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Rapid Diagnosis of Herpes Simplex Encephalitis - Immunocytochemical Localisation of Antigen in Cytological Preparations

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Abstract

Role of immunocytochemical localization of Herpes Simplex Viral antigen in cytological preparations viz., brain biopsy smears and CSF cytospin, for a rapid and specific diagnosis of the encephalitis was evaluated. The antigen could be localised in the neurons of the brain biopsy and mononuclear cells in CSF, in three cases, thus helping in a rapid diagnosis and timely antiviral treatment. Failure to localise the antigen in another brain biopsy alerted to seek alternative diagnosis and avoid the expensive Acyclovir therapy. Immunocytochemical detection of HSV antigen in cytological preparations appears to be a very useful technique for diagnosis in neurological practice.

Key words -

Herpes Simplex Encephalitis,
Rapid diagnosis,
Cytological preparations,
Immunohistochemistry

Herpes Simplex type I Virus (HSV I) is recognised to be the commonest identifiable cause of sporadic fatal encephalitis in humans [1], [2], [3], [4]. The existing laboratory methods for diagnosis of Herpes Simplex Encephalitis (HSE) include the demonstration of viral antibodies in CSF and isolation of the virus [5]. However antibody detection is of limited value in the diagnosis of HSE unless paired samples of serum/CSF are available and a rise or fall in the titre is demonstrable in them [6]. Isolation of the virus from the brain biopsy specimens constitute the definitive diagnostic criterion [5], [7]. However, this is time consuming, requiring a minimum of 4-7 days. Therefore antigen detection in the inflammatory cells of CSF cytospin, or biopsied brain tissue, which requires 24 hours or less has gained importance in the diagnosis of HSE

[8]. Immuno fluorescence has been used for the demonstration of HSV antigen in various tissues and is considered a method for diagnosis of viral infections [9].

The present study was undertaken to immunologically localise HSV antigen in cytological preparations of central nervous system tissue and to evaluate the usefulness of the method as a specific and rapid diagnostic aid. Thus the usual emperically used expensive antiviral therapy, based only on clinical, electrophysiological and radiological findings, can be rationalised.

Immunostaining was carried out, on smears of brain biopsies in two cases and CSF cytospin preparation in another three cases of suspected HSE, in addition to other immunological, serological tests. Definitive diagnosis could be established the same day in 3 cases and appropriate therapy could be instituted immediately.

Material and Methods

The study was carried out on five patients admitted to the neurological services of NIMHANS between 1988 and 1992. Detailed clinical and laboratory data suggested a probable diagnosis of Herpes simplex Encephalitis in all of them (Tables I and II). Serum and CSF samples were obtained from all these subjects for immunological studies. Diagnostic brain biopsy was performed in 2 cases (cases 1 and 2). The site of biopsy in both cases was the posterior temporal region.

Table I - Clincal data

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Table II - Laboratory data

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POS/+=Positive
NEG/-=Negative
B=Brain biopsy specimen
C=CSF Cytology
ND=Not done (Figures in parentheses indicate the day immuno cytochemistry was carried out for antigen detection, from the day of onset of clinical illness).

Antibodies to HSV I

Antibodies to HSV I were estimated both in serum and CSF by ELISA as per the procedure of Lenette and Schmidt (1979) [10].

HSV-I antigen localisation

For immuno cytological study, squash smear preparation from the fresh brain tissue in cases 1 and 2 and cytospin preparation from the CSF in the other three cases were made. Even though CSF was obtained in all 5 patients, cytospin preparation of the same for antigen localisation in cells was possible only in 3 patients (cases 3, 4, 5). All the smears (2 of brain biopsy and 3 CSF Cytospin smears) were fixed in 90% alcohol and stained immunohistochemically with affinity purified rabbit anti HSV-I antibody (DAKO product) by PAP technique of Sternberger et al [11], using swine anti rabbit serum as the link antibody. On the control smears, the primary antibody was replaced by normal rabbit serum.

Results

Antibody studies

In case 1, two consecutive samples of serum and CSF were obtained on the 18th and 68th days after the onset of clinical symptoms. The first sample was found to be positive for anti HSV-I antibodies in the serum and CSF. The second sample obtained after 50 days showed a significant fall in the antibody titres both in the serum and CSF. In cases 2,3, and 5, only one sample of serum and CSF was available for study. These showed high titres in the serum for HSV antibodies. Since a second sample was not available for analysis, the test was considered equivocal (Table II). In case 4, there was an initial high titre of HSV-I antibodies in the CSF. The second sample obtained after 4 days showed a five-fold decrease in the antibody titres. This finding pointed to a possible decrement of Herpes Encephalitic process also. A third sample obtained 60 days after showed a further fall in the antibody levels.

