EVALUATION OF INTRATHECAL IMMUNOGLOBULIN PRODUCTION IN CASES OF CHILDHOOD BACTERIAL MENINGITIS

İsmail H. KARA, MD.1 Bünyamin DİKİÇİ, MD. 2 Mehmet BOŞNAK, MD. 3 Fuat GÜRKAN, MD. 4 Kenan HASPOLAT, M.D. 5

ABSTRACT

The aim of this study was to evaluate the IgG, IgM and IgE indices of different types of childhood bacterial meningitis.

This prospective cohort study was performed in Dicle University, Paediatric Clinic of Medical Faculty from August 1996 to 1997. There were 18 patients with bacterial meningitis including eight patients with meningococcal meningitis (MM), five patients with purulent (PM) and five patients with tuberculous (TBM) in the studied group. IgG index was calculated by formula of Link and Tibbling (IgG ratio/albumin ratio of CSF to serum). Age of patients ranged between 11 and 156 months with a mean of 65.1±49 months. One of patients with TBM died; shunting operation was performed to three of the rest. Neurological sequelae developed in all patients with TBM, and in 2 patients with PM. temporary visual impairment was determined in only one patient with MM. The mean IgG, IgM and IgE indices in our cases with TBM were 1.2, 0.8 and 6.9, respectively. The mean CSF IgG and CSF IgE indices were slightly higher in patients with TBM than in other subjects (p<0.01 and p=0.01, respectively). CSF of patients with PM showed significantly high titres of circulating IgE antibodies than in other subjects (p=0.001).

The patients with CNS inflammation, especially TBM, had elevated IgG and IgE indices compared to other groups. Moreover, CSF of patients with PM showed significantly high titres of circulating IgE antibodies than in other subjects. These results showed that brain blood barrier damage was most severe and neurological sequelae was higher in cases of TBM compared MM and PM in our series.

Key Words: Blood-brain barrier, IgG, IgM and IgE indices, meningitis.

INTRODUCTION

Meningitis is an infection of the subarachnoid space and leptomeninges caused by a variety of pathogenic organisms. Granulomatous meningitis and bacterial meningitis are the most important forms worldwide in terms of incidence, sequelae, and ultimate loss of productive life (1-3). frequency of granulomatous meningitis caused by M. tuberculosis has been reported as 0.74 % in Southeast Anatolian Region and 0.38 % in general of Turkey (4).
Meningitis due to *N. meningitides* is most often encountered in children and young adults and may occur in epidemics. Meningococcal meningitis (MM) epidemics can occur anywhere in the world. However this risk is particularly high during the dry season in the Southeast Anatolia Region of Turkey and this is the area characterized by hyperendemicity that regularly gives rise to epidemics. At least 30 countries worldwide, including the United States, have reported serious outbreaks of MM in recent years (5, 6). Although clinical signs are often apparent, not all cases are diagnosed by clinical examination unless gravity is taken into account. Individuals with MM without overwhelming meningococcemia have a case fatality rate of only 3%. The only hope of survival is early institution of appropriate antimicrobial therapy (even prior to hospitalization) (1, 5).

As a general rule, once the diagnosis of meningitis is suspected, immediate examination of the CSF is indicated. A number of tests measuring levels of various CSF proteins, enzymes, and mediators have been proposed as potential discriminators between viral and bacterial meningitis. One of them is CSF/serum antibody index, which can be calculated using the following formula of Tibbling and Link (7). Normal CSF IgG index is lower than 0.7, but it was elevated in some conditions (meningitis, parainfectious encephalomyelitis, neoplasm and demyelinating disease) (6, 8, 9, 10).

The aim of this study was to evaluate the IgG, IgM and IgE indices of different types of childhood bacterial meningitis.

**PATIENTS AND METHODS**

This prospective cohort study was performed in Dicle University, Paediatric Clinic of medical faculty, from August 1996 to 1997. Informed consent was obtained from parents of the study patients, and the Ethical Committee approved the research protocol.

