Diagnostic value of cerebrospinal fluid levels of D-lactate, tumour necrosis factor-alpha and interleukin-6, -8, and -17 in suspected nosocomial meningitis

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Abstract

Introduction: This study aimed to determine the diagnostic value of interleukin (IL)-6, IL-8, IL-17, tumour necrosis factor-alpha (TNF-α) and D-lactate levels in the cerebrospinal fluid (CSF) of nosocomial meningitis patients.

Methods: The CSF levels of cytokines and D-lactate were compared across 29 episodes of nosocomial meningitis, 38 episodes of pleocytosis (without meningitis) and 54 control subjects.

Results: The CSF levels of IL-6, IL-8, and D-lactate were higher in the group with nosocomial meningitis compared to the control group and the group with pleocytosis without meningitis (P < 0.05). For IL-6 levels (threshold: >440 pg/mL), the sensitivity and specificity were 55.17% and 94.74%, respectively. For IL-8 levels (threshold: >1,249 pg/mL), the sensitivity and specificity were 44.83% and 84.21%, respectively. In patients with nosocomial meningitis, when the threshold of D-lactate levels was >1.05 μmol/mL, the sensitivity and specificity were 75.86% and 63.16%, respectively. In pleocytosis (without meningitis) CSF samples and in nosocomial meningitis CSF samples, the highest area under the receiver operating characteristic curve (AUC) was calculated for triple combination model of IL-6, IL-8 and D-lactate levels (AUC 0.801, P < 0.001) and double combination model of IL-6 and IL-8 (AUC 0.790, P < 0.001).

Conclusion: Our study findings suggest that IL-6, IL-8 and D-lactate levels could be diagnostic markers for nosocomial meningitis.

Keywords: Cytokine, D-lactate, IL-6, IL-8, IL-17, nosocomial meningitis, TNF-α

INTRODUCTION

Acute nosocomial meningitis is an uncommonly encountered hospital-acquired infection. Though rare, it is a clinical presentation with high rates of morbidity and mortality (20%–50%) among nosocomial infections.[1] Therefore, rapid diagnosis and treatment are essential. However, diagnosis is often challenging, as it is difficult to differentiate between nosocomial meningitis and pleocytosis without meningitis.[2,3] The classical clinical manifestations of meningitis, such as fever, meningismus and altered state of consciousness, are also observed in pleocytosis without meningitis and other underlying diseases; thus, they provide limited value in the diagnosis of nosocomial meningitis.[4] Other markers such as changes in cerebrospinal fluid (CSF) cell counts and abnormal levels of protein, lactate and glucose may not always be due to an infection, but can also result from surgical interventions.[3,5]
Moreover, antibiotics that have been used preoperatively for other purposes may also influence the results of CSF bacterial cultures.\[6\]

The presence of D-lactate in sterile body fluids indicates the presence of invading bacteria.\[6\] There are numerous in vitro studies that have investigated the use of lactate to differentiate between bacterial and aseptic meningitis, but the data for D-lactate measurements for the rapid diagnosis of nosocomial meningitis are limited.\[7,8\] Cytokines are a suitable marker to detect inflammation of the meninges, and they have a considerable role in the pathophysiology of meningitis.\[9,10\] In human and animal studies, investigation of interleukin (IL)-6, IL-8, IL-17 and tumour necrosis factor-alpha (TNF-α) in CSF have revealed that the levels of these proinflammatory cytokines are significant in differentiating between bacterial meningitis, aseptic meningitis and non-infectious pleocytosis.\[9,10,14\] A few studies have also evaluated D-lactate and IL-17 levels in the CSF of patients with bacterial meningitis.\[6,7,14\]

In this study, we aimed to determine the diagnostic value of proinflammatory cytokines and D-lactate levels in CSF for the diagnosis of nosocomial meningitis by comparing the results of the following investigations: CSF cell counts, CSF biochemistry (glucose/concomitant blood glucose and protein levels in CSF), CSF Gram and Giemsa staining, and bacterial cultures.

