Cerebrospinal Fluid Cytology Using a Cytocentrifuge in the Early Diagnosis of Cause of Meningitis

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Summary: A cytocentrifuge was found to be an effective and rapid tool preparing cytological samples from cerebrospinal fluid for diagnosing bacterial, neoplastic and viral meningitis as well as herpes simplex encephalitis. Using the centrifuge, we have been able to make early diagnoses for various type of the disease and to give prompt treatments for patients. Some of our successful cases using the system were listed as follows: (1) After observing vacuolated phagocyte with coccus which was definitely bacterial meningitis, patients could be administered immediately antibiotics. (2) A patient of herpes simplex encephalitis whose phagocytes either with hemosiderin and erythrocytes or hemosiderin showed excellent result with adenosine arabinoside. (3) Tumor cells in the first tap from the lumbar of a patient of meningeal carcinomatosis were readily demonstrated with this system. (4) Only in the viral meningitis, lymphocytes with multilobulated nuclei which resembled abnormal adult T-cell leukemia cells and atypical lymphocytes were observed.

Key words: cerebrospinal fluid—cytology—cytocentrifuge—bacterial meningitis—herpes simplex encephalitis—meningeal carcinomatosis—viral meningitis

Introduction

The mortality in many types of meningitis has been reduced in the past two decades. However, patients who have struck with bacterial, tuberculous and neoplastic meningitis and herpes simplex encephalitis would die or be left incapacitated in sequela. It is due to delay in diagnosing the cause and instituting therapy. An early diagnosis is often difficult because it takes some time isolating organisms from cerebrospinal fluid (CSF) and determining serum viral titer. We have studied the possibility of using a cytocentrifuge for cytological examination of CSF that would be effective in making an early diagnosis of various type of meningitis.

Patients and Methods

1. Patients

In this study, 28 meningitis patients who were from 15 to 64 years old, an average of 34, were involved. Among them, we determined serologically five cases of varicella-zoster and one herpes simplex virus infections. CSF from 21 patients were found to have enterovirus in 19 and
Streptococcus pneumoniae in two cases. Malignant cells were identified from CSF in a patient who was having gallbladder cancer.

II. Methods

In order to study CSF cytology, cells were collected with a Shandon cytocentrifuge (the Shandon Scientific Inc., Great Britain) (Fig. 1). A rotor for the centrifuge and its assembly are shown in Figs. 2, 3 and 4. Some details are described as follows:

Fig. 1. Overall view of the Shandon cytocentrifuge.

Fig. 2. The Shandon cytocentrifuge is supplied with a 12 position sealed head.

Fig. 3. Assembly of the glass slide, filter paper, slide clip and sample chamber.
There are 12 chambers in a rotor. Each chamber accepts an assembly in which a glass slide covered with a filter paper bearing two 7 mm perforations is inserted as shown in Fig. 3. A perforation is aligned with a channel located at the bottom of the assembly.

CSF is placed in a sample chamber whose end is connected to the channel shown in Fig. 4. CSF is centrifuged at 900 rpm for 10 min. As the rotor rotates, CSF flows through the channel. The liquid portion of CSF is absorbed on the paper and the cells are collected in the hole on the slide. After air-dried, the cells are stained with the May-Giemsa and examined under a light microscope.

Results

I. Bacterial Meningitis:
Case 1.
A 34-year-old man was admitted to the hospital with fever and stiff neck. The spinal fluid contained 1,880 cells per 3 c. mm and sugar, 5 mg per 100 ml. Its culture for bacteria showed Streptococcus pneumoniae. Before the isolation of the bacteria from CSF, CSF cytology revealed that vacuolated phagocytes with diplococcus were present (Fig. 5). Despite antibiotics was administered, the patient died 9 hours after.

Case 2.
A 34-year-old woman was admitted to the hospital because of fever with unknown origin. Two weeks after admission, she became drowsy. The CSF contained 38,400 cells per 3 c. mm, protein 620 mg, sugar 229 mg per 100 ml. Before Streptococcus pneumoniae was identified in CSF, CSF cytology revealed that vacuolated phagocytes with diplococcus were present (Fig. 6). Although antibiotics was administered immediately, the patient died 12 hours after.