18 patients with bacterial meningitis including eight patients with meningococcal meningitis (MM), five patients with purulent meningitis (PM) and five patients with tuberculous meningitis (TBM) were enrolled in study. CSF and serum Ig levels of patients were obtained at the first day of hospitalisation. The diagnosis was confirmed by bacteriologic examination (cultures and gram's stain), clinical, physical, and laboratory examination and was supported by computed tomography (CT). Criteria for exclusion from the study were one of the following: Parainfectious encephalomyelitis, neoplasm and demyelinating disease, juvenile rheumatoid arthritis, and other collagen diseases, and immunodeficiency syndromes.

Case definition of CNS Tuberculosis by either microbiologic or clinical criteria (8):

A. Microbiologic case definition. One of the following:

1. Isolation of *M. tuberculosis* from CSF.

2. Abnormal neurologic signs and symptoms, CSF, or cranial CT consistent with CNS tuberculosis, and isolation of *M. tuberculosis* from any site.
B. Clinical case definition. Abnormal neurologic signs and/or symptoms, and 2 of the following:

1. Discovery of adult source case with contagious tuberculosis who had significant contact with child.

2. Presence of Mantoux (5 IU) skin test reaction 10 mm of induration, or 5 mm of induration if child had close contact with infected adult.

*(15 mm of induration was considered positive for children with BCG. BCG is in routine immunisation programme in Turkey).*

3. CSF abnormalities without evidence of other infectious cause.

4. Abnormalities on cranial CT consistent with CNS tuberculosis.

Enzymun-Test (Boehringer Mannheim Immunodiagnostics ES 700.) by Nephelometer (Behring 100 Analyser-Germany) was used to measure the Ig levels. Immunoglobulin indices were calculated by formula of Link and Tibbling (7). IgG index was calculated as (IgG ratio/albumin ratio of CSF to serum). CSF IgM index was calculated as (IgM ratio/albumin ratio of CSF to serum), and CSF IgE index was calculated as (IgE ratio/albumin ratio of CSF to serum).

SPSS/PC 7.5 program was used for statistical analysis. Kruskal-Wallis 1-Way Anova, (Mann-Whitney U test was used to compare of two independent samples) Pearson's R, and chi-square tests were used for data analysis. Statistical significance was defined as p<0.05. The results are expressed as mean±SD.

RESULTS

The demographic and clinico-laboratory characteristics of patients by meningitis type are summarised in table 1. Meningitis groups in figure 1 showed the mean IgG, IgM and IgE indices. The ages of patients ranged between 11 and 156 months with a mean of 65±49 months. The mean duration of hospitalisation of patients was 15±17 (8-80) days. This duration was significantly higher in patients with TBM than in other patients (p=0.01).

CSF of patients with TBM showed high titres of circulating IgG and IgM antibodies, and the mean CSF IgG and CSF IgE indices were higher in patients with TBM than in other subjects (respectively, p<0.01 and p=0.01), (Figure 1). CSF of patients with PM showed higher titres of circulating IgE antibodies than in other subjects, and it was statistically significant (p=0.001).
**Table 1.** The demographic and clinico-laboratory features of patients by meningitis types.

