Interferon-Gamma Inducing Factor (IL-18), Interferon-Gamma and Cystatin C Levels of Children with Meningitis

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Abstract:

The objective of this study was to measure the levels of Interleukin (IL)-18, Interferon (IFN) - γ and cystatin C in the cerebrospinal fluid (CSF) of children with meningitis. The study was carried out on 30 patients (12 males and 18 females) admitted to in-patient pediatric department in El Minya Fever Hospital and EL-Minya University Hospital, for evaluation and treatment from meningitis during the period from January 2004 to December 2004. Within the entire group of the study, the most common signs of meningitis were included. Thirteen children had septic meningitis (positive CSF culture) and 17 children had aseptic meningitis (negative CSF culture and lymphomonocytic pleocytosis in CSF analysis). Twenty children of matched age and sex, having normal CSF during evaluation for meningitis, served as a control group. All CSF samples were obtained under aseptic conditions. CSF samples were cultured, analyzed for glucose, protein and WBCs. Another part from CSF samples were immediately frozen at −70°C until Cystatin C, IFN-γ and IL-18 in CSF were assayed by using a two–site enzyme linked immunosorbent assay for Cystatin C, IL-18 and IFN-γ.

The results showed that septic meningitis group had significantly higher CSF WBCs and protein levels than in aseptic meningitis and control groups (P<0.005). IL-18 was significantly higher in septic group, it was detected in 95% of children with septic meningitis and only in the CSF of 48% of aseptic meningitis group. In control subjects, 10% had detectable levels of IL-18 in the CSF. IFN-γ was significantly higher in aseptic group, it was detected in 96% of aseptic meningitis and 35% of children with septic meningitis, while 21% was detected in CSF of control subjects. Cystatin C was detected in 44% of children with septic meningitis and in 56% of aseptic meningitis group, while cystatin C was detected in CSF of all control subjects (100%). CSF concentrations of both IL-18 and IFN-γ were significantly higher in children with septic and aseptic meningitis, compared with control children (P<0.001), while cystatin C was significantly decreased in septic and aseptic groups. No significant correlation was found between IL-18, IFN-γ and cystatin C levels and total leukocyte count in the CSF of children with meningitis.

Conclusions: Our data suggest that IL18, IFN-γ and cystatin C are significantly changed in the CSF of children with meningitis. IL18 had greatest elevations while Cystatin C was significantly decreased in those children with septic meningitis. IFN-γ had greatest elevation seen in those children with aseptic meningitis. So, Cystatin C and IL18 could be considered as sensitive and specific parameters for detection of septic meningitis, while IFN-γ could be considered as sensitive and specific parameter for detection of aseptic meningitis.

Introduction:

Despite effective antibiotic treatment, CNS infections especially meningitis is still an infection with a high mortality rate in children. Furthermore, patients who recover, may suffer from neurological sequelae, including cerebral infarction, recurrent seizures, hearing loss, learning disabilities, and/or mental retardation. When the bacteria have entered the subarachnoidal space, endogenous cells of the CNS and infiltrated leukocytes are triggered to produce proinflammatory cytokines like TNF-α, IL-1β, and IL-6, which lead to an increased permeability of the blood-brain barrier (BBB). As a result of this enhanced permeability state, leukocytes, predominantly granulocytes, are recruited to the CNS. Activated granulocytes play a major role in bacterial clearance and also secrete a variety of inflammatory mediators like IL-18 and IFN-γ that may not only kill bacteria, but also cause damage to the CNS. Also, the released lysosomal enzymes as lysosomal cysteine proteases play the same damaging role to the CNS. IFNs, especially IFN-γ are an essential part of both the innate and adaptive cytokine responses to CNS infections especially the viral infection, having important functions in the regulation of the immune system. In addition to inducing an antiviral state, IFNs are noted for their function in many immunoregulatory...
processes, including up-regulation of major histocompatibility complex (MHC) class I and II molecules, activation of macrophages and natural killer cells, augmentation of dendritic cell responses, and promotion of proliferation and survival of activated lymphocytes. 