**METHODS**

The samples of patients’ CSF were obtained for diagnostic analysis from the Department of Neurosurgery, Uludag University Faculty of Medicine Hospital, Turkey. The CSF samples were cryogenically stored at −80°C until the commencement of the study, in four portions to avoid the effects of freezing and thawing. Patients who had not taken steroid and antibiotics were eligible for the study. A total of 121 CSF samples from 104 patients were included in the study. The IL-6, IL-8, IL-17, TNF-α and D-lactate levels in the CSF samples were measured.

Patient data obtained included age, gender, clinical findings, type of operation, Glasgow Coma Score (GCS), presence of intracranial catheters (external ventricular drainage, intracranial pressure, ventriculoperitoneal shunt), direct microscopic examination, Gram/Giemsa staining of CSF, and CSF biochemistry values. Mortality rates were calculated within the first 28 days after CSF sampling. This study was approved by Uludag University Faculty of Medicine Ethical Committee (Ref: 2013-19/7) on 19 November 2013. Informed consent was not obtained because of the retrospective study design.

Nosocomial central nervous system (CNS) infection was defined based on the definitions suggested by the Center for Disease Control and Prevention, which define healthcare-associated nosocomial meningitis as comprising at least one of the following criteria: (a) organism cultured from CSF; (b) patient has at least two of the following: fever >38°C or headache, or meningeal or cranial nerve sign(s); (c) increased white cells, elevated protein and decreased glucose in CSF; (d) organism seen on Gram stain of CSF; and (e) organism cultured from blood.\[15\] Non-culture-based diagnostic laboratory tests, specific antibody titres or molecular tests for bacteria (e.g., multiplex polymerase chain reaction) were not performed in our hospital patients. As most patients had undergone an intracranial surgery, the corrected leucocyte count in CSF was calculated assuming that in a person with normal findings, the blood passing into CSF contains 1 leucocyte per 700 erythrocytes per mm\(^3\).\[16\]

The CSF samples were allowed to reach room temperature and thereafter, cytokine (IL-6, IL-8, IL-17a and TNF-α) and D-lactate levels were measured using enzyme-linked immunosorbent assay (ELISA) and colorimetric assay, respectively according to the instructions provided by the manufacturers. The CSF levels of IL-6, IL-8 and TNF-α were determined using BosterImmunoleader® ELISA kit (Boster Biological Technology, Pleasanton, CA, USA) specific for each cytokine, whereas IL-17 levels were determined using Human IL-17A/F Heterodimer ELISA kit and DuoSet® Ancillary Reagent Kit (R&D Systems, Inc., Minneapolis, MN, USA). Spectrophotometric measurements were done at 450 nm. The resulting absorbance values were calculated according to the standard curve plot to determine the concentration of D-lactate present in CSF samples. D-lactate levels of the patient were expressed in μmol/mL after normalising the data to the sample volume used.

Statistical analyses were performed using IBM SPSS Statistics version 23.0 for Windows (IBM Corp, Armonk, NY, USA). Shapiro–Wilk test was used to examine whether the data showed normal distribution. For the data that did not show normal distribution, Mann–Whitney U test was used for comparison of two groups and Kruskal–Wallis test for comparison across more than two groups. Categorical data were examined using Pearson’s chi-square test, Fisher’s exact test and Fisher–Freeman–Halton test. Receiver operating characteristic (ROC) curves were plotted for the CSF cytokine and D-lactate levels and the cut-off values were determined. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for the optimal cut-off value. The performance of the CSF cytokine and D-lactate levels in predicting nosocomial meningitis was determined using ROC curves, with the area under the curve (AUC) being of primary interest. MedCalc® Statistical Software version 19.5.3 (MedCalc...
Table 1. Demographic and surgical factors characteristics of patient and control groups.