<table>
<thead>
<tr>
<th>Variable (Mean± SD)</th>
<th>TBM (n=5)</th>
<th>PM (n=5)</th>
<th>MM (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (mo)</td>
<td>60±54</td>
<td>58±50</td>
<td>72±44</td>
<td>NS</td>
</tr>
<tr>
<td>Hospitalisation Time (Day)</td>
<td>28±30</td>
<td>11±3</td>
<td>9±1</td>
<td>=0.01</td>
</tr>
<tr>
<td>Blood Leukocytes /mm3</td>
<td>6.900±8.700</td>
<td>16.100±10.100</td>
<td>19.500±7.000</td>
<td>NS</td>
</tr>
<tr>
<td>Blood PNL/mm3</td>
<td>8.800±6.000</td>
<td>12.000±9.800</td>
<td>13.400±4.600</td>
<td>NS</td>
</tr>
<tr>
<td>ESR mm/hours</td>
<td>48±26</td>
<td>54±34</td>
<td>42±24</td>
<td>NS</td>
</tr>
<tr>
<td>CSF cells/mm3</td>
<td>34±35*</td>
<td>940±134</td>
<td>680±450</td>
<td>=0.008</td>
</tr>
<tr>
<td>Lymphocytes in Frotti %</td>
<td>75±30*</td>
<td>30±30</td>
<td>15±10</td>
<td>=0.04</td>
</tr>
<tr>
<td>CSF PNL %/mm3</td>
<td>35±35</td>
<td>70±30</td>
<td>85±10</td>
<td>NS</td>
</tr>
<tr>
<td>CSF Albumin (mg/dl)</td>
<td>80±87</td>
<td>122±76</td>
<td>90±85</td>
<td>NS</td>
</tr>
<tr>
<td>CSF Protein (mg/dl)</td>
<td>183±105</td>
<td>218±125</td>
<td>164±96</td>
<td>NS</td>
</tr>
<tr>
<td>CSF Glucose (mg/dl)</td>
<td>58±29</td>
<td>39±38</td>
<td>73±25</td>
<td>NS</td>
</tr>
<tr>
<td>CSF Chloride (mg/dl)</td>
<td>95±10</td>
<td>109±18</td>
<td>108±13</td>
<td>NS</td>
</tr>
<tr>
<td>CSF IgG mg/dl</td>
<td>34±47</td>
<td>19±15</td>
<td>16±30</td>
<td>NS</td>
</tr>
<tr>
<td>CSF IgM mg/dl</td>
<td>5.8±4.4</td>
<td>2.6±2.2</td>
<td>3.8±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>CSF IgE IU/mL</td>
<td>4.9±3.2</td>
<td>124±157*</td>
<td>3.8±1.1</td>
<td>=0.001</td>
</tr>
<tr>
<td>Serum IgG mg/dl</td>
<td>832±370</td>
<td>855±246</td>
<td>847±260</td>
<td>NS</td>
</tr>
<tr>
<td>Serum IgM mg/dl</td>
<td>152±51</td>
<td>103±26</td>
<td>92±92</td>
<td>NS</td>
</tr>
<tr>
<td>Serum IgE IU/mL</td>
<td>195±231</td>
<td>308±257</td>
<td>46±45</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Figure 1.** The mean IgG, IgE and IgM indices of cases with tuberculous, purulent and meningococcal meningitis.
Table 2. Physical and neurological examination findings of patients.

<table>
<thead>
<tr>
<th></th>
<th>Tuberculous Meningi. (n=5)</th>
<th>Purulent Meningi. (n=5)</th>
<th>Meningococcal Meningitis (n=8)</th>
<th>$\chi^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>1.32</td>
<td>NS*</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Convulsion</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Stiff neck</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>3.06</td>
<td>NS</td>
</tr>
<tr>
<td>Meningeal irritation</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>5.85</td>
<td>NS</td>
</tr>
<tr>
<td>Babinski sign</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>1.04</td>
<td>NS</td>
</tr>
<tr>
<td>Petechiae / ecchymoses</td>
<td>1**</td>
<td>0</td>
<td>8</td>
<td>14.8</td>
<td>&lt;0.00</td>
</tr>
</tbody>
</table>

*NS=Not significant,
**Allergic reaction was developed against to Isoniazid in a case with Tuberculous meningitis.

There were statistically significant differences in only the mean CSF white cell count Per mm$^3$ and lymphocytes Percent among meningitis groups (p=0.008 and p=0.04, respectively). There were no statistically significant differences in the clinical findings reflecting meningitis as fever, headache, vomiting, convulsion, stiff neck, meningeal irritation and Babinski sign except petechiae/ecchymoses among meningitis groups. An allergic reaction developed against to isoniazid in only one case with TBM (Table 2).

