cases were the patients whose clinical findings indicated the need for lumbar puncture to exclude the diagnosis of meningitis in the Emergency Clinic.

Laboratory Studies: Leukocyte counts, protein and glucose levels in CSF, CSF to blood ratio of glucose, gram-stained smears of CSF and routine cultures were done in all cases. Latex agglutination tests for pneumococcus, Haemophilus influenzae type b and meningococcus in the CSF were done in all patients. CSF samples were stored at -40 °C until analysis.

Detection of TNF-α and IL-1b: The levels of TNF-α and IL-1β in the CSF samples obtained during the diagnosis were evaluated. TNF-α concentrations were measured by an ELISA kit (Cistron TNF-α kit) with a sensitivity of ≤ 20 pg/ml. First, the wells were coated with 100 µL standard, control and test sera and incubated for two hours at 37 °C; the wells were then washed three times with washing buffer. 100 µL TNF-α antisera was then added to each well, incubated for two hours at 37 °C, and the washing was repeated three times. 100 µL anti-Ig G-HRP conjugate was added to each well, incubated for 30 minutes at room temperature and washed again. 100 µL substrate was then added to each well and incubated at room temperature without closing the tips of the wells. 50 µL of 4N sulfuric acid was added to each well, and the OD was read at 490 nm. The optic density values were averaged to obtain the mean values and their standard errors. Titers were expressed as mean concentration ± standard error for each group.

IL-1β levels were detected by an ELISA kit (Cistron TNF-α kit) with the same sensitivity. The same procedure was repeated, but instead of TNF-α antisera, IL-1β antisera were used.

Statistical Analysis: Student's t-test and Mann-Whitney U tests were used to compare the groups with respect to TNF-α and IL-1β levels.

Results

A total of 112 patients were analyzed; 38 of the patients had bacterial and 36 had nonbacterial meningitis. Since the incidence was low, we found only four cases with tuberculous meningitis. The remaining 34 patients were the control subjects. The ages of the patients ranged from two months to 13 years in the control group (mean 3.42 years), one month to 14 years in the nonbacterial meningitis group (mean 4.14 years), three months to 15 years in the bacterial meningitis group (mean 3.65 years), and three to 11 years in the tuberculous meningitis group (mean 6 years).

The CSF findings of the patients are sumarized in Table I. S. pneumoniae was isolated in seven patients, N. meningitidis in five patients and H. influenzae in two patients. A diagnosis of bacterial meningitis was suggested by CSF cytochemistry in 24 patients.

Table I: Cytochemical Findings of the CSF of Patients

	Groups			
	Control	Nonbacterial m.*	Bacterial m.	Tuberculous m.
Cell count (/mm^3)	0.82 ± 0.61	82.71 ± 72.20	175.60 ± 233.12	429.00 ± 148.94
PMNL (/mm^3)	0	31.08 ± 52.17	141.11 ± 158.44	71.50 ± 82.56
Lymphocyte (/mm^3)	0.82 ± 0.61	51.63 ± 48.36	34.49 ± 27.63	357.50 ± 163.28
Protein (mg/dl)	32.28 ± 17.41	79.95 ± 43.18	103.75 ± 43.07	169.25 ± 104.16
Glucose (CSF/blood)	0.81 ± 0.23	0.69 ± 0.17	0.36 ± 0.23	0.17 ± 0.08
Culture positivity	–	–	14/38**	–

* m.: meningitis.

** P.: pneumonia was isolated in 7 patients, N. meningitidis in 5 patients and H. influenzae in 2 patients.

TNF-α levels are summarized in Table II. The high standard deviation values were due to the significant differences between the cases. In the bacterial and tuberculous meningitis groups, the concentrations were significantly higher than in the control and nonbacterial meningitis groups. The TNF-α levels of the control and nonbacterial meningitis groups did not differ significantly ($p = 0.39$). The TNF-α levels were significantly higher in the bacterial and tuberculous meningitis groups than in the control and nonbacterial meningitis groups ($p < 0.0001$). There was no statistically significant difference in TNF-α levels between the bacterial and tuberculous meningitis groups ($p = 0.39$).

Table II: TNF-α Levels in the CSF

Groups	n	TNF-α (pg/ml) Mean ± Standard Deviation (Range)	p		
Control	34	0.57 ± 1.08 (0-4.36)	0.39	< 0.0001	< 0.0001
Nonbacterial meningitis	36	0.79 ± 1.09 (0-4.60)			
Bacterial meningitis	38	76.41 ± 58.10 (12.01-246.30)			0.39
Tuberculous meningitis	4	47.13 ± 16.78 (36.4-71.60)			

IL-1β levels were correlated with TNF-α levels in all groups (Table III). IL-1β levels were not significantly different between the control and nonbacterial meningitis groups ($p = 0.39$), but were significantly higher in the bacterial and tuberculous meningitis groups than in the control and nonbacterial meningitis groups ($p < 0.0001$). There was no statistically significant difference in IL-1β levels between the bacterial and tuberculous meningitis groups ($p = 0.73$).

