Recent Advances in the Pathophysiology of Meningitis in Children

Magdy M.K. Mahmoud,1 Abdel-Raouf Omar,2 Gharib Fawy,3 and Khaled Salah4
From the Departments of Pediatrics,1 Neurology2,3 and Clinical Pathology,4 El-Minia1,2,4 and Sohag3 Universities

Abstract:
Over the past decade, much has changed on the landscape of meningitis. The purpose of this study was to investigate the involvement of nitric oxide (NO) and tumor necrosis factor alpha (TNF-α) in the pathogenesis of childhood meningitis. We measured the concentration of NO₂ (a stable metabolite of NO) and TNF-α in serial samples of cerebrospinal fluid (CSF) from 21 children with septic and 18 with aseptic meningitis and 20 control patients without meningitis. Significantly higher CSF NO₂ concentrations were detected in those with bacterial meningitis than those with aseptic meningitis (27.6 ± 26.8 versus 12.2 ± 12.3 µmol/l; P<0.001) or among non-meningitis subjects (13.2 ± 24.2 µmol/l; P<0.0001). Clinical and laboratory improvement following administration of antibiotics and dexamethasone was associated with a fall in CSF (NO₂) to normal levels in these patients. The mean (±SD) of concentration in septic meningitis was 148.74 ± 338.77 pg/ml. There was significantly more TNF-alpha than aseptic meningitis (6.85 ± 17.93 pg/ml; P<0.001) or non-meningitis (7.67 ± 16.07 pg/ml; P<0.001). We did not find a correlation between CSF nitrate/nitrite levels and TNF-α (r = 0.046). Our findings indicate that NO and TNF-α production are enhanced in the CSF compartment of children with septic meningitis and support the hypothesis that both markers are involved in the pathophysiology of septic meningitis.

Abbreviations: NO: Nitric oxide, NOS: Nitric oxide synthases, TNF-α: Tumor Necrosis Factor Alpha.

Introduction:
Nitric oxide (NO) is a labile free radical produced by a variety of cells and is involved in such physiologic processes as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated cytotoxicity.1-3 NO is formed by the oxidative deamination of the amino acid L-arginine by nitric oxide synthases (NOS). Three isoforms of this enzyme are described. Neuronal NOS (nNOS or NOS1) is constitutively present in both the central and peripheral nervous systems, where NO acts as a neurotransmitter. Endothelial NOS (eNOS or NOS3) is constitutively expressed by endothelium and other cell types and is involved in cardiovascular homeostasis. In contrast, inducible NOS (iNOS or NOS2) is absent in resting cells, but the gene is rapidly expressed in response to stimuli such as proinflammatory cytokines. Once present, iNOS synthesises 100–1000 times more NO than the constitutive enzymes and does so for prolonged periods. This high concentration of NO may inhibit a large variety of microbes, but may also potentially damage the host, thereby contributing to pathology.4-7 The antimicrobial effect stems not from NO itself, but from reactive nitrogen intermediates formed by the oxidation of NO. Reaction between NO and the free radical superoxide (OD⁻) results in the formation of the unstable molecule peroxynitrite (OONO⁻), while that between NO and thiol groups produces nitrosothiols. These reactive nitrogen intermediates inactivate key microbial enzymes, such as ribonucleotide reductase and aconitase, by reacting with iron containing groups in these enzymes.6,9 In bacterial meningitis, meningeal inflammation is initiated by local production of proinflammatory cytokines, especially tumor necrosis factor alpha (TNF-α) and interleukin 1β,10,11 which are both potent stimuli of iNOS expression in leucocytes, glia, and neurons. Whether NO acts as a neurotoxic or neuroprotective molecule may be dependent on its redox state; in its oxidized form (NO +) inactivates glutamate receptors and reduces the neurotoxicity of excitatory amino acids, whereas the reduced form (NO⁻) can form peroxynitrate, a potent neurotoxin.5,7 The therapeutic use of NOS inhibitors in bacterial meningitis has been suggested, but as NO is likely to have profound effects on cerebral blood flow and other important physiological processes, our understanding of the role of NO in meningitis seems inadequate to justify this intervention at present.12-14