| Variable             | Meningitis (n=21) | Pleocytosis* (n=29) | Control† (n=54) | Overall P | Pairwise P* |
|----------------------|-------------------|---------------------|----------------|-----------|-------------|
| Female gender        | 8 (38.1)          | 13 (44.8)           | 27 (50.0)      | 0.641     | 0.634       |
| Age (yr)             | 46.1±14.93        | 48.10±15.22         | 57.6±14.79     | 0.039*    | 0.652       |
| Type of surgery      |                   |                     |                |           |             |
| Tumour               | 10 (47.6)         | 10 (34.5)           | 17 (31.5)      |           |             |
| Intracranial bleeding| 8 (38.0)          | 8 (27.6)            | 11 (20.4)      |           |             |
| Ventriculoperitoneal shunt | 1 (4.7)      | 2 (6.9)             | 11 (20.4)      |           |             |
| Intraorbital foreign body | 1 (4.7)    | 0 (0)               | 0 (0)          |           |             |
| Vertebral            | 1 (4.7)           | 1 (3.4)             | 0 (0)          |           |             |
| No surgery           | 0 (0)             | 8 (27.6)            | 15 (27.8)      |           |             |

*Data presented as mean±standard deviation. *Pleocytosis without meningitis. †Patients without meningitis or pleocytosis. *Pairwise comparison between Meningitis and Pleocytosis groups. Although overall P is statistically significant, there is no significant difference in pairwise comparisons.

Table 2. Comparison of risk factors in patient and control groups.

| Variable             | Meningitis (n=21) | Pleocytosis* (n=29) | Control† (n=54) | P          |
|----------------------|-------------------|---------------------|----------------|------------|
| Craniotomy           | 14 (66.7)*        | 11 (37.9)**         | 10 (18.5)*     | <0.001*    |
| Dural defect         | 16 (76.2)         | 19 (65.5)           | 32 (59.3)      | 0.384      |
| ICP catheter         | 4 (19.0)**        | 0 (0)**             | 4 (7.4)**      | 0.043*     |
| EVD catheter         | 9 (42.8)          | 6 (20.7)            | 21 (38.9)      | 0.169      |
| VP shunt             | 3 (14.3)          | 3 (10)              | 13 (24.1)      | 0.264      |

*Pleocytosis without meningitis. Patients without meningitis or pleocytosis. Results of pairwise comparisons between groups. Values with unlike letters were significantly different among groups. *Statistically significant. EVD: external ventricular drainage, ICP: intracranial pressure, VP: ventriculoperitoneal

There were 29 patients (38 CSF samples) with pleocytosis due to reasons other than meningitis (trauma, intervention, tumour, haemorrhage), in whom meningitis was not considered. Fifty-four patients (54 CSF samples) who had neither pleocytosis nor meningitis were enrolled as controls.

Table 3. Culture results in meningitis group (N=16).

| Microorganism               | n (%)            |
|-----------------------------|------------------|
| Coagulase-negative Staphylococcus | 11 (68.75) |
| Acinetobacter baumannii      | 2 (12.5)         |
| Enterococcus faecalis        | 1 (6.25)         |
| Klebsiella pneumoniae        | 1 (6.25)         |
| Staphylococcus aureus        | 1 (6.25)         |

In the CSF examination, leucocyte count and the levels of protein and glucose were evaluated for the diagnosis of nosocomial meningitis. Our comparison of the meningitis and pleocytosis groups showed no significant difference in CSF leucocyte counts (P = 0.112) [Table 4]. The median glucose level was 56 (5–133) mg/dL in the meningitis group and 73 (38–136) mg/ dL in the control group. Glucose levels were significantly lower in the meningitis and pleocytosis groups as compared to the control group (P = 0.001). However, the glucose levels did not differ significantly between the meningitis and pleocytosis groups (P = 0.715) [Table 4]. The lowest glucose level (5 mg/dL) was detected in a patient with Acinetobacter baumannii growth in CSF.

Protein concentration in CSF differed significantly across all groups (P < 0.001). In all CSF samples evaluated, the median protein concentration was 44.8 (4.97–1468.4) mg/dL. Median protein concentration was 141.6 (13.6–1468.4) mg/dL in the group with nosocomial meningitis and 34.9 (6.8–392) mg/dL.

