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The Relation of Cerebrospinal Fluid Nitric Oxide Levels to Prognosis and Differential Diagnosis of Meningitis*

Abstract: This study was designed to investigate the role of nitric oxide (NO) in the differential diagnosis of bacterial, tuberculous and viral meningitis, and the relation between cerebrospinal fluid (CSF) NO levels and meningitis prognosis.

Twenty patients with bacterial meningitis, 9 with tuberculous meningitis, 11 with viral meningitis/meningoencephalitis and 21 control patients were included in the study. CSF NO levels were investigated by measuring the levels of nitrite with a colorimetric test.

Mean CSF nitrite levels were 3.9 - 2.0 µmol/l in bacterial meningitis, 2.7 - 1.9 µmol/l in tuberculous meningitis, 1.9 - 1.7 µmol/l in viral meningitis/meningoencephalitis and 1.4 ± 1.1 µmol/l in control groups. The patients with bacterial and tuberculous meningitis had higher CSF nitrite levels than the control group (p < 0.05), but the patients with viral meningitis/meningoencephalitis did not (p > 0.05). However, there was no significant difference between bacterial and tuberculous meningitis or between tuberculous meningitis and viral meningitis/meningoencephalitis groups.Nitrite levels were correlated with white blood cell (WBC) counts (r = 0.567, p = 0.000), protein (r = 0.548, p < 0.001) and glucose levels (r = -0.271, p < 0.05).

In conclusion, although the measurement of CSF nitrite levels is helpful for the differential diagnosis of meningitis, this parameter is not superior to other routine parameters. However, it may have a characteristic effect on prognosis.

Key Words: Meningitis, nitric oxide, sequela, prognosis

Introduction

Meningitis is an inflammation of the cerebrospinal membranes that may develop from infectious or non-infectious etiology. Although the etiologies are different, clinical symptoms and signs are similar in meningitis. Thus, macroscopic appearance, examination of white blood cells (WBC), biochemical tests (protein, glucose, lactate), serological tests, culture and direct microbiological examination of cerebrospinal fluid (CSF) are very important in the differential diagnosis of the meningitis. In spite of these examinations, because of normal, almost normal or atypical CSF findings, difficulties in differential diagnosis are often confronted (1,2).

Meningitis, especially bacterial, has characteristics of rapid progression and a high mortality rate, as well as having the potential of forming permanent neurological or audiological sequelae. Therefore, specific treatment must be started rapidly. However, despite effective treatment, there is no important difference in the mortality and sequelae of meningitis for the last 30 years (3). Recently, attention has shifted to the pathophysiology of meningitis and adjuvant therapy. Several studies suggest that various inflammatory mediators including cytokines, platelet activating factor, arachidonic acid metabolites and reactive oxygen species contribute to the pathological process of meningitis, and the development of neuronal injury (4-9). Recent research suggests that nitric oxide (NO) may have some important pathophysiological effects during bacterial meningitis (10-13). However, the role of this molecule is unclear in other types of meningitis (14,15). NO is produced from L-arginine by nitric oxide synthase (NOS) in neutrophils, macrophages, vascular endothelial cells, astrocytes, microglias and neurons in response to several immunological stimulations and cytokines. NO is not a stable substance, and it converts into nitrite and nitrate in 5-10 seconds. It was established that there are roles of NO in microvascular injury, cerebral edema, CSF.

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pleocytosis, neurological injury and necrotic cell death in oligodendroglia (9,10,12,13,16).

In this study, we aimed to investigate the role of NO in the differential diagnosis and prognosis of meningitis by determining the CSF levels of nitrate and nitrite, the stable end products of the NO metabolism.

Materials and Methods

Forty patients with meningitis and 21 control subjects were included in this study between January 1998 and March 2000. The study was approved by the institutional ethics committee.