Interleukin-18 (IL-18), a member of the IL-1 cytokine superfamily, IL-18 produced by activated macrophages and is considered to play a key role in activation of the cell-mediated immunity and clearance of invading microorganisms. IL-18 stimulates T cells and possibly macrophages for interferon-gamma (IFN-gamma) production. IL-18 also has been associated with autoimmune disease, that is, expression of its messenger RNA was to be a cell-mediated autoimmune disease. Cystatin C (7-trace) is a basic protein with a molecular weight of 13,000-14,500 belonging to the cystatin superfamily, which is present as a constituent of normal plasma, cerebrospinal fluid, urine, and seminal fluid. Cystatin C is known to bind tightly to lysosomal cysteine proteases such as cathepsins B, H, and L. Cystatins may play a defensive role in extracellular fluids by protecting organs from the cysteine proteases of invading pathogens and from endogenous cysteine proteases that escape from lysosomes.

The distinction between immune-regulatory and effector cytokines and chemokines, and neural growth and survival factors (neurotrophins) becomes increasingly blurred. We discuss here the role of immune cytokines IL-18 and IFN-γ as mediators of innate glial responses in the central nervous system, also study cystatin C as protease inhibitor in children with CNS infectious (bacterial and viral meningitis).

**Subjects and Methods:**

The study was carried out on 30 patients (12 males and 18 females) admitted to in-patient pediatric department in El Minya Fever Hospital and EL-Minia University Hospital, for evaluation and treatment from meningitis during the period from January 2004 to December, 2004. Within the entire group of the study, the most common signs of meningitis were included: fever (99%), lethargy or irritability (94%), poor feeding or food intolerance (80%) or respiratory decompensation (30%). Their age ranged from 1 to 12.8 years (3.6 ±3.8 years). According to CSF cultures, the patients were classified into 2-groups: Group A, septic meningitis group: 13 patients having positive CSF culture and group B, aseptic meningitis group: 17 patients having negative CSF culture and lymphomonocytic pleocytosis in CSF analysis. Twenty children of matched age and sex, having normal CSF during evaluation for meningitis, served as a control group. All CSF samples were and WBCs. Another part from CSF samples were immediately frozen at –70 °C until Cystatin C, IFN-γ and IL-18 in CSF were assayed by using a two –site enzyme linked immunosorbent assay for Cystatin C, IL-18 and IFN-γ.

All patients and controls were subjected to the following:

1. Thorough history taking with special emphasis on age, dietetic history, vaccination history.
2. Careful clinical examination for meningeal signs and other signs of infections like high grade fever, lethargy, irritability, disturbed conscious level, poor feeding, food intolerance and respiratory decompensation.
3. Laboratory investigations: included complete blood picture, CSF culture to detect the causative organism, CSF examination for glucose level, protein level, cells, Cystatin C, IL-18 and IFN-γ levels.

**Statistical Analysis:**

Data are indicated as mean ± standard error of mean. Statistical analysis involved the Mann-Whitney U test with a Bonferroni correction and calculation of the Spearman’s correlation coefficient.

**Results:**

The results are illustrated in the following tables I-VII and figure 1.
Table II: CSF parameters of septic meningitis group versus control group:

<table>
<thead>
<tr>
<th>CSF parameters</th>
<th>Septic meningitis (n=13)</th>
<th>Control (n=20)</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>24±18</td>
<td>49±13</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>375±325</td>
<td>66±38</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>WBCs count</strong></td>
<td>3375±7826</td>
<td>7±10</td>
<td>&lt; 0.05</td>
<td>Highly Significant</td>
</tr>
<tr>
<td><strong>Cystatin C</strong></td>
<td>1.9±7.2</td>
<td>4.3±1.3</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>IL-18</strong></td>
<td>75.51 ± 23.25</td>
<td>0.10 ± 0.04</td>
<td>&lt; 0.001</td>
<td>Highly Significant</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>13.85 ± 5.51</td>
<td>3.98 ± 2.04</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table III: CSF parameters of aseptic meningitis patients group versus control group

<table>
<thead>
<tr>
<th>CSF parameters</th>
<th>Aseptic meningitis group (n=17)</th>
<th>Control group (n =20)</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>43±5</td>
<td>49±13</td>
<td>&lt; 0.1</td>
<td>Non Significant</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>66±28</td>
<td>66±38</td>
<td>&lt; 0.25</td>
<td>Non Significant</td>
</tr>
<tr>
<td><strong>WBCs count</strong></td>
<td>360±351</td>
<td>7±10</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Cystatin C</strong></td>
<td>2.3±1.6</td>
<td>4.3±1.3</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>IL-18</strong></td>
<td>3.23 ± 1.37</td>
<td>0.10 ± 0.04</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>36.28 ± 19.45</td>
<td>3.98 ± 2.04</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table IV: CSF parameters of septic meningitis group versus aseptic meningitis groups