Table III: IL-β Levels in the CSF

Groups	n	IL-β (pg/ml) Mean ± Standard Deviation (Range)	p		
Control	34	0.42 ± 0.58 (0-1.90)	0.39	< 0.0001	< 0.0001
Nonbacterial meningitis	36	1.04 ± 3.02 (0-17.90)			
Bacterial meningitis	38	54.91 ± 27.98 (18.20-148.20)			0.73
Tuberculous meningitis	4	61.22 ± 40.43 (28.91-118.12)			

The lowest levels of both TNF-α and IL-1β in the bacterial and tuberculous meningitis groups were significantly higher than the highest levels of both the control and nonbacterial meningitis groups. The highest TNF-α and IL-1β concentrations of the control and nonbacterial meningitis groups were 4.60 and 17.90 pg/ml, respectively, and the lowest values of the other two groups were 12.01 and 18.20 pg/ml, respectively.

Discussion

The role of TNF-α and IL-1β in the pathogenesis of meningitis has been demonstrated by many studies in the recent years. This progress has led to an understanding of the pathogenesis of bacterial meningitis, the usage of these cytokines in the diagnosis of meningitis and new modes of therapy aiming to inhibit the synthesis and secretion of these cytokines.

TNF-α and IL-1β concentrations are found to be increased in the CSF sampled just after the onset of meningitis symptoms[3, 6, 8, 19, 20]. We also observed that both TNF-α and IL-1β levels were increased in the bacterial meningitis group, whereas they were normal in children with nonbacterial meningitis and in control subjects. The increase in these two cytokines is an intermediate step in the CSF inflammation triggered by the teichoic acid in the gram-positive bacterial cell wall and the lipooligosaccharide in gram-negative bacteria. These components of bacteria first activate the natural killer cells, especially the macrophages, and then the Kuppfer cells, CNS astrocytes and glial cells; these cells synthesize TNF-α in response. TNF-α in return increases the synthesis of IL-1β by stimulating macrophages, endothelial cells, astrocytes and glial cells[21, 22]. TNF-α and IL-1β are in part responsible for the cytochemical findings of bacterial meningitis and some of its complications, such as hearing loss, by their direct effects and by increasing the synthesis of prostaglandins, platelet-activating factor and other inflammatory mediators[23].

Despite some studies demonstrating an increase in the secretion of TNF-α by blood mononuclear cells during viral infections, no increase in TNF-α levels were found in clinical studies of nonbacterial meningitis[20, 24-26]. In our study there was no significant difference between the CSF TNF-α levels of nonbacterial meningitis and control subjects.

We found that the lowest values of both mediators in the bacterial meningitis group were higher than the highest of the nonbacterial meningitis and control cases, indicating 100 percent specificity in distinguishing bacterial and nonbacterial cases. This specificity is higher than in the other reported studies[3, 8, 20].

Helminen et al.[27] showed that IL-1β levels were increased in bacterial meningitis but not in nonbacterial meningitis cases. Viruses also have an inhibitory effect on IL-1β synthesis[28, 29]. IL-1β levels were significantly higher in our bacterial meningitis group, confirming the former studies. The finding of high IL-1β levels in two patients with nonbacterial meningitis in a former study can be explained by ythe late sampling of CSF in these patients[20].

The exact role of inflammatory mediators in tuberculous meningitis is still unclear. One of the aims of this study was to determine the roles of TNF-α and IL-1β in tuberculous meningitis. The levels of both TNF-α and IL-1β were found to be

elevated in these cases, as in cases of bacterial meningitis. It was demonstrated that the level of TNF-α produced by monocytes from patients with pulmonary tuberculosis was related to the disease state of pulmonary tuberculosis; monocytes from patients with chronic refractory tuberculosis released significantly lower amounts of TNF-α[30]. The moderate increase in TNF-α in tuberculous meningitis cases in the present and former studies may be due to this fact. The number of cases is too low to draw a conclusion, but the findings suggest that tuberculous meningitis must also be considered in cases with increased CSF levels of TNF-α and IL-1β.