In the presence of clinical evidence of meningitis, the patients were examined for peripheral blood WBC count, serum CRP levels, CSF pleocytosis, macroscopic appearance and biochemical characteristics of CSF. The patients were divided into three groups as bacterial meningitis, tuberculous meningitis and viral meningitis/meningoencephalitis. (i) Bacterial meningitis was defined as the presence of clinical signs of meningitis, highly elevated serum C-reactive protein (CRP), polymorphonuclear pleocytosis in CSF and/or positive bacterial culture of CSF. (ii) The diagnosis of tuberculous meningitis was based on the relevant clinical and laboratory findings, pleocytosis in CSF (which, depending on the duration of the disease, was dominated by polymorphonuclear or mononuclear cells), negative culture for other bacteria and fungi, plus two or more of the following - showing of acid-fast bacilli on microscopy or isolation of Mycobacterium tuberculosis from a culture of CSF, positive tuberculin skin test, evidence of tuberculosis in another site of the body and clinical response to anti-tuberculous treatment. (iii) The diagnosis of viral meningitis/meningoencephalitis was based on clinical manifestations, negative or slightly elevated serum CRP, mononuclear pleocytosis in CSF, and negative bacterial and fungal cultures. The control group consisted of subjects who presented meningeal irritation signs that required analysis of CSF to exclude the presence of meningitis or diseases of central nervous system. They were later found to be free of such diseases based on the results of CSF examinations and negative clinical findings during follow up. Patients treated with antibiotics or anti-inflammatory agents before lumbar puncture were excluded from the study. CSF samples, obtained on the first day of admission, were divided into two parts: one part was used in routine examinations, and the other (3 ml) was stored at -20 °C for study.

The measurement of CSF nitrate and nitrite levels

The concentrations of nitrite and nitrate, the stable end products of NO in CSF, were measured using a colorimetric test (17). In the first step, nitrite levels in CSF were determined by Griess reactive, which converts nitrite into a deep purple azo compound (Nitrite/nitrate Colorimetric Assay Kit Boehringer, Mannheim, Germany). The absorbance of standards and samples were measured by spectrophotometer at 550 nm. Later, the combined levels of nitrite and nitrate were determined by the same method after the enzymatic reduction of nitrate to nitrite using nitrate reductase (1 U/ml). The CSF nitrate concentration was calculated by subtracting the nitrite level from the combined level. The detection limits of nitrite and nitrate were 0.3 and 0.2 µmol/l respectively.

Statistical analysis

Statistical analysis was made by variant analysis in SPSS version 10.0. LSD multiple comparison test was used for the determination of differences between the groups.

Correlations between parameters were evaluated in all study subjects and controls, and were calculated by Pearson’s method. A p value < 0.05 denoted the presence of statistical significance.

Results

Forty patients (23 males, 17 females) were included in this study. The mean ± SD age of the patients was 34.7±18 (median: 31.0, range: 14-78 years). Twenty patients had bacterial meningitis, nine tuberculous meningitis and 11 viral meningitis/meningoencephalitis.

Twenty-one subjects with a mean age of 36 ± 18 (median: 30, range: 14-73, 12 males and 9 females) were included as a control group.

In the bacterial meningitis group, Streptococcus pneumoniae was isolated in four, Neisseria meningitidis in two, and Bacillus anthracis in one of the patients. No causative agent was detected in the other 13 cases. In the tuberculous meningitis group, Mycobacterium tuberculosis was detected by culture and/or direct examination of CSF in four cases. In the viral meningitis/meningoencephalitis group, although virus isolation was not conducted, it was assumed that viruses
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were the causative agents. According to clinical features, two patients were diagnosed as having Herpes simplex encephalitis and two mumps meningitis.

Six patients (3 bacterial, 2 tuberculous, 1 viral meningitis/meningoencephalitis) died, and three patients developed neurological or audiological sequelae (2 bacterial and 1 tuberculous meningitis).

The CSF nitrite, nitrate, protein, glucose and WBC values of the patients and control subjects are shown in Table 1.

Nitrite levels in the CSF samples of the patients with bacterial and tuberculous meningitis were significantly elevated in comparison with the control group (p < 0.01, p < 0.05, respectively). However, no significant elevations were found in patients from the viral meningitis/meningoencephalitis group (p > 0.05). The highest mean value of nitrite (3.9±2.0 µmol/l) was detected in the bacterial meningitis group. It was observed that CSF nitrite levels had a more homogeneous distribution than nitrate levels (Table 1).

