Biopsy Histopathology in Herpes Simplex Encephalitis and in Encephalitis of Undefined Etiology

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Received November 15, 1983

The histopathology of herpes simplex encephalitis (HSE) has been described principally from postmortem studies which reveal end-stage disease. Biopsy material, which selects an earlier stage in disease development, has been used principally to isolate virus, identify viral particles, and locate viral antigens. Further, little attention has been paid to the histopathology of biopsies of encephalitis of undefined etiology. In the present study, sections from biopsies which yielded virus and those which were negative for virus were evaluated in a systematic and controlled manner. Biopsies yielding virus were characterized by meningeal inflammation, perivascular infiltrates, and glial nodules. Biopsies which did not yield virus and which failed to reveal another diagnosis were characterized by nonspecific gliosis. Thus the early histopathology of HSE is characterized by early signs of inflammation in the absence of necrosis and generally differs from biopsies in which virus is not isolated.

The pathology of herpes simplex encephalitis (HSE) has been most extensively studied in postmortem material [1]. Postmortem material, however, represents an end-point in the evolution of the disease. It would be of interest to analyze the histopathological changes early in the course of proven human herpes simplex virus (HSV) infection. Such a study gains importance from the need to make therapeutic decisions in the course of a suspected HSE [2]. The practice of obtaining brain biopsy prior to the initiation of antiviral therapy affords the opportunity to compare the histopathology of virologically proven HSE early in the disease with that in which no agent is isolated. In order to compare the histopathological characteristics of viral-positive specimens with those from which no virus was isolated, we collected cases which were biopsied for the possibility of HSE. The histologic sections were evaluated with standardized criteria but without knowledge of whether the biopsies yielded HSV. Subsequently the results were grouped into viral-positive and viral-negative categories and the observations analyzed.

PATIENTS AND METHODS

Patients

Cases biopsied for focal encephalitis in a period of 78 months at the West Haven VA Medical Center and the Yale-New Haven Hospital were reviewed. The medical
records of these patients were reviewed for clinical, laboratory, and neurodiagnostic test characteristics. Various neurodiagnostic tests were used. These included the electroencephalogram, isotope brain scan, computerized tomography, and angiography. Histopathologic biopsy material was available for review (as discussed later) for 12 of the 14 identified patients. Of the 12 patients, five had virus-positive biopsies. Two patients whose biopsies were negative were later shown by other studies to have had HSE. One had autopsy-confirmed viral isolation [2] whereas the other patient developed a diagnostic level of CSF anti-HSV antibody.

**Histopathologic Studies**

Routine sections of formalin-fixed, paraffin-embedded, and hematoxylin- and eosin- (H and E) stained biopsy specimens were reviewed for the 12 cases. Sections with special stains were reviewed when available. In the current study, however, these stains contributed no information beyond that obtained with routine H and E stained sections. The sections were read without the benefit of clinical or virological data. Changes in both gray and white matter as well as the meninges were evaluated for each case (Table 2). After the standardized evaluation, an overall diagnostic impression was rendered. Subsequently the readings were assigned to the appropriate patients and arranged according to whether the biopsy specimen had yielded HSV in culture.

**RESULTS**

**Clinical Characteristics**

Cases with HSV positive and negative biopsies analyzed retrospectively revealed no discriminative clinical differences (Table 1). Symptoms, signs, or abnormalities in neurodiagnostic tests referable to temporal or adjacent parietal or frontal lobes were present in all patients. Fever prior to biopsy was also found in all patients. CSF pleocytosis was found in all but one, an HSE proven patient. Localization by neurodiagnostic test was obtained in six of seven patients with proven HSE and in three of five patients negative for HSE. Of these tests, the pre-biopsy EEG was localizing in five positive cases and in two negative cases. Two of the positive cases demonstrated periodic discharges before biopsy.

**Histopathological Findings**

The sections from all cases were read in the absence of clinical and virological data. Of the 12 cases selected for study, five yielded HSV from the biopsy whereas

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**TABLE 1**

Clinical Characteristics of Patients Biopsied for Herpes Simplex Encephalitis

| HSV Isolation | Clinical Focality | Fever | CSF Pleocytosis | Focality on Neurodiagnostic Test* |
|---------------|-------------------|-------|----------------|----------------------------------|
| Positive      | 5/5*              | 5/5   | 4/5            | 4/5                              |
| Negative†     | 5/7               | 7/7   | 7/7            | 5/7                              |

*Electroencephalogram, isotope brain scan, computerized axial tomography, angiography
†Number positive/Number tested
‡Contains two cases shown to be HSE by other evidence (one by virus isolation at autopsy, one by CSF antibody studies)
seven did not. The histopathological findings are shown in Table 2. Non-specific reactive gliosis was found in white and gray matter in all HSV positive and negative biopsies. However, signs of inflammation were found predominantly in biopsies which yielded HSV. Thus, meningeal infiltrates were found in all positive biopsies which included portions of meninges. Perivascular infiltrates were found in negative as well as positive biopsies; however, in positive biopsies they were more frequently found in both gray and white matter. Glial nodules were found only in positive biopsies. Intranuclear inclusion bodies were found in one virus-positive biopsy. In the two cases in which the biopsies were negative for virus but which were shown by autopsy or CSF antibody studies to be HSE, no inflammation was found in the biopsies. Four of the five virus-positive specimens were histopathologically diagnosed as encephalitis, whereas only one of seven of the virus-negative specimens was diagnosed as encephalitis (Table 2). No etiology was found in the one negative case histopathologically diagnosed as encephalitis.