REFERENCES

1. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumour necrosis factor. Lancet 1989; 1: 1122-1126.
2. Cerami A, Beutler B. The role of cachectin/TNF in endotoxic shock and cachexia. Immunol Today 1988; 9: 28-31.
3. McCracken GH Jr, Mustafa MM, Ramilo O, Olsen KD, Risser RC. Cerebrospinal fluid interleukin1-beta and tumor necrosis factor concentrations and outcome from neonatal gram-negative enteric bacillary meningitis. Pediatr Infect Dis J 1989; 8: 155-159.
4. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1987; 1: 355-357.
5. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 1989; 169: 333-338.
6. Mustafa MM, Ramilo O, Saez-Llorens X, Mertsola J, McCracken GH Jr. Role of tumour necrosis factor alpha (cachectin) in experimental and clinical bacterial meningitis. Pediatr Infect Dis J 1989; 8: 907-908.
7. Meningitis Study Group. Report of a workshop. Pathophysiology of bacterial meningitis-implications for new management strategies. Pediatr Infect Dis J 1987; 6: 1143-1171.
8. Nadal D, Leppert D, Frei K, Gallo P, Lamche H, Fontana A. Tumour necrosis factor-alpha in infectious meningitis. Arch Dis Child 1989; 64: 1274-1279.
9. Ramilo O, Mustafa MM, Saez-Llorens X, et al. Role of interleukin 1-beta in meningeal inflammation. Pediatr Infect Dis J 1989; 8: 909-910.
10. Dinarello CA. Biology of interleukin 1. FASEB J 1988; 2: 108-115.
11. Mustafa MM, Lebel MH, Ramilo O, et al. Correlation of interleukin-1-beta and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. J Pediatr 1989; 115: 208-213.
12. Kern JA, Lamb RJ, Reed JC, Daniele RP, Nowell PC. Dexamethasone inhibition of interleukin 1-beta production by human monocytes. Posttranscriptional mechanisms. J Clin Invest 1988; 81: 237-244.
13. Lebel MH, Freji BJ, Syrogiannopoulos GA, et al. Dexamethasone therapy for bacterial meningitis. Results of two double-blind, placebo-controlled trials. N Engl J Med 1988; 319: 964-971.
14. Glimoker M, Kragsbjerg P, Forsgren M, Olcen P. Tumor necrosis factor-alpha (TNF alpha) in cerebrospinal fluid from patients with meningitis of different etiologies: high levels of TNF alpha indicate bacterial meningitis. J Infect Dis 1993; 167: 882-889.

15. Kanra G, Özen H, Seçmeer G, Ceyhan M, Ecevit Z, Belgin E. The beneficial effects of dexamethasone in children with pneumococcal meningitis. Pediatr Infect Dis J (in press).

16. Barnes PF, Fong SJ, Brennan PJ, Twomey PE, Mazumder A, Modlin RL. Local production of tumor necrosis factor and IFN-gamma in tuberculous pleuritis. J Immunol 1990; 145: 149-154.

17. Zhang Y, Doerfler M, Lee TC, Guillemin B, Rom WN. Mechanisms of stimulation of interleukin-1 beta and tumor necrosis factor-alpha by Mycobacterium tuberculosis components. J Clin Invest 1993; 91: 2076-2083.

18. Spanos A, Harrell FE Jr, Durack DT. Differential diagnosis of acute meningitis. An analysis of the predictive value of initial observations. JAMA 1989; 262: 2700-2707.

19. Ramilo O, Mustafa MM, Porter J, Olsen KD, Luby J, McCracken GH. Concentrations of IL-1β and TNF-α in CSF of infants and children with bacterial meningitis. APS-SPR Meeting, Washington D.C., U.S.A., 1989, abstract no: 1108.

20. Ramilo O, Mustafa MM, Porter J, et al. Detection of interleukin of 1 beta but not tumor necrosis factor-alpha in cerebrospinal fluid of children with aseptic meningitis. Am J Dis Child 1990; 144: 349-352.

21. Beutler B. The tumor necrosis factors: cachectin and lymphotoxin. Hosp Pract 1990; 25: 45-56.

22. Vane JR, Anggord EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990; 323: 27-36.

23. Meningitis Study Group. Report of a second workshop. Pathophysiology of bacterial meningitis. Pediatr Infect Dis J 1989; 8: 899-933.

24. Beutler B, Crochin N, Milsark IN, Cerami A. Induction of cachectin (tumor necrosis factor) by influenza virus: deficient production by endotoxin-resistant (c3H/HeJ) macrophages. Clin Res 1986; 34: 491A.

25. Aderka D, Holtman H, Toker L, Hahn T, Wallach D. Tumor necrosis factor induced by Sendai virus. J Immunol 1987; 136: 2938-2942.

26. Arditi M, Monogue KR, Yogev R. CSF cachectin activity in children with bacterial and aseptic meningitis. Pediatr Res 1989; 25: 171A.

27. Helminen M, Vesikari T. Spontaneous and inducible interleukin 1 production from peripheral blood monocytes in bacterial and viral infection in children. Pediatr Infect Dis J 1987; 6: 1102-1106.

28. Rodgers BC, Scott DM, Mundin J, Sissons JG. Monocyte-derived inhibitor of interleukin 1 induced by human cytomegalovirus. J Virol 1985; 55: 527-532.

29. Wainberg MA, Numazaki K, Destaphano L, Wong I, Goldman H. Infection of human thymic epithelial cells by human cytomegalovirus and other viruses: effect on secretion of interleukin 1-like activity. Clin Exp Immunol 1988; 72: 415-421.

30. Takashima T, Ueta C, Tsyuguchi I, Kishimoto S. Production of tumor necrosis factor alpha by monocytes from patients with pulmonary tuberculosis. Infect Immun 1990; 58: 3286-3292.