In this study, we investigated the role of NO production in childhood meningitis by determining the levels of NO₂ in cerebrospinal fluid samples. We also investigated the relationship between CSF nitrates/nitrite and TNF-alpha levels.
Subjects and Methods:
The study population consisted of 40 patients: 21 children with septic meningitis (12 boys and 9 girls) with an age range from 2 months to 8 years (mean ± SD, 3.2 ± 2.2 years) and 19 children with aseptic meningitis (11 boys and 8 girls) with an age range from 6 months to 7 years (2.6 ± 2.1 years). Septic meningitis was diagnosed by the presence of positive bacterial cultures of CSF and blood, in addition to clinical manifestations of meningitis, highly elevated CRP, and polymorphonuclear pleocytosis in CSF. Children with septic meningitis were treated with intravenous beta-lactam antibiotics (e.g. ampicillin, cefotaxime, ceftriaxone) with dexamethasone (0.6 mg/kg/day in 4 divided doses) at least for 2 weeks and were observed, clinically and laboratory, for another 2 weeks. All patient showed clinical and laboratory response to the treatment. The diagnosis of aseptic meningitis depended on the clinical manifestations, negative CRP, mononuclear pleocytosis in CSF, and negative bacterial cultures of CSF.

The control group consisted of 20 children who presented with fever that required analysis of CSF to exclude the presence of meningitis. They were 12 boys and 8 girls aged 2.8 ± 1.9 years (range from 3 months to 6.5 years). Informed consent was obtained from the parents of all patients enrolled in this study. CSF Samples were obtained on the first day of admission and were kept at -70°C. Determination of nitrate/nitrite level was performed by use of the nitrate/nitrite Colorimetric Assay Kit (LDH Method) (Cayman Chemical, USA). This kit provided the measurement of total nitrate/nitrite concentration in 2-step process. The first step was the conversion of nitrate to nitrite by the enzyme nitrate reductase. The second step was the addition of Griess reagent, which converts nitrite into a deep purple azo compound. The absorbance of standards and samples was read at 540 nm. TNF-α levels were assayed in CSF by the use of an ELISA kit (Quantikine, UK) according to the manufacture’s instruction.

Statistical Analysis:
Results were expressed as mean ± SD. Statistical analysis was performed using unpaired and paired two-tailed t test. Pearson’s correlation was used to measure how variables or rank orders are related. A p value of <0.05 denoted the presence of statistical significance.

Results:
The cerebrospinal fluid characteristics of the studied groups (septic meningitis, aseptic meningitis and controls) were illustrated in table I.

<table>
<thead>
<tr>
<th></th>
<th>Septic meningitis (n = 21)</th>
<th>Aseptic meningitis (n = 19)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>3.2 ± 2.2</td>
<td>2.6 ± 2.1</td>
<td>2.8 ± 1.9</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Boys</td>
<td>12</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>- Girls</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>CSF:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- WBC/mm³</td>
<td>4010 ± 380</td>
<td>100 ± 2.03</td>
<td>0</td>
</tr>
<tr>
<td>- Glucose (mg/dl)</td>
<td>24.6 ± 22.7</td>
<td>70.5 ± 11.2</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>- Protein (mg/dl)</td>
<td>264.3 ± 100.5</td>
<td>110.3 ± 21.4</td>
<td>42 ± 5.2</td>
</tr>
<tr>
<td>- TNF-α (pg/ml)</td>
<td>148.74 ± 338.77</td>
<td>6.85 ± 17.93</td>
<td>7.67 ± 16.07</td>
</tr>
<tr>
<td>- NO--2 (µmol/l)</td>
<td>27.6 ± 26.8</td>
<td>12.2 ± 12.3</td>
<td>13.2 ± 24.2</td>
</tr>
<tr>
<td>Significance</td>
<td>P1 = 0.0001**</td>
<td>P2 = 0.05*</td>
<td>P3 = 0.0003**</td>
</tr>
<tr>
<td></td>
<td>P4 = 0.001*</td>
<td>P5 = 0.01*</td>
<td>P6 = 0.001*</td>
</tr>
</tbody>
</table>