RESULTS

Of the 104 patients who provided the 121 CSF samples for analysis, 48 (46.2%) were female and 56 (53.8%) were male. Patient demographics and types of surgery that the patients underwent are summarised in Table 1. The 121 samples were divided into three groups: Group 1: nosocomial meningitis (29 samples, 24.0%); Group 2: pleocytosis without meningitis (38 samples, 31.4%); and Group 3: controls with neither meningitis nor pleocytosis (54 samples, 44.6%).
In the control group. In pairwise comparisons, the protein levels were significantly higher in the group with nosocomial meningitis compared to the control group \( (P < 0.001) \). Protein levels were significantly higher in the group with nosocomial meningitis compared to the pleocytic group without meningitis \( (P = 0.002) \) [Table 4].

In all the CSF samples investigated, the median concentration of IL-6 was found to be 18 (2–7390) pg/mL. In the group with nosocomial meningitis, the median value for IL-6 was 469 (5–7390) pg/mL, and IL-6 level was significantly higher in the meningitis group compared to the control group \( (P < 0.001) \). IL-6 level was noticeably higher in the group with pleocytosis without meningitis compared to the control group \( (P < 0.001) \). A significant difference in IL-8 level was found between the group with nosocomial meningitis and the group with pleocytosis without meningitis \( (P = 0.045) \) [Table 5].

As patients with intracranial haemorrhage had high levels of proinflammatory cytokines in CSF due to inflammation, only those patients who experienced haemorrhage were evaluated. When the groups with and without meningitis (control group and the group with pleocytosis without meningitis) were compared, significance was found only in the IL-6 levels among the cytokines \( (P = 0.02) \). No significant difference was found between these two groups in terms of IL-8 levels in CSF \( (P = 0.084) \) [Table 6].

### Table 4. Comparison of cerebrospinal fluid (CSF) examination results between patient groups and control.

| Variable                  | Median (range) | Overall \( P^* \) | Pairwise \( P^* \) |
|---------------------------|----------------|-------------------|-------------------|
| CSF leukocyte count (/mm\(^3\)) |               |                   |                   |
| Meningitis (1) (\(n=29\)) | 160 (0–48,000) | <0.001            | 1 vs. 2 0.112     |
| Pleocytosis\(^a\) (2) (\(n=38\)) | 25 (10–4600)  |                   | 1 vs. 3 <0.001    |
| Control\(^b\) (3) (\(n=54\)) | 0 (0–110)     |                   | 2 vs. 3 <0.001    |
| CSF erythrocyte count (/mm\(^3\)) |             |                   |                   |
| Meningitis (1) (\(n=29\)) | 6900 (0–230,000) | 0.008            | 1 vs. 2 0.041     |
| Pleocytosis\(^a\) (2) (\(n=38\)) | 1320 (0–70,200) |                   | 1 vs. 3 0.004     |
| Control\(^b\) (3) (\(n=54\)) | 245 (0–141,000) |                   | 2 vs. 3 0.135     |
| Glucose (mg/dL)           | 56 (5–133)    | 0.001             | 1 vs. 2 0.715     |
| Protein (mg/dL)           | 141.6 (13.6–1468.4) | <0.001          | 1 vs. 2 0.002     |

\(^a\)Pleocytosis without meningitis. \(^b\)Patients without meningitis or pleocytosis. \(^c\)Corrected CSF leukocyte count; secondary to operation or caused by bloody touching. *Statistically significant at \(P<0.05\).

### Table 5. Comparison of serum cytokines and D- lactate levels between patient groups and control.

| Variable      | Median (range) | Overall \( P^* \) | Pairwise \( P^* \) |
|---------------|----------------|-------------------|-------------------|
| IL-6 (pg/mL) | 469 (5–7390) | <0.001            | 1 vs. 2 <0.001    |
| IL-8 (pg/mL) | 907 (0.01–1610) | <0.001          | 1 vs. 3 <0.001    |
| D-lactate (µmol/mL) | 2.45 (0.332–22.04) | <0.001      | 1 vs. 3 <0.001    |
| IL-17 (pg/mL) | 0.01 (0.01–1203) | 0.077            | 1 vs. 3 0.012     |
| TNF-\(\alpha\) (pg/mL) | 0.01 (0.01–16,428) | 0.024             | 1 vs. 3 0.015     |