When comparing nitrite levels, a significant difference was found between the bacterial and viral meningitis/meningoencephalitis groups (p < 0.01), but there was no difference between bacterial - tuberculous meningitis groups (p > 0.05), and the viral meningitis/meningoencephalitis - tuberculous meningitis groups (p > 0.05).

No significant difference was found between the CSF nitrite levels of the patients who died or those who recovered (p > 0.05). In bacterial meningitis group, however, the CSF nitrite levels of three patients who died (4.5, 5.3, 9.1 µmol/l) and one patient who had sequela (4.4 µmol/l) were higher than the mean value (3.9 µmol/l) of this group, but the others were not. The CSF nitrite level of the patient who had sequela in the tuberculous meningitis group (5.5 µmol/l) was also higher than the mean levels of this group (2.7 µmol/l). (Table 2).

Although mean protein and glucose levels of the bacterial and tuberculous meningitis groups were significantly different from the viral meningitis/meningoencephalitis and control groups (p < 0.05 when comparing the glucose levels of the bacterial meningitis and control group, and p < 0.01 for the

Table 1. Clinical and laboratory characteristics of the patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>BM (^a) (mean ± SD) n = 20</th>
<th>TM (^b) (mean ± SD) n = 9</th>
<th>VM (^c) (mean ± SD) n = 11</th>
<th>Control (mean ± SD) n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>34 ± 18</td>
<td>40 ± 19</td>
<td>30 ± 10</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>(15 - 78)</td>
<td>(16 - 65)</td>
<td>(14 - 73)</td>
<td>(14 - 73)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>14</td>
<td>2</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (^d)/mm(^3)</td>
<td>3606.5 ± 2135.6 (1390 - 8500)</td>
<td>287.8 ± 168.6 (50 - 510)</td>
<td>108.2 ± 149.6 (10 - 500)</td>
<td>10 ± 8.4</td>
</tr>
<tr>
<td>(700 - 7250)</td>
<td>(20 - 270)</td>
<td>(0 - 130)</td>
<td>(0 - 20)</td>
<td></td>
</tr>
<tr>
<td>PNL (^e)/mm(^3)</td>
<td>2807.5 ± 1737.0 (220 - 3000)</td>
<td>91.1 ± 73.4 (20 - 400)</td>
<td>15.5 ± 38.6 (10 - 370)</td>
<td>0</td>
</tr>
<tr>
<td>(700 - 7250)</td>
<td>(20 - 400)</td>
<td>(0 - 130)</td>
<td>(0 - 20)</td>
<td></td>
</tr>
<tr>
<td>MNL (^f)/mm(^3)</td>
<td>799 ± 717.3 (6 - 75)</td>
<td>196.7 ± 138.1 (6 - 75)</td>
<td>92.7 ± 114.6 (5 - 151)</td>
<td>56.7 ± 9.7</td>
</tr>
<tr>
<td>(700 - 7250)</td>
<td>(20 - 400)</td>
<td>(0 - 130)</td>
<td>(0 - 20)</td>
<td>(38 - 76)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>42.8 ± 15.3 (6 - 75)</td>
<td>27.3 ± 19.2 (6 - 75)</td>
<td>68.2 ± 40.8 (5 - 151)</td>
<td>56.7 ± 9.7</td>
</tr>
<tr>
<td>(1390 - 8500)</td>
<td>(20 - 400)</td>
<td>(10 - 370)</td>
<td>(5 - 151)</td>
<td></td>
</tr>
<tr>
<td>Protein, mg/dl</td>
<td>244.95 ± 141.3 (244.95 - 141.3)</td>
<td>190.78 ± 94.4 (99 - 358)</td>
<td>45.27 ± 20.0 (21 - 87)</td>
<td>31.57 ± 9.4</td>
</tr>
<tr>
<td>(60 - 570)</td>
<td>(20 - 400)</td>
<td>(10 - 370)</td>
<td>(5 - 151)</td>
<td></td>
</tr>
<tr>
<td>Nitrata, µmol/l</td>
<td>7.7 ± 4</td>
<td>14.7 ± 18.6 (1.3 - 14.3)</td>
<td>6.6 ± 4.7 (1.9 - 62.6)</td>
<td>5.5 ± 3.9</td>
</tr>
<tr>
<td>(4 - 7)</td>
<td>(1.9 - 62.6)</td>
<td>(0.6 - 17.0)</td>
<td>(0.2 - 12.9)</td>
<td></td>
</tr>
<tr>
<td>Nitrite, µmol/l</td>
<td>3.9 ± 2.0 (1.4 - 9.1)</td>
<td>2.7 ± 1.9* (1.4 - 9.1)</td>
<td>1.9 ± 1.1 (1.4 - 9.1)</td>
<td>1.37 ± 0.8</td>
</tr>
<tr>
<td>(1.3 - 14.3)</td>
<td>(0.8 - 6.2)</td>
<td>(0.6 - 3.4)</td>
<td>(0.3 - 2.9)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)BM: Bacterial meningitis, \(^b\)TM: Tuberculous meningitis, \(^c\)VM: Viral meningitis/meningoencephalitis, \(^d\)WBC: White blood cell, \(^e\)PNL: Polymorphonuclear leukocyte, \(^f\)MNL: Mononuclear leukocyte, * p < 0.05 compared with control group