P1: Comparison between CSF TNF-α levels in septic meningitis and controls.
P2: Comparison between CSF TNF-α levels in aseptic meningitis and controls.
P3: Comparison between CSF TNF-α levels in septic and aseptic meningitis.
P4: Comparison between CSF NO--2 levels in septic meningitis and controls.
P5: Comparison between CSF NO--2 levels in aseptic meningitis and controls.
P6: Comparison between CSF NO--2 levels in septic and aseptic meningitis.
* Significant, ** highly significant P value (< 0.05.)

The mean NO--2 in CSF samples obtained during the early stages of the disease in patients with septic meningitis (27.6 ± 26.8 µmol/l) was significantly higher than control group (13.2 ± 24.2 µmol/l, p<0.001) (figures 1,2).

Following clinical and laboratory improvement, the CSF NO--2 diminished to normal range (table II). The CSF NO--2 in patients with aseptic meningitis remained below the normal upper limit level throughout the observation period (12.2 ± 12.3 µmol/l, P<0.001).
bactericidal antibiotics. Although important advances in clarifying the pathophysiological events in meningitis, the mechanism responsible for damage of central nervous system are not yet completely understood. Recently, it has been suggested that nitric oxide (NO), a labile free radical, contributes to pathological processes in bacterial meningitis.\(^7,9,11\)

In our study, we found that the CSF NO\(_2\) level in children with septic meningitis before treatment was significantly higher than that in aseptic meningitis and the controls, while after clinical and laboratory improvement the CSF NO\(_2\) level was insignificantly differ in the three groups of the study. These results indicate that the septic meningitis in children leads to increase production of NO in CSF and the fall in its level after response to treatment is another evidence for this effect. In contrast, children with aseptic meningitis did not show high NO\(_2\) concentrations, suggesting that viral factors do not cause a substantial induction of NO production in children.

Our observations were in agreement with those of Korneliss et al.,\(^15\) in 1996, Uysal et al.,\(^16\) in 1999, Murawaska et al.,\(^17\) in 2000 and Azumagaura et al.,\(^18\) in 2003, who reported a significantly high levels of NO\(_2\) in CSF samples obtained from children during the early stages of septic meningitis support the presence of an enhanced production of NO in the CSF compartment. Milstein et al.,\(^1\) in 1994 and Hockett et al.,\(^19\) in 1995, reported increased CSF levels of NO metabolites in adult patients with bacterial meningitis.

NO synthesis may be immuno-induced by bacterial lipopoly-saccharides and inflammatory cytokines in a variety of cells, including neutrophils, microglia/macrophages, microvascular endothelial cells, astrocytes and neuronal cells in the CSF compartment.\(^17,18\)

The mechanism by which high levels of NO contribute to the blood-brain and blood-CSF barrier permeability changes is not fully understood. One plausible scenario is that excessive levels of NO disrupt various enzyme systems associated with mitochondrial respiration, DNA replication and citric acid cycle in microvascular endothelial cells, thereby causing cellular destruction or alteration and loss of integrity of these barriers.\(^5,7,9\) Also, we found that the CSF level of TNF-\(\alpha\) was significantly higher in septic meningitis than in aseptic meningitis and the controls. Our results were in accordance with those reported by others.\(^16,19,20\)

### Table II: CSF levels of both NO\(_2\) and TNF-\(\alpha\) levels before and after treatment in patients with septic and aseptic meningitis and controls

<table>
<thead>
<tr>
<th></th>
<th>Septic meningitis</th>
<th>Aseptic meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td>NO(_2) ((\mu)mol/l)</td>
<td>27.6 ± 26</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>901 ± 101</td>
<td>201 ± 101</td>
</tr>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td>NO(_2) ((\mu)mol/l)</td>
<td>12.2 ± 10</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>301 ± 201</td>
<td>211 ± 23a</td>
</tr>
</tbody>
</table>