\(^a\)Pleocytosis without meningitis. \(^b\)Patients without meningitis or pleocytosis. *Statistically significant at \(P<0.05\). IL: interleukin, TNF: tumour necrosis factor.
As TNF-α and IL-17 levels were too low to be measured in most CSF samples, these two parameters were studied by comparing the three groups. There was no statistically significant difference in IL-17 levels among the three groups ($P = 0.077$) [Table 5]. However, there was statistically significant difference in the levels of TNF-α among the groups ($P = 0.024$). TNF-α levels were especially higher in the group with nosocomial meningitis than in control patients without pleocytosis ($P = 0.010$) [Table 5].

We evaluated the potential use of the proinflammatory cytokines IL-6, IL-8, TNF-α, and IL-17 as biomarkers for a new diagnostic test that can be used for the diagnosis of nosocomial meningitis and pleocytosis without meningitis. The sensitivity, specificity, PPV, NPV, cut-off and AUC values for IL-6, IL-8, and D-lactate were shown in Table 7. Accordingly, when the performance of IL-6 to differentiate between the group with nosocomial meningitis and the group with pleocytosis and without meningitis was examined, AUC for IL-6 was 0.774 ($P < 0.001$). For IL-8, the AUC was 0.643 ($P = 0.037$) [Figure 1]. When the performance of IL-6 for differentiating between the group with meningitis and the control group was examined, AUC for IL-6 and IL-8 was statistically significant (AUC 0.894 vs. 0.832, $P < 0.001$).

In addition, D-lactate level in CSF of patient, which was calculated using colorimetric assay, was found to be statistically significant for the diagnosis of meningitis ($P < 0.001$). When all samples were evaluated, median D-lactate level was found to be 0.807 (0.003–22.04) μmol/mL. In the group with nosocomial meningitis, median D-lactate level was 2.45 (0.332–22.04) μmol/mL. D-lactate level was significantly higher in the group with meningitis compared to the control group ($P < 0.001$). D-lactate level was found to be higher in the group with meningitis compared to the group with pleocytosis without the diagnosis of meningitis ($P = 0.002$) [Table 5]. For D-lactate, AUC was 0.807 ($P < 0.001$) [Table 7]. On comparing the AUC values given in Table 7, no statistically significant difference was found.

For D-lactate, AUC was 0.723 ($P < 0.001$) (the cut-off value for D-lactate is >1.05) [Figure 1]. For IL-17 and TNF-α tests, ROC analysis did not yield a significant threshold ($P = 0.339$ and $P = 0.392$, respectively). Thus, in our study, the potential to use IL-17 and TNF-α as values to differentiate between the group with nosocomial meningitis and the group with pleocytosis without the diagnosis of meningitis was inconclusive.

In the CSF samples with pleocytosis without the diagnosis of meningitis and in the CSF samples diagnosed with nosocomial meningitis, different combinations of IL-6, IL-8, and D-lactate levels were compared. When the individual examination and the combined examination of IL-8 and D-lactate were compared, there was no statistical difference between AUC of both approaches. Also, when the individual examination and the combined examination of IL-6 and D-lactate were compared, there was no statistical difference between AUC

### Table 6. Comparison of cytokine and D-lactate levels of patients with meningitis and pleocytosis patients without meningitis.

| Parameter       | Median (range) | Meningitis (n=8) | Pleocytosis (n=19) | P     |
|-----------------|----------------|------------------|-------------------|-------|
| IL-6 (pg/mL)    | 448 (9–760)    | 94 (9–423)       | 0.020*            |
| IL-8 (pg/mL)    | 1370 (29–1518) | 633 (0.01–1468)  | 0.084             |
| D-lactate (μmol/mL) | 3.59 (0.525–5.74) | 1.375 (0.11–5.945) | 0.058           |
| IL-17 (pg/mL)   | 0.01 (0.01–1203) | 0.01 (0.01–2378) | 0.481             |
| TNF-α (pg/mL)   | 0.01 (0.01–1168) | 0.01 (0.01–413)  | 0.515             |