II. Herpes Simplex Encephalitis:
A 34-year-old man was admitted to the hospital due to fever and loss of consciousness. There was no abnormal finding by both the four vessel cerebral angiography and brain CT scan. CSF on the fifth day was slight xanthochromic and its cell count was 78 per 3 c. mm, with protein content of 64 mg per 100 ml. CSF cytology showed phagocytes with hemosiderin or erythrocytes (Fig. 7). Immediately, the patient was placed on parenteral vidarabin 15 mg per kg. of body weight which was continued for 2 weeks. He was afebrile and regained his consciousness after administration of the drug and discharged without any neurological deficits 2 months later. Serologic tests revealed a rise in titer of complement fixing antibodies against the herpes simplex virus from 1:4 on admission to 1:32 after 14 days.

III. Neoplastic Meningitis:
A 64-year-old woman was admitted
Fig. 5. Case 1 of bacterial meningitis. Vacuolated neutrocytes with or without diplococcus are seen. May-Giemsa stain. ×1,000

Fig. 6. Case 2 of bacterial meningitis. Cytoplasm of some neutrocytes has vacuoles with or without diplococcus. May-Giemsa stain. ×40
Fig. 7. A case of herpes simplex encephalitis. Phagocytes either with hemosiderin and erythrocytes or hemosiderin are seen. May-Giemsa stain. ×250

Fig. 8. A case of meningeal carcinomatosis. Tumor cells with a large and hyperchromatic nucleus are seen. Nucleus/cytoplasmic ratio increased. May-Giemsa stain. ×500
with complaint of headache of 2 weeks duration. Examination showed neck stiffness and Kernig's sign. The CSF pressure was 210 mmH₂O. It contained 580 cells per 3 c.mm, 68 mg protein and 11 mg sugar per 100 ml. The CSF cytology showed adenocarcinoma cells (Fig. 8). After intrathecal methotrexate therapy, 20 mg weekly, began, gradual improvement of the CSF findings was observed. Malignant cells disappeared from CSF 2 weeks after therapy. Cancer of the gallbladder was found during the therapy and she died from acute renal failure one month later.

IV. Herpes Zoster Meningitis:

Table 1 shows CSF cytological observations of five patients with herpes zoster meningitis. The specimens indicated increasing number of lymphocytes which

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Cell c. (/3mm³)</th>
<th>Differential cell picture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>M</td>
<td>1,370</td>
<td>Lymphocyte 76.0</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>222</td>
<td>Atypical lymphocyte 14.5</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>F</td>
<td>31</td>
<td>Monocyte 12.5</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>F</td>
<td>67</td>
<td>Neutrocyte 0</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1**

CSF cytology of herpes zoster meningitis (Differentiation of 200 cells)

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Pathogen</th>
<th>Cell c. (/3mm³)</th>
<th>Differential cell picture (%)</th>
</tr>
</thead>
<tbody>
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<td>echo 30</td>
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<tr>
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<td>Atypical lymphocyte 2.0</td>
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<tr>
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<td>16</td>
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<td>262</td>
<td>Monocyte 4.0</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>M</td>
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<td>Neutrocyte 47.0</td>
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<td>50</td>
<td>M</td>
<td>echo 30</td>
<td>158</td>
<td></td>
</tr>
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<td>8</td>
<td>28</td>
<td>F</td>
<td>echo 30</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td>9</td>
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<td>F</td>
<td>echo 30</td>
<td>880</td>
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<tr>
<td>17</td>
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<tr>
<td>19</td>
<td>24</td>
<td>F</td>
<td>entero**</td>
<td>375</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

CSF cytology of enterovirus meningitis (Differentiation of 200 cells)

*: Multilobulated nuclei **: Undetermined type of enterovirus except echo 30
Fig. 9. Case 3 of enterovirus meningitis. An atypical lymphocyte is seen. May-Giemsa stain. ×1,000

Fig. 10. Case 3 of enterovirus meningitis. Lymphocytes with multilobulated nuclei are seen. May-Giemsa stain. ×1,000
V. Enterovirus meningitis

Results of cytological study of enterovirus meningitis are listed in Table 2. Echovirus type 30 was isolated from CSF in 17 cases. Atypical lymphocytes were found in 16 cases (Fig. 9). In addition to the atypical lymphocytes, lymphocytes with multilobulated nuclei resembling abnormal cells of adult T-cell leukemia were observed in 12 patients (Fig. 10). Furthermore, the lymphocytes with multilobulated nuclei were seen in a meningitis patient with undetermined type enterovirus. These two type of lymphocytes were not observed in other type of meningitis.