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others), no difference was found between the bacterial -
tuberculous meningitis groups and the viral
meningitis/meningoencephalitis - control groups
(p>0.05).

We investigated the correlations between nitrite levels
and WBC counts, and protein and glucose levels of CSF. A
positive correlation was found between nitrite levels and
WBC counts (r = 0.567, p < 0.001), and nitrite levels and
protein levels (r = 0.548, p < 0.001). A negative
correlation was found between nitrite and glucose levels
(r = -0.271, p < 0.05). No correlation was found
between nitrite and nitrate levels, or age and sex.

Discussion

The first step of the inflammatory process in
meningitis, which is triggered by entry of the pathogen
into subarachnoid space, is the releasing of cytokines,
interferons, arachidonic acid metabolites, platelet-
activating factor and complement components by
stimulation of lipopolysaccharide, lipoteichoic acid,
peptidoglican, bacterial toxins or viral components
(7,8,18). By induction of these inflammatory mediators,
a variety of effector final mediators including reactive
oxygen species (superoxide, peroxynitrite, etc.), reactive
nitrogen species (NO) and excitatory amino acids
(glutamate, aspartate, taurine, alanine) are produced
(4,19). These mediators are responsible for changes in
the central nervous system during meningitis. However,
the mechanisms responsible for damage in the central
nervous system are not yet completely understood. In
recent experimental studies, it was observed that NO
destroyed the blood-brain barrier either directly or
mediately by the effect of TNF-α (9,20,21). By
administration of NO synthase (NOS) inhibitors, an
increase in regional blood flow and destruction of the
blood-brain barrier was prevented. Furthermore,
decreasing brain edema, intracranial pressure and CSF
leukocyte count were observed (13,22).

However, Leib et al. (23) demonstrated that
production of NO was reduced by the administration of
aminoguanidin, a NOS inhibitor, in early and late stages of
experimental meningitis, but CSF bacteria count and
convulsion incidence increased. Based on these results,
they claimed that NO was useful in reducing cerebral
ischemia. In addition to experimental studies, several
clinical studies showed increased CSF-NO levels in
bacterial meningitis (11,12,15,24). Similar results were
reported in studies concerning tuberculous meningitis
(25,26).

Although the role of NO is more evident in bacterial
meningitis, its role in viral meningitis is not clear.
Shigemoto et al. (14) reported that there was inducible
nitric oxide synthase (iNOS) induction and an elevation of
NO levels in the brain tissue of rats, and clinical healing
was observed after the administration of iNOS inhibitors
in the experimental model of encephalitis with Herpes
simplex type-1. Milstien et al. (27) observed an elevation
of CSF nitrite/nitrate levels in a small group of patients
with viral meningitis. However, an increase in CSF-NO
levels was not observed in the other clinical studies
concerning viral meningitis (15,24,26). In this study, we
did not observe an elevation in the CSF nitrite levels of
the patients with viral meningitis/meningoencephalitis
compared with the control subjects.