*Statistically significant. IL: interleukin, TNF: tumour necrosis factor

### Table 7. ROC analysis of cerebrospinal fluid cytokine and D-lactate levels between groups.

| Variable                  | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Cut-off value | AUC   | P*  |
|---------------------------|-----------------|-----------------|---------|---------|---------------|-------|-----|
| **Nosocomial meningitis group vs. pleocytosis without meningitis group** |                 |                 |         |         |               |       |     |
| IL-6 (pg/mL)              | 55.17           | 94.74           | 88.9    | 73.5    | >440          | 0.774 | <0.001|
| IL-8 (pg/mL)              | 44.83           | 84.21           | 68.4    | 66.7    | >1249         | 0.643 | 0.037|
| D-lactate (μmol/mL)       | 75.86           | 63.16           | 61.1    | 77.4    | >105          | 0.723 | <0.001|
| IL-6 and IL-8             | 55.17           | 94.74           | 88.9    | 73.5    | >0.5317       | 0.790 | <0.001|
| IL-6 and D-lactate        | 62.07           | 89.47           | 81.8    | 75.6    | >0.5740       | 0.779 | <0.001|
| IL-8 and D-lactate        | 82.76           | 60.53           | 61.5    | 82.1    | >0.3134       | 0.730 | <0.001|
| IL-6 and IL-8 and D-lactate| 55.17         | 94.74           | 88.9    | 73.5    | >0.60364      | 0.801 | <0.001|
| **Nosocomial meningitis group vs. control group** |                 |                 |         |         |               |       |     |
| IL-6 (pg/mL)              | 79.31           | 87.27           | 76.7    | 88.9    | >70           | 0.894 | <0.001|
| IL-8 (pg/mL)              | 89.66           | 63.64           | 56.5    | 92.1    | >90           | 0.832 | <0.001|
| D-lactate (μmol/mL)       | 79.31           | 72.73           | 60.5    | 87.0    | >0.861        | 0.807 | <0.001|

*Model refers to multivariate receiver operating characteristic (ROC) analyses of the combinations of IL–6 (pg/mL) and IL–8 (pg/mL). *Statistically significant. AUC: area under the ROC curve, IL: interleukin, NPV: negative predictive value, PPV: positive predictive value.
of both approaches. On comparing AUC of IL-6 and IL-8, there was statistically significant difference between them. But there was no statistical difference between the combined examination of IL-6 and IL-8 and the individual examination.

In cases of nosocomial meningitis, assuming the threshold of D-lactate level to be >1.05 µmol/mL, the sensitivity and the specificity were found to be 75.86% (95% confidence interval [CI] 56.5–89.7) and 63.16% (95% CI 46.0–78.2), respectively [Table 7]. Also, upon comparison of cytokine values between male and female gender in the meningitis and pleocytosis without meningitis groups, there was no statistically significant difference found.

**DISCUSSION**

Majority of postneurosurgical CNS infections are of bacterial origin and lead to the development of nosocomial meningitis.[17] Although not commonly seen, the incidence of nosocomial meningitis among neurosurgery patients in the postoperative period is approximately 4%.[1,3] Clinical signs of meningitis in neurosurgery patients, such as new-onset fever and/or consciousness alterations, are also observed following neurosurgical operations. Therefore, in neurosurgery patients, the diagnosis of nosocomial meningitis may be mistaken or delayed.[18] A definitive diagnosis of nosocomial meningitis could be made based on the isolation of bacteria from CSF samples. However, in patients with a clinical suspicion of nosocomial meningitis, bacterial growth in CSF may not always be observed, and the inability to differentiate between nosocomial meningitis and aseptic meningitis results in unnecessary long-term antibiotic use.[1-4,19,20] In some patients, due to the previous use of antibiotics, the negative culture results may be insufficient to exclude the possibility of bacterial meningitis.[6]