<table>
<thead>
<tr>
<th>CSF parameters</th>
<th>Septic meningitis patient (n=13)</th>
<th>Aseptic meningitis patients (n=17)</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>24±18</td>
<td>43.5±5</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>375±325</td>
<td>66±28</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>WBCs count</strong></td>
<td>3375±7826</td>
<td>360±351</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>Cystatin C</strong></td>
<td>1.9±7.2</td>
<td>2.3±1.6</td>
<td>&lt; 0.002</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>IL-18</strong></td>
<td>75.51 ± 23.25</td>
<td>3.23 ± 1.37</td>
<td>&lt; 0.045</td>
<td>Significant</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>13.85 ± 5.51</td>
<td>36.28 ± 19.45</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table V: The difference between percentages of the presence of IL-18 in different groups

<table>
<thead>
<tr>
<th>Patient &amp; Control</th>
<th>Percentage</th>
<th>Chi Square</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic &amp; Aseptic</td>
<td>95% &amp; 48%</td>
<td>7.632</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td>Septic &amp; Control</td>
<td>35% &amp; 21%</td>
<td>4.532</td>
<td>&lt; 0.01</td>
<td>Significant</td>
</tr>
<tr>
<td>Aseptic &amp; Control</td>
<td>48% &amp; 10%</td>
<td>6.587</td>
<td>&lt; 0.05</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table VI: The difference between percentages of the presence of IFN-γ in different groups

<table>
<thead>
<tr>
<th>Patient &amp; Control</th>
<th>Percentage</th>
<th>Chi Square</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic &amp; Aseptic</td>
<td>35% &amp; 96%</td>
<td>6.982</td>
<td>&lt; 0.02</td>
<td>Non Significant</td>
</tr>
<tr>
<td>Septic &amp; Control</td>
<td>35% &amp; 21%</td>
<td>4.532</td>
<td>&lt; 0.9</td>
<td>Non Significant</td>
</tr>
<tr>
<td>Aseptic &amp; Control</td>
<td>96% &amp; 21%</td>
<td>7.351</td>
<td>&lt; 0.01</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table VII: The difference between percentages of the presence of Cystatin C in different groups

<table>
<thead>
<tr>
<th>Patient &amp; Control</th>
<th>Percentage</th>
<th>Chi Square</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic &amp; Aseptic</td>
<td>44% &amp; 100%</td>
<td>9.742</td>
<td>&lt; 0.001</td>
<td>Significant</td>
</tr>
<tr>
<td>Septic &amp; Control</td>
<td>44% &amp; 100%</td>
<td>9.742</td>
<td>&lt; 0.001</td>
<td>Significant</td>
</tr>
<tr>
<td>Aseptic &amp; Control</td>
<td>56% &amp; 10%</td>
<td>7.234</td>
<td>&lt; 0.03</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Figure (1): different CSF parameters in different groups

![Figure (1): different CSF parameters in different groups](image-url)
Discussion:

In CNS infections, inflammation is initiated by local production of several soluble mediators. Many different cytokines and chemokines control the inflammatory response via activation and migration of white blood cells (WBCs). The physiologic functions of those cytokines were inferred from the biological activity observed in vitro and confirmed by in vivo experiments. The results of those experiments indicated that the production of proinflammatory cytokines (interleukin [IL]-1, IL-18 and interferon [IFN-γ]) and anti-inflammatory cytokines (IL-4, IL-10 and transforming growth factor-β1) are involved in the migration of WBCs into the infected area and that inflammation is terminated by anti-inflammatory cytokines (IL-4, IL-10 and transforming growth factor-β1, IL-18 and interferon [IFN-γ]) and antilysosomal released substances such as cystatin-C that are produced for elimination of the microorganisms. In humans, however, the induction of inflammation by cytokines at the initial stage of infection has not been demonstrated.