\(S = \text{significant p value} < 0.05\), \(NS = \text{Non-significant p value}\)

\(\alpha\)

The mean (± SD) of TNF-\(\alpha\) concentration in septic meningitis was 148.74 ± 338.77 pg/ml. There was significantly more TNF-\(\alpha\) than aseptic meningitis (6.85 ± 17.93 pg/ml; P<0.0003) or non-meningitis (7.67 ± 16.07 pg/ml; P<0.0001)(figures 1,3). Mean CSF nitrate/nitrite levels did not correlate with TNF-\(\alpha\) (\(r = 0.046\)), white blood count (\(r = 0.217\)), or protein (\(r = 0.263\)). However, there was a moderate negative correlation with CSF glucose levels (\(r = -0.46, p = 0.056\)).

**Discussion:**

Bacterial meningitis continues to be an important cause of morbidity and mortality during infancy and childhood despite the availability of effective bactericidal antibiotics. Although important advances have been made in clarifying the pathophysiological events in meningitis, the mechanism responsible for damage of central nervous system are not yet completely understood. Recently, it has been suggested that nitric oxide (NO), a labile free radical, contributes to pathological processes in bacterial meningitis.\(^7,9,11\)

In our study, we found that the CSF NO\(_2\) level in children with septic meningitis before treatment was significantly higher than that in aseptic meningitis and the controls, while after clinical and laboratory improvement the CSF NO\(_2\) level was insignificantly differ in the three groups of the study. These results indicate that the septic meningitis in children leads to increase production of NO in CSF and the fall in its level after response to treatment is another evidence for this effect. In contrast, children with aseptic meningitis did not show high NO\(_2\) concentrations, suggesting that viral factors do not cause a substantial induction of NO production in children.

Our observations were in agreement with those of Korneliss et al.,\(^15\) in 1996, Uysal et al.,\(^16\) in 1999, Murawaska et al.,\(^17\) in 2000 and Azumagaura et al.,\(^18\) in 2003, who reported a significantly high levels of NO\(_2\) in CSF samples obtained from children during the early stages of septic meningitis support the presence of an enhanced production of NO in the CSF compartment. Milstein et al.,\(^1\) in 1994 and Hockett et al.,\(^19\) in 1995, reported increased CSF levels of NO metabolites in adult patients with bacterial meningitis.

NO synthesis may be immuno-induced by bacterial lipopoly-saccharides and inflammatory cytokines in a variety of cells, including neutrophils, microglia/macrophages, microvascular endothelial cells, astrocytes and neuronal cells in the CSF compartment.\(^17,18\)

The mechanism by which high levels of NO contribute to the blood-brain and blood-CSF barrier permeability changes is not fully understood. One plausible scenario is that excessive levels of NO disrupt various enzyme systems associated with mitochondrial respiration, DNA replication and citric acid cycle in microvascular endothelial cells, thereby causing cellular destruction or alteration and loss of integrity of these barriers.\(^5,7,9\) Also, we found that the CSF level of TNF-\(\alpha\) was significantly higher in septic meningitis than in aseptic meningitis and the controls. Our results were in accordance with those reported by others.\(^16,19,20\)
In contrast to the previous data, we found no correlation between nitrate/nitrite and TNF-α levels of CSF. The main product of NO in CSF is nitrate and this may be a cause for the absence of a correlation with TNF-α level. We found no correlation between CSF nitrate/nitrite levels and CSF white blood count or protein levels. There was, however, a moderate negative correlation with CSF glucose levels, confirming previously reported data. NO suppresses cellular oxidative metabolism through the inhibition of tricarboxylic acid cycle and electron transport chain enzyme and enhances anaerobic glycolysis. This mechanism may contribute to the decreased CSF glucose concentration in patients with bacterial meningitis. In addition, inhibition of carrier-mediated transport across the blood-brain barrier causes low CSF glucose levels.

**Conclusion:**
Our observations indicate that NO and TNF-α production is enhanced in the CSF compartment of children with septic meningitis and support the hypothesis that both markers contribute to the pathophysiology of septic meningitis.

**References:**