CT revealed 3rd and 4th ventricle dilatation, cortical atrophy, and periventricular hypodense areas in 3 of 5 cases with TBM. However, CT revealed 3rd and 4th ventricle dilatation in one case, and periventricular hypodense areas in another case with PM. Tomographic findings were normal in all patients with MM, in one patient with TBM and in three patients with PM.

After discharge, our patients were monthly controlled in pediatric outpatients clinic. One of patients with TBM died, and shunting operation was performed in three of the rest. Neurological sequelae developed in all patients with MM, in one patient with TBM and in three patients with PM.

DISCUSSION

Most viral and bacterial infections of the central nervous system (CNS) are associated with intrathecal synthesis of antibody (7). Cerebrospinal fluid (CSF) protein concentration is usually elevated, in the range of 100 to 500 mg/dl, but as elevated protein reflects alteration in the blood-brain barrier (BBB). Elevations in CSF Ig may occur as a result of breakdown in the blood-brain barrier (11,12).

Hong (2) reported that levels of CSF IgG and IgM in patients with PM were 9 mg/dl, and 4 mg/dl. But, in our study, the levels of CSF IgG, and IgM in
patients with PM were 19 mg/dl, and 2.6 mg/dl, respectively (Table 1).

Hung et al (7,11) reported that they evaluated the BBB function in childhood CNS infections, using CSF in 74 patients including 34 cases with aseptic meningitis, 24 with encephalitis and 16 with PM. They suggested that the quantitation of IgG and other derivatives in CSF might have given a sensitive supplementary reference for the detection of CNS infection and the patients with encephalitis, especially chronic encephalitis, had a selectively elevated IgG index compared to other groups. But, BBB damage was most severe in cases of PM in that series.

Tipold et al (13) performed an experimental study and reported that a surprisingly high number of animals with encephalitis, including dogs with protracted diseases such as chronic distemper encephalitis and granulomatous meningoencephalomyelitis, showed an elevated IgG production with an additional evidence of intrathecal IgM and IgA production.

Forsberg et al (14) stated that 11 patients with bacterial meningitis, all had an increased CSF IgM index, (CSF/serum IgM):(CSF/serum albumin), indicating intrathecal IgM production. Seven patients had a slightly increased CSF IgG index, and 7 a slightly increased IgA index.

Mathai et al (15) reported that CSF of patients with TBM showed high titres of circulating antimycobacterial antibodies than in nontuberculous subjects. The CSF IgG index was found to be significantly higher in patients with TBM. In cases with multiple sclerosis and various infections of the CNS (TBM, etc.), the mean IgG and IgE indices were reported as 0.45 and 0.29, respectively (16).

The mean IgG, IgM and IgE indices in all our cases with meningitis were 0.7, 0.6 and 4.9 units, respectively. The mean IgG index of our cases with TBM, PM and MM was 1.2, 0.6 and 0.4 units (p<0.01), respectively (Figure 1). However, it is suggested that most patients with TBM had clearly an increased IgE index suggesting a local biosynthesis (16), but we failed to detect high levels of IgE in the CSF and increased IgE indices in patients with TBM. In contrast, we detected significantly higher levels of IgE in the CSF in patients with PM (p=0.001), but IgE index was not significantly higher (p>0.05). We found statistically significant difference in the mean IgG and IgE indices between TBM and other bacterial meningitis (respectively, p<0.01 and p=0.01), (Figure 1).

The CT scan is useful to demonstrate the presence or absence of focal intracranial suppurative collections, subdural effusions, or progressive ventricular enlargement that may require shunt surgery. Certain findings of CT scan, such as evidence of diffuse microcytic degeneration with associated cortical atrophy or infarctional patterns can be prognostic (1). CT revealed 3rd and 4th ventricle dilatation, cortical atrophy, and periventricular hypodense areas in 3 of 5 cases with TBM. However, CT revealed 3rd and 4th ventricle dilatation in only one case, and periventricular hypodense areas in another case with PM.