Discussion

Cytologic techniques of CSF have not been useful in diagnosing meningitis due to its available amount, low protein content and its fragile cells. Several techniques have been presented for preserving better cellular detail (Bennet et al. 1968; Bots et al. 1964; Den Hartog Jager, 1969; Kline, 1962; Koelmel, 1977; Kolar and Zeman, 1968; Komp and Cox, 1972; Krentz and Dyken, 1972; McCormach et al. 1953; Seal, 1956, 1964; Watson, 1966). In recent years, using the Shandon cytocentrifuge has been proved to be a rapid, accurate technique for diagnosing meningeal leukemia or lymphoma (Drewinko et al. 1973; Ducos et al. 1979). Although centrifugal force would cause morphologic changes in cells, this method would lose less cells than Sayk's spontaneous sedimentation (Sayk, 1962).

Diagnosis and therapy for bacterial meningitis are depended on the results of isolation and identification of organisms in CSF. In order to reduce the mortality rate and sequelae, the therapy should be started before the organisms are identified. Early diagnosis had been made by the counter-immunoelectrophoresis (Myhre, 1974) and the limulus lysate test (Jorgensen and Lee, 1978). By using a cytocentrifuge, vacuolated phagocytes with coccus were identified only within 30 minutes in two cases of pneumococcal meningitis. Detection of vacuolated phagocyte with coccus in CSF are useful for confirming the diagnosis of bacterial meningitis regardless of its type.

Herpes simplex encephalitis has been thought to be the most important cause of the fatal sporadic encephalitis. Since it would require days to develop antibody against the virus, an early diagnosis and therapy are essential for survival of the patients. Using the centrifuge enabled us to achieve the objectives. In this study, we were able to detect phagocytes either with hemosiderin and erythrocytes or hemosiderin from a patient in cytological study. These phagocytes give us a fairly reliable diagnostic value for encephalitis with hemorrhage such as herpes simplex encephalitis. As Whittley et al. (1977) reported the high efficacy of adenosine arabinoside for this particular encephalitis, it demonstrated an excellent result on the patient.

The clinical presentations of meningeal carcinomatosis have been variable, and its diagnosis entirely depends upon the demonstration of malignant cell in CSF. Antemortem diagnosis is being facilitated by the improved techniques such as Sayk's method and membrane filtration. Our case in this study strongly indicated that a cytocentrifuge would be useful in making diagnosis of meningeal carcinomatosis as well as meningeal leukemia and lymphoma (Sato et al. 1983).

In aseptic meningitis with enterovirus, there has been reported existing pronounced CSF granulocytosis in the initial stage, more often somewhat later followed by a
typical lymphocytosis. In order to distinguish the enterovirus meningitis from partially treated bacterial, tuberculous, fungal and neoplastic meningitis and granulomatous diseases such as sarcoidosis, both clinical and laboratory studies are fundamental. However, it requires a certain period of time to perform them. In the present study, CSF lymphocytes with multilobulated nuclei resembling abnormal cells in adult T-cell leukemia and atypical lymphocytes were found in enterovirus meningitis. They were not observed in other types of meningitis except for herpes zoster meningitis. CSF atypical lymphocytes were observed in all cases of zoster meningitis. Atypical lymphocyte in CSF was reported in a case of cerebellitis in relation to Epstein-Barr infection (Shoji et al. 1983). These lymphocytes were thought to be T cells according to their morphological characteristics. Therefore, they might reflect the morphological changes of CSF T cell in viral infection. The presence of lymphocytes with multilobulated nuclei or atypical lymphocytes in CSF with meningitis indicating the particular therapy would be unnecessary.

References


68. 491-501.