Therefore, diagnostic tests that ensure rapid diagnosis of nosocomial meningitis are required. Other than Gram staining, a reliable and rapid test for the diagnosis of nosocomial meningitis has not been established for clinical use.[6] In our study, Gram staining of CSF revealed the presence of bacteria in 11 (9.0%) subjects. Increase in leucocyte counts and protein levels and decrease in glucose levels of CSF are the commonly used parameters to evaluate nosocomial bacterial meningitis. In a study performed by Ross et al.[4] among neurosurgery patients, CSF leucocyte count above 1000/mL had a sensitivity of 61% and a specificity of 68% for the diagnosis of nosocomial meningitis. On the other hand, although the sensitivity of polymorphonuclear leucocyte (PMNL) predominance in CSF was 94%, the specificity was found to be only 28%. In most patients who experienced intracranial haemorrhage, PMNL predominance in CSF may be observed. Therefore, leucocyte counts are not sufficient for differentiating nosocomial meningitis and pleocytosis without meningitis.[1,10] In our study, the sensitivity and specificity of the CSF leucocyte count for the diagnosis of nosocomial meningitis were 89% and 74%, respectively. Based on the CSF leucocyte count determined using direct microscopic examination, no significant difference was found across the groups. When the group with nosocomial meningitis and the group with pleocytosis without meningitis were compared, direct microscopic examination did not reveal any significant difference in terms of leucocyte counts.

In CNS infections, bacteria and bacterial products cause the production of intrathecal proinflammatory and anti-inflammatory cytokines.[11,21] TNF-α, IL-1, IL-6 and IL-8 are cytokines that occur early in infection.[21,22] IL-17 is a potent proinflammatory cytokine produced by CD4 memory Th17 cells.[14,23] In a study that demonstrated elevated levels of IL-6 in acute meningitis, the threshold value of IL-6 for the diagnosis of meningitis was 1065.96 pg/mL, and IL-6 had a sensitivity of 76.2% and specificity of 100%.[24] However, in some studies conducted in children, the elevation observed in the level of IL-6 was not
found to be statistically significant for differentiation between aseptic meningitis and bacterial meningitis.\(^\text{9,25}\) In another study, with a CSF IL-6 level of >90 pg/dL, the specificity and sensitivity for bacterial meningitis were 100% and 95%, respectively.\(^\text{10}\) Similarly, IL-6 level was significantly higher in the nosocomial meningitis group compared to the control group in our study. IL-6 level was substantially higher in the group with nosocomial meningitis compared to the group with pleocytosis without meningitis.

Studies have demonstrated increased levels of the proinflammatory cytokines TNF-\(\alpha\), IL-1 and IL-6 in traumatic and ischaemic intracranial cases during normal recovery period. On the other hand, it was thought that IL-8 levels were high in nosocomial meningitis due to other causal factors because IL-8 leads to PMNL chemotaxis.\(^\text{26,27}\) However, in two studies performed in patients with bacterial meningitis, no correlation was observed between IL-8 levels and granulocyte counts in CSF.\(^\text{14}\) In the study performed by Seki et al.\(^\text{22}\) to investigate the level of IL-8 in bacterial meningitis, it was found that IL-8 levels (224 \(\pm\) 2.57 pg/mL) were significantly different between the group with bacterial meningitis and the group with aseptic meningitis, and that the diagnostic value of leucocyte counts was as significant as that of protein and glucose levels. In the same study, IL-6 was also found to be high in aseptic meningitis and bacterial meningitis cases, and this was considered a sign of meningeal inflammation. In the study performed by López-Cortés et al.,\(^\text{28}\) in bacterial meningitis, IL-8 level was high and the threshold value was 2.5 ng/dL. In several studies, the threshold values vary by the method and the commercial kit used. IL-8 may be significantly useful as a secondary parameter to reinforce the fast diagnosis of bacterial meningitis in addition to standard markers discussed.\(^\text{13,29}\) In our study, for IL-8 testing, when the threshold was considered to be >90 pg/mL, the sensitivity and specificity were 89.66% and 63.6%, respectively. In addition, NPV was found to be 92.1%.