**DISCUSSION**

The present study appears to be the first controlled evaluation of the histopathology in biopsies from patients with suspected HSE. Originally the histopathology of HSE had been established in autopsy material by Haymaker [1]. More recently Nahmias et al. reported that 85 percent of HSV positive biopsies were histopathologically diagnosed as encephalitis whereas only 36 percent of HSV negative biopsies were diagnosed as encephalitis [3]. However, the incidence of the individual characteristics of the histopathology were not given. In addition, the histology was not performed in a controlled fashion. In the present study the histopathologist was denied access to the clinical and virological data associated with the histological sections. Specific characteristics and a diagnostic impression were noted. Four of five HSV positive biopsies were scored as encephalitis. These were associated with meningeal inflammation, perivascular infiltration, and glial nodules. Inclusions were found in one case. Necrosis and hemorrhage were not part of the pathology. In the virus-negative group one was evaluated as encephalitis and another was read as stroke. However, the five remaining biopsies revealed only nonspecific gliosis.

Autopsy studies have tended to demonstrate rather severe histopathological changes. Necrosis characterized portions of the cortex of each of the three cases reported by Haymaker [1]. Large mononuclear and compound granular cells were found to replace superficial cortical laminae. Perivascular infiltrates and neuronophagic nodules were also described. In an elegant immunoperoxidase study of HSE by Esiri the cases came to autopsy from four days to three years after the onset

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**TABLE 2**

Histopathological Characteristics of Biopsies Taken for Suspected Herpes Simplex Encephalitis

| HSV Isolation | Meningeal Infiltrate | Gliosis | PVI* | Glial Nodules | Inclusions | Diagnosis |
|---------------|----------------------|---------|------|---------------|------------|-----------|
| Positive      | 4/4*                 | 5/5     | 5/5  | 4/5           | 5/5        | 3/5       | 1/5       | 4/5 Encephalitis |
| Negative      | 2/6                  | 7/7     | 6/6  | 2/7           | 2/7        | 0/7       | 0/7       | 5/7 Gliosis      |

*G = Gray matter, W = White matter
*Perivascular infiltrate
*Number tested/Number in which assessment possible
of neurological disease [4]. The four cases examined in the first week after the onset of neurological signs are the most comparable to cases biopsied in the course of management of suspected HSE. Signs of inflammation were scanty and seen as perivascular cuffs in the meninges and superficial cortex. In these patients viral antigen was found in both hemispheres, with slightly more on the more affected side. Inflammation and necrosis was found to peak in the third week, at which time viral antigen was declining. Our findings in this biopsy study correspond to those found by Esiri in the first week of neurological dysfunction. Meningeal and perivascular infiltrates were found but necrosis was not found in biopsies which yielded HSV.

Comparison of isolation-positive versus isolation-negative biopsies in the National Collaborative Study revealed a morphologic diagnosis of encephalitis in 85 percent of positive cases [3]. Four of the five virus-positive biopsies in the present series were read “blindly” as encephalitis. In the National Collaborative Study 56 percent of HSV positive biopsies and 14 percent of HSV negative biopsies contained intranuclear inclusions. In the present series only one case was found to contain intranuclear inclusions. Although Haymaker required inclusions as well as the isolation of HSV for the diagnosis of HSE [1], it is clear that the observation of inclusions is inconsistent. In a study of ten cases of HSE, White and Taxy found inclusions to be absent in one, rare in six, and numerous in three [5]. In addition the type of fixative influences their visualization; Bouin's fixative facilitates their identification. Thus, while the observation of intranuclear inclusions is useful, it is neither consistent nor diagnostic and is technically variable.

Considerable interest attaches to the group of biopsies from which virus was not isolated. Of paramount therapeutic importance is the category of other processes which can mimic HSE. In the National Collaborative Study, other infections, including several other viral infections, vascular disease, and tumors were found [6]. In the present group negative for virus isolation one case of vascular disease and one case histopathologically identified as encephalitis were found. Another category are those biopsies which fail to yield HSV but in which HSE can be diagnosed by other means. In the present series one case had HSV isolated at autopsy (previously reported [2]), and another was shown to be HSE by the development of antibody in the CSF. Each of these cases demonstrated only nonspecific gliosis on histopathological examination of the biopsy specimens. There remain the biopsies from which virus is not isolated, in which the histopathology does not yield an alternative diagnosis, and in which a diagnosis is not reached by other tests. Clinically they would be termed encephalitis of undetermined etiology. Three such biopsies are in the present series. The histopathology in each of these cases was read as nonspecific gliosis. On occasion this type of biopsy will be obtained in HSE in which the appropriate sampling was not achieved. However, this is a group in which other etiologies must be sought by careful epidemiologic, immunologic, and immunohistological studies.

ACKNOWLEDGEMENTS

We are grateful to G.D. Hsiung for access to the records of the Diagnostic Virology Laboratory of the West Haven VA Medical Center; to L.G. Miller, W.A. Andiman, and G. Tucker for access to the records of the Virology Laboratory of the Yale-New Haven Hospital; to Virginia Wilson and Elizabeth Mullaly for assistance with the histopathological material; to the Laboratory Division of the Connecticut State Department of Health for viral antibody determinations; to J. Pawlisch and S. Livingston for assistance with medical records of the Yale-New Haven Hospital; to M. Murray, Deborah Beauvais, and Sarah Murphy for manuscript preparation; and to Ms. Fran Bernstein and the staff of the West Haven VA Medical Center Laboratory for assistance with searching the literature.
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