HERPES SIMPLEX ENCEPHALITIS: REPORT OF TWO FATAL CASES IN SINGAPORE

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Studies in recent years have shown that Japanese encephalitis virus is one of the major causes of acute encephalitis in children in Singapore. It is estimated that 40 per cent of viral encephalitis cases among children is due to this arbovirus (Chan, 1965). The aetiology for the remaining cases has not been investigated, but it is believed that agents such as enteroviruses, mumps and herpes simplex viruses would be involved in a proportion of these cases. A case of encephalitis attributed to mumps virus (based on serological test) has, in fact, been reported (Institute for Medical Research, 1957), and a coxsackie B-4 virus has also been isolated from the brain of a fatal case of encephalitis (Lee, 1961). In this paper, we report two fatal cases of encephalitis caused by herpes simplex virus. The viruses were isolated in mice during routine examination of post-mortem brain specimens for Japanese encephalitis virus. This is the first report of herpes simplex encephalitis in Singapore.

CASE 1

The patient was a 6-year-old female Chinese child admitted on June 12, 1959 to the Paediatric Unit of the Singapore General Hospital, with a history of fever, vomiting, and headache for 5 days. There was no history of convulsions.

On examination, the child was febrile (103°F) and drowsy. The tonsils were injected, and the cervical nodes were palpable on both sides. The muscle tone was slightly increased, there was neck rigidity, and Kernig's sign was positive. There was no apparent loss of sensation, and the reflexes were normal. No abnormal findings were detected in the other systems.

A lumbar puncture showed a mildly raised cerebrospinal fluid pressure; the cell count of the fluid was 180 per c. mm., being made up of numerous lymphocytes and some polymorphs. The chlorides, glucose, and protein were