In the study performed by López-Cortés et al.,\(^\text{30}\) following a neurosurgical operation, the sensitivity and the threshold value of TNF-\(\alpha\) were 74% and 150 pg/mL, respectively, in differentiating between pleocytosis related to aseptic meningitis in CSF and pleocytosis due to a bacterial CSF infection.

Levels of TNF-\(\alpha\) were markedly higher in children with meningitis compared to those without meningitis.\(^\text{9,25}\) However, 84.6% of aseptic meningitis patients were positive for TNF-\(\alpha\).\(^\text{9}\) It was observed that the levels of TNF-\(\alpha\) were decreased following antibiotic therapy.\(^\text{25}\) In some studies, TNF-\(\alpha\) was not found to be correlated with bacterial meningitis.\(^\text{9,31-33}\) In our study, among the patients with nosocomial meningitis, TNF-\(\alpha\) levels did not yield significant results when the group with aseptic meningitis and the group with nosocomial meningitis were compared with the control group.

Contrary to previous studies, the study performed by Asano et al.\(^\text{14}\) reported high levels of IL-17 in bacterial and aseptic meningitis cases. As compared to the other cytokines, there are few studies studying IL-17 in bacterial meningitis. In our study, despite high levels of IL-8 and IL-6 found in the patients, IL-17 levels were very low and had no significant threshold value. Accordingly, the performance of IL-17 and TNF-\(\alpha\) tests to differentiate between the group with nosocomial meningitis and the control group was not significant in our study.

D-lactate is a parameter that can be helpful for the fast diagnosis of bacterial meningitis.\(^\text{31}\) High concentrations of D-lactate in CSF lead to altered mental status and encephalopathy.\(^\text{34}\) In addition, CSF levels of lactate do not vary by the presence of erythrocytes.\(^\text{1}\) In a study performed by Chen et al.,\(^\text{27}\) D-lactate was found to have sensitivity and specificity of 94.7% and 79.7%, respectively, for the diagnosis of bacterial meningitis. Similarly, in our study, concentrations of D-lactate were higher in the patients with nosocomial meningitis compared to other groups.

When IL-6 and D-lactate were concurrently evaluated, the specificity (89.47%) and PPV (81.8%) were higher, whereas the concomitant evaluation of IL-8 and D-lactate showed higher NPV (82.1%). In the pleocytosis without meningitis CSF samples and in the nosocomial meningitis CSF samples, when the different combinations of IL-6, IL-8 and D-lactate levels were compared, there was statistically significant difference between them (\(P<0.001\)). We opined that more significant results could be obtained in terms of the diagnosis if the parameters were collectively evaluated. Zhang et al.\(^\text{15}\) also evaluated the combination of CSF procalcitonin, lactate, IL-8 and IL-10 concentrations for the diagnosis of postneurosurgical bacterial meningitis; they concluded that the combination of several markers may improve the diagnostic accuracy in detecting postneurosurgical bacterial meningitis.

Consequently, we investigated the contributions of the IL-6, IL-8, IL-17 and TNF-\(\alpha\) cytokines and D-lactate to the diagnosis of nosocomial meningitis. Although IL-6 and IL-8 levels were high in pleocytosis resulting from neurosurgical interventions, we observed that this increase was greater in bacterial CNS infections. In neurosurgery patients, the specificity and sensitivity of D-lactate, IL-8 and IL-6 were found to be high in differentiating between aseptic and infection-related pleocytosis. We believe that these parameters are valuable for the fast differential diagnosis between nosocomial meningitis and aseptic meningitis. However, studies conducted with larger patient groups are required to obtain definitive results.

Our study has several limitations that need to be considered. First, the sample size was small. Second, non-culture-based diagnostic laboratory tests, specific antibody titres or molecular tests for bacteria (e.g., multiplex polymerase chain reaction)
were not used in patients because the molecular tests are not used routinely in our hospital.

In conclusion, our study findings suggest that IL-6, IL-8 and D-lactate levels could be diagnostic markers for nosocomial meningitis.

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**Conflicts of interest**
There are no conflicts of interest.

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