Traditionally, CSF leukocyte count has been considered a useful tool for the differential diagnosis, with a low pleocytosis or a relative lymphocytosis considered a sign of nonbacterial cause. However, Negri et al. in 2000, reported that a CSF pleocytosis of >1000/mL only had a sensitivity of 61% and a specificity of 68% as a diagnostic marker of CSF infection in neurosurgical patients. Moreover, although 94% of patients with bacterial meningitis had CSF polymorphonuclear predominance, this criterion only had a specificity of 28%. Our data show similar results. Using a cutoff point of ≥1000/mL, CSF leukocyte count discriminated between CSF infection and aseptic pleocytosis with a sensitivity of 65% and a specificity of 89%. A cutoff point of ≥1700 leukocytes/mL had to be selected to attain a specificity of >95%, lowering its sensitivity to 51%. CSF polymorphonuclear predominance was not a useful measurement. Thus, there were nonbiochemical CSF criteria sensitive and specific enough to reliably differentiate aseptic from bacterial meningitis.

This study demonstrates that IL-18 is released in high amounts in patients with bacterial meningitis but not in those with viral meningitis or in healthy subjects. Since IL-18 is considered to be macrophage specific, its release in bacterial meningitis can be attributed to mononuclear phagocytes that are, along with polymorphonuclear leukocytes, recruited in large number into infected subarachnoidal space. IL-18 could be involved in clearance of invading pathogens, as suggested by the protective effect of administration of this cytokine in animal models of infection or the impaired natural killer cell activity and reduced Th1 cell response in experimental infection in IL-18-deficient mice. This is in agreement with Fassbender et al. in 1999, who reported that IL-18 was significantly increased in inflammatory CNS diseases especially meningitis.

There was no a significant correlation between IL-18 and IFN-γ concentration in CSF of patients with bacterial meningitis and viral meningitis, and the interesting observation that IFN-γ was highly significant elevated in viral meningitis rather than in bacterial meningitis, this in accordance with Dinarello et al. in 2002, who suggests that IL-18, rather than being a simple inducer of IFN-γ synthesis, plays a more complex role in the inflammatory host response or of apoptotic pathways, which both cause tissue damage.

There was no significant correlation between IL-18 concentrations in CSF and CSF white blood cells in bacterial meningitis can be explained by the fact that CSF cell counts reflect recruitment of granulocytes rather than that of monocytes, which are, in contrast, the source IL-18, this is in agreement with Sareneva, in 2000. This could also explain the observation of a significant correlation between IL-18 levels and CSF white blood cells in viral meningitis, cytologically characterized by a lymphomonocytic cellular picture. Immunohistochemical studies have demonstrated cystatin C immunoreactivity in microglial cells, astrocytes, the choroid plexus, and some neurons, where Cystatin C levels in CSF are 5.5 times higher than those in plasma, and Cystatin C is the dominant cysteine protease inhibitor.

Cystatin C may also play an important role in inflammatory neurologic diseases (IND), where the proteolytic enzymes are involved in demyelination and the breakdown of the blood–CSF barrier. Gelatinases and matrix metalloproteinases may be involved in the progress of meningitis.

In this study, in spite of the blood–brain barrier is damaged in septic meningitis with overflow of the peripheral circulation into the intrathecal space, there was a significant decrease in CSF cystatin C levels in septic group. This result is in accordance with Nagai et al. in 2000, who explained the downregulation of cystatin C secretion by reduced production or increased degradation of cystatin C in septic meningitis.

Bollengier, in 2002, explained this decrease in CSF cystatin C level by inflammatory cytokines as one reason for the low CSF cystatin C levels in IND, where the levels of two inflammatory cytokines, IFN-γ and tumor necrosis factor (TNF-α), which were hardly detected in the CSF of inflammatory neurologic diseased (IND) patients. Warfel et al. in 2003, studied Cystatin C in hereditary cerebral hemorrhage with amyloidosis-I (HCHWA-I), and explained that the lowering of CSF cystatin C levels...
may be due to decreased secretion by CNS cells. Culture studies showed that monocytes/macrophages, microglial cells, and astrocytes secrete cystatin C, and that the secretion is decreased by addition of lipopolysaccharide or IFN-γ.\textsuperscript{28}

**Conclusion:**

The previous data suggested that IL18, IFN-γ and cystatin C are significantly changes in the CSF of children with meningitis. IL-18 had a greatest elevations while Cystatin C had significant decreased in those children with septic meningitis. IFN-γ had greatest elevation seen in those children with aseptic meningitis.

So, Cystatin C, IL-18 could be considered as sensitive and specific parameter for detection of septic meningitis while IFN-γ could be considered as sensitive and specific parameter for detection of aseptic meningitis.

**References:**

22. Sareneva T, Matikainen S, Kurimoto M, Julkunen I. Influenza virus-induced IFN-alpha/beta and IL-18