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Detection of Japanese Encephalitis Virus Cell Associated Antigen in CSF by Indirect Immunoflourescence

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ABSTRACT

Japanes encephalitis [JE] is a mosquito-transmitted virus disease. A large number of people with JE die during the early phase of illness. We carried out a study for rapid detection of cell-associated antigen in the cerebrospinal fluid [CSF] samples of patients clinically diagnosed to have viral encephalitis. One hundred twenty patients clinically diagnosed as viral encephalitis were included in the study. Fifty five patients with various other illness based on culture and serology were taken as controls. An indiect immunoflourescence test was employed to detect the cell-associated antigen in CSF cells. The cell associated antigen was detected in 53 of the 120 patients clinically diagnosed as viral encephalitis. The distinct advantage of cell-associated antigen detection test is that it could be completed in 2-3 hours. Although the diagnostic value of antigen detection is less than demonstration of IgM antibodies in CSF, it is useful during the first week of illness when IgM antibodies cannot be demonstrated in CSF.

Key words: Japanese encephalitis, cell-associated antigen, CSF, diagnosis

INTRODUCTION

Japanese encephalitis [JE] is the most common form of epidemic viral encephalitis in India and several South East Asian countries (1). Morbidity rate varies from 0.3 to 1.5 per 100,000 population with case fatality of 10%-60% (2). A rapid and definite laboratory test is necssary to identify JE among patients clinically diagnosed as viral encephalitis. Virus isolation and serology are time consuming. Immuno fluorescence assay is a rapid and specific test for virus identification (3). A study was carried out to demonstrate the cell-associated Japanese encephalitis virus [JEV antigen in the cerebrospinal [CSF] cells of patients clinically diagnosed to have viral encephalitis.

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MATERIAL AND METHODS

Patients

One hundred twenty patients clinically diagnosed to have viral encephalitis, aged 2 years to 28 years, admitted to the Paediatrics and Medicine wards of Jawaharlal Institute of Postgraduate Medical Education and Research [JIPMER] hospital, Pondicherry during the period of one year [May '97-April '98] were included in the study. Patients were admitted with moderate to high grade fever, headache, altered sensorium, meningeal signs and convulsions. Altered sensorium was seen in 88 patients [73.3%]. Of these 88 patients, 36.4% of patients had Grade I sensorium, 40.9% patients had Grade II sensorium and 22.7% had Grade III sensorium.

In all the cases CSF was clear, sterile for bacterial and fungal culture. In 18.2% of patients CSF pleocytosis was observed, of which lymphocyte predominance was seen in 81.2% cases.

CSF glucose was mildly elevated [79-136 mg %] in 30.2% of cases, normal [45-72 mg %] in 48.3% of patients and decreased in 23.3% cases. In 59.3% of patients CSF protein was normal [range 15-40 mg %] and increased in 40.5% [range 48-152 mg %]. Signs of meningeal irritation [neck rigidity and Kerning's sign] were seen in 14.8% of the patients. Clinical diagnosis of viral encephalitis was made based upon these data.

Control Group

CSF was procured from 55 patients with various illness such as bacterial meningitis, tuberculous meningitis, herpes encephalitis and non-infectious neurological conditions who constituted the control group.

Cells

Vero cells infected with JEV [P 20778] and positive CSF sample were used as positive control in each batch of the test.

Mouse ascitic fluid

The lyophilised mouse immune peritoneal fluid [M 61107, 84151-1] was obtained from National Institute of Virology, Pune.

Immunofluorescent assay

Aseptically collected CSF samples were subjected to cytocentrifugation at 1000 rpm for 10 minutes. [Sakura Fine Technical Co. Ltd., Japan]. The cell smears were air-dried and fixed in cold acetone for 20 minutes. Indirect immunofluorescence technique was employed to detect the JEV antigen in cells (4).

Briefly, JEV specific immune peritoneal fluid was used at a dilution of [1:10] in phosphate buffered saline. The immunofluorescent antibody titre was determined by using JEV infected vero cells. Goat-anti mouse fluoroscene isothiocyanate [FITC] conjugate [Dakopatts, Netherlands] was used as secondary antibody [1:10 in PBS]. The smears were quenched with 1% Evan's blue to stain the cells to obtain a good background.