According to the results of several studies, Gram-
negative bacteria induce TNF-α stronger than Gram-
positive bacteria, and cause higher CSF-NO levels
(10,15,28). Since we detected Gram-positive bacteria
only in two cases, we could not perform such an
evaluation. The highest nitrite level (9.1 µmol/l) in our
study was measured in the CSF of the patient with
anthrax meningitis who died 6 hours after
hospitalization. The cause of the highest level might be
due to the severe clinical progress of the patient or the
hemorrhagic characteristic of the CSF.

Nitrate is the major metabolic end product of NO in
the circulation, and it was suggested that elevated nitrate
levels of CSF during bacterial meningitis were the result

<table>
<thead>
<tr>
<th>Table 2. CSF nitrite levels (µmol/l) of the patients who died and those who had sequelae.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>who died</td>
</tr>
<tr>
<td>n = 6</td>
</tr>
<tr>
<td>BM</td>
</tr>
<tr>
<td>TM</td>
</tr>
<tr>
<td>VM</td>
</tr>
</tbody>
</table>

BM: Bacterial meningitis, TM: Tuberculous meningitis,
VM: Viral meningitis/meningoencephalitis
of either diffusion from the disrupted blood-brain barrier or enhanced production of NO in bacterial meningitis (10). However, CSF is comparable to an oxygen containing aqueous solution, a condition in which NO is oxidized primarily to nitrite with little or no formation of nitrate, and the CSF concentration of nitrite per se may provide a more specific parameter for gauging endogenous NO production in the central nervous system (15). In addition, according to previous studies (10,12,15,24,26,29), CSF nitrate levels during bacterial meningitis are more heterogeneous than nitrite levels, as shown in this study. Therefore, it was considered that the CSF nitrite level is an indicator of endogenous NO production in CSF. For these reasons, we took nitrite levels into consideration as an indicator of NO production in central nervous system.

Murawaska et al. (24) reported the correlations between CSF nitrite-leukocytes, and nitrite-protein levels in bacterial meningitis, as we observed in this study. If it is kept in mind that NO contributes to meningeal inflammation, destroys the blood-brain barrier and facilitates protein transporting to CSF, it is logical to assume the presence of a correlation between CSF nitrite and protein. In studies that did not detect a correlation between CSF nitrite level and leukocyte count, it was emphasized that a "major source of nitrite was not inflammatory cells" (10,30). We think this opinion is correct. Although we found a significant correlation between these two parameters, the correlation was not strong. We also found a negative correlation between CSF nitrite and glucose levels. Similar results have been reported in other studies (10,29). The low glucose levels may be explained by the inhibition of mitochondrial respiration that enhances anaerobic glycolysis through excessive NO production (31,32). In addition, inhibition of the carrier-mediated transport system across the blood-brain barrier causes decreased CSF glucose levels in bacterial meningitis.

Recent studies have suggested that NO may be responsible for neurological and audiological sequelae in bacterial meningitis (10,33). In our study, two patients with bacterial meningitis and one patient with tuberculous meningitis developed neurological or audiological sequelae. Two of the three patients (one bacterial and one tuberculous meningitis) had higher levels of nitrite than the median levels of their own groups. No significant difference was found between the CSF nitrite levels of the patients who died and those who recovered (p > 0.05). However, the CSF nitrite levels of three patients who died in the bacterial meningitis group were higher (4.5, 5.3, and 9.1 µmol/l) than the mean value of this group. The absence of significant differences between the patients who died and those who recovered may be due to the limited number of patients. Further studies are required to evaluate the relationship between NO and prognosis.

According to our data, CSF glucose and protein levels were not helpful for the differentiation of bacterial and tuberculous meningitis. However, our case numbers were limited, and our results suggest that CSF nitrite measurements were also not helpful for the differential diagnosis of bacterial and tuberculous, or tuberculous and viral meningitis.

In conclusion, although CSF nitrite measurement is no more useful than other routine examinations in the differential diagnosis of meningitis, enhanced NO production may have an important effect on prognosis and may contribute to the pathophysiology of bacterial and tuberculous meningitis.

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