Focal motor and sensory deficits, visual impairment, hearing loss,
seizures, hydrocephalus, and a variety of cranial nerve deficits can result from meningitis. the immune and inflammatory response in the brain to certain pathogens such as M. tuberculosis may be responsible for much of the morbidity of this disease in that organ system (3,6). In our study, fever, vomiting, Babinski sign and stiff neck were found in almost all patients (p>0.05), nevertheless, petechiae/ecchymoses were found only in patients with MM (p<0.0001) (Table 2). There were not statistically significant differences in neurological examination findings of patients between groups (p>0.05).

After discharge, patients were monthly controlled in pediatric outpatients clinic. after six months follow up, one patient with TBM died, in three of five patients were performed shunting operation because of hydrocephalus. We did not observed to the morbid sequelae in any patients with MM. Neurological sequelae developed in all surviving patients with TBM and in two patients with PM. There was statistically significant difference in sequelae rate between TBM group and other groups (X²=9.59, df=2, p=0.008).

Nowadays, vaccination is a major prophylactic modality in prevention of meningitis especially for MM. Untreated the disease is always fatal. The usefulness of plain group A plus C polysaccharide vaccines is limited because of poor effectiveness in young children who constitute the highest risk group. During epidemics, mass vaccination should be carried out as early as possible according to the state of alert defined for the area. More recent conjugate vaccines against group A and C, which are effective in young children and provide long-term protection by induction of immunologic memory, may allow routine vaccination in the future. (5).

As a conclusion, the CSF-serum antibody index may be useful for differential diagnosis of bacterial meningitis. The patients with CNS inflammation, especially TBM, had elevated IgG and IgE indices compared to other groups. On the other hand, CSF of patients with PM showed significantly high titres of circulating IgE antibodies than in other subjects. These results showed that BBB damage was most severe in cases of TBM and neurological sequelae were higher in cases of TBM compared MM and PM in our series.

ÖZET

ÇOCUKLUĞA ÇAĞININ BAKTERİYEL MENENJİTLİ OLGULARINDA İNTRATEKAL İMMÜNOGLOBULİN ÜRETİMİNİN DEĞERLENDİRILMESİ

Bu çalışma amacını, çocukluğa çağın farklı tipteki bakteriyel menenjetlerinde, immünoglobulün G (IgG), IgM ve IgE indekslerini değerlendirirmektir.

Bu prospektif kohort çalışma, Dicle Üniversitesi Tıp Fakültesi Pediatri Kliniğinde, Ağustos 1996 ile 1997 tarihleri arasında gerçekleştirildi. Çalışma grubunda, sekiz meningokossik menenjit (MM), beş pürülan (PM) ve beş tüberkülüoz (TBM) olmak üzere toplam 18 hasta bulunuyordu. Immünoglobulin indeksleri, Link ve Tibbling (Ig orani/albumin orani, beyin omurilik sıvısı) BOS/serum) formülüne göre hesaplandı.
Hastaların yaşları 11 ile 156 ay arasında değişmekte olup, ortalama yaş 65±49 aydır. TBMI'li bir olgu eksi̇tus olurken; diğer üç hastaya şan̄t operasyonu uygulandı. Nörolojik sekel TBMI'li bütün otlarda gelişirken, PM'Ili iki olgu gelişti. Geçici görme kaybı, yalnız MM'Ili bir olguna geli̇ti. TBMI'li otlarda ortalama IgG, IgM ve IgE indeksleri sırasıyla 1.2, 0.8 ve 6.9'du. TBMI'li otlarda, ortalama, ortalama IgG ve IgE indeksleri diğer bireylerden hafifçe daha yüksekti (sırasıyla, p<0.01 ve p=0.01). PM'Ili hastaların BOS'u diğer bireylerden, belirgin olarak daha yüksek tıtrede IgE antikor düzeyleri göstermektediydi (p=0.001).


Anahtar Kelimeler: Kan-beyin bariyeri, IgG, IgM ve IgE indexi, Çocukluğun bakteriyel menenjitleri.

REFERENCES