The smears were screened using a BHF Fluorescent Microscope [Olympus, Japan]. The smears were graded from [+] to [++++] based upon the percentage of cells showing fluorescence and amount of fluorescence emitted by the cells [Figure 1]. The cells sharing $\geq$ ++ were considered positive Non-specific reaction was ruled out by checking the antibody with normal CSF cells.

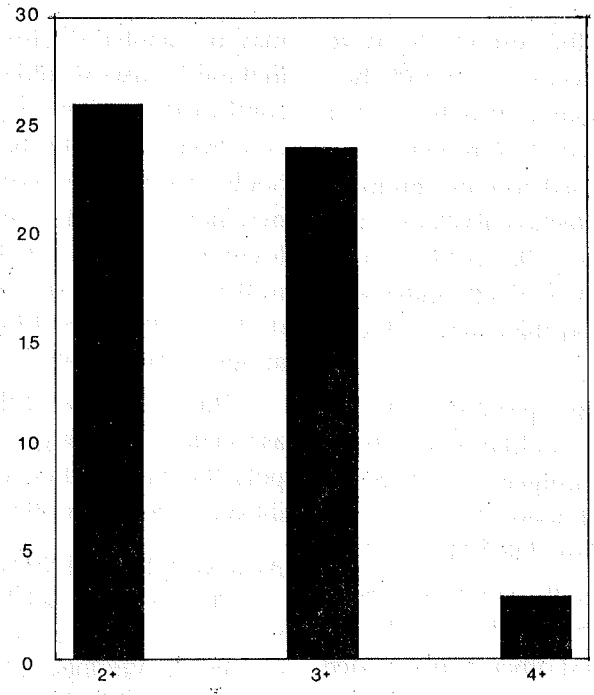

Figure 1. JEV cell-associated antigen grades in CSF cells

RESULTS

Out of 120 CSF sample tested for JEV cell-associated antigen, 53 samples were positive [44.2%] by indirect immuno-flourescence. The infected cells showed extranuclear apple green fluorescence which were clearly distinguished from the uninfected or control normal cells. The duration of fever on admission was available for 76 patients which was in the range of 3-5 days.

Of the 53 patients tested positive for cell-associated antigen in CSF, 10 expired [18.9%] during their hospital stay.

Ten patients [18.9%] were seriously ill during their hospital stay and were taken against medical advice [AMA]. Fourteen patients [26.1%] who recovered had neurological deficit at discharge.

DISCUSSION

Japanese encephalitis is prevalent in and around Pondicherry (5,6). The mortality in this disease varied from 20%-40% in different parts of India (7).

Immunoflouresence was introduced Coons et al in early 1940 (4). After the development of non-destructive conjugation of FITC to antibody, its use has considerably broadened in diagnostic virology (8).

In the present study we have used indirect immunoflouresence test to detect cell-associated JEV antigen in human CSF cells using polyclonal antibodies raised in mice. In 53 out of 120 cases [44.2%] JEV antigen could be detected in CSF, while in the remaining 67 [55.8%] which were clinically diagnosed as viral encephalitis were negative for JEV antigen.

In ten patients the test results were inconclusive [fluoroscence <++] and they were considered negative. It is likely that these are cases of secondary response i.e., they have been exposed to other group B viruses prior to the infection. Earlier studies have shown that West Nile and Dengue 2 viruses are prevalent in South India and these could have been the cause of broad antibody response (9).

The findings of the present study are comparable with those of Matur et al, 1990, where JEV specific antigen was detected by indirect immunofluoescence technique in 15 out of 31 patients [48.4%].

Negative test results could have been due to following reasons. Firstly, clinical presentation of several other diseases such as encephalitis caused by other viruses, cerebral malaria, tuberculous meningitis may reresemble JE (10) and the diagnosis in these 67 cases would have probably been confirmed if other diagnostic tests were employed. Secondly, the method used may not be sensitive. Secondly, the method used may not be sensitive enough to detect the level of antigen or JEV would have multiplied with in the CSF cells and destroyed them resulting in the absence of antigen bearing cells (11).

Thus, detection of the presence of cell-associated JEV antigen in CSF cells using polyclonal antibodies seems to be a rapid, inexpensive diagnostic procedure.

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