Antigen studies

Immunocytochemical staining of the brain biopsy tissue in case-1 showed the presence of viral antigen within both the cytoplasm and the nucleus of some neurons (Figure I). However, smears from the other brain tissue (case 2) did not stain for the viral antigen. On further histological study, this tissue revealed characteristic pathological features of tuberculous meningitis with border-zone reaction and arteritis in the temporal cortex.

- ***Immunocytochemical localisation of HSV antigen in the brain biopsy smear - Case 1 : PAP technique (× 120) - Insert a. Higher magnification showing intracytoplasmic HSV antigen in neurons (× 240) - b. Intranuclear inclusion in the neuron (arrow) smear fixed in alcohol and stained with haematoxylin eosin (× 180)***

Immunostaining of cytospin preparations of the CSF in cases 3 and 5 revealed the presence of viral antigen within mononuclear cells (Figure 2) but not in case 4.

- ***CSF cytospin preparation showing positively stained mononuclear cell for HSV antigen (double arrow). Note unstained lymphocyte in the same field. Case 3. PAP technique (× 360)***

In all the above cases, the results of the antigen detection studies were communicated to the clinicians within 6 hours. In the three positive cases, treatment with acyclovir was started immediately. Virus was subsequently isolated from the brain tissue in case 1 at a later date. Virus isolation was not attempted on CSF samples because of insufficient sample available.

Of the two antigen negative cases, one showed tuberculous pathology on histological examination of the brain biopsy specimen (case 2). In case 4, the diagnosis of HSE was considered equivocal as immunostaining for the viral antigen on two CSF samples collected at an interval of 3 days was negative inspite of high levels of IgG by ELISA. However, empirically acyclovir was started by the clinician from the 4th day of admission.

The first patient (case 1), diagnosed as a case of HSE both by immunostaining and later by virus isolation, recovered following acyclovir therapy within 2 weeks and remained asymptomatic even at 30 months follow up and has resumed his profession. Case 2 succumbed to her primary pathology viz, tuberculous meningitis and arteritis. Case 3 showed clinical improvement with acyclovir therapy but was not available for subsequent follow up. Case 4 received the antiviral therapy for HSE and was discharged with partial improvement. He returned to the Neurology service two months later in a vegetative stage. The repeat CSF examination revealed increased levels of antibodies to HSV. He is

receiving supportive therapy but has not shown much improvement and continues to be in the same state. Case 5 also showed clinical improvement with acyclovir therapy and was subsequently not available for follow - up.

Discussion

There are a number of treatable conditions which clinically mimic acute viral encephalitis [5], especially tuberculous meningitis and cerebral malaria. In view of this, it is desirable that early detection and identification of the causative agent be made in encephaliticis, for timely specific therapy. Antigen detection in CSF has proved to be rapid, sensitive and specific in encephalitis caused by a variety of viruses such as Mumps, Measles, Herpes Simplex and Herpes Zoster [12]. Recent reports from India have also indicated that detection of both soluble and cell bound viral antigen in CSF is very useful in the early diagnosis of Japanese Encephalitis [13], [14].

Laboratory diagnosis of Herpes Encephalitis remains a difficult problem. For instance, an international collaborative antiviral study group on Herpes Simplex Encephalitis in the USA concluded that false positive results are not uncommon with immunological methods of diagnosis, irrespective of whether antigen or antibody detection systems are used, either on serum or CSF samples. To-date, the most specific method available for the diagnosis of HSE is isolation of the virus from brain tissue. Performing an invasive procedure such as brain biopsy is not feasible at most centers in India. Hence it has become quite clear that non invasive methods of diagnosis are needed. Antigen detection either in the cells of the CSF or in the soluble form is a valuable substitute to virus isolation chiefly because of the ease of performance and the rapidity with which results can be obtained. There are very few studies wherein rapid diagnosis of HSE has been attempted utilising antigen localisation method in the cells of CSF. To the best of our knowledge, this is the first report from India detailing the use of a rapid antigen localisation technique on cytological preparations from both brain biopsy tissue and CSF for the diagnosis of HSE. The results of our study indicate that the immunoperoxidase method which has the advantage of being simple and sensitive, may find wide application for the rapid diagnosis of HSE.

The inclusions in HSE are typically intranuclear within the neurons. However, the virus replication occurs both within the nucleus and cytoplasm. Therefore the antigen can be detected in either of these locations. In CSF samples, the antigen is localised within mononuclear cells. Though the localisation of HSV antigen in the CSF cells, similar to the brain cells is diagnostic of HSE, failure to demonstrate the antigen does not exclude the diagnosis. Other limitations of the antigen localisation in CSF cells is obtaining sufficient number of cells in the sample for immunocytochemical localisation. Despite these limitations, antigen localisation in CSF cells is an useful adjunct to clinical diagnosis especially when a positive result is obtained. The maximum beneficial effect of antiviral therapy in HSE can be achieved only when a definite diagnosis is made early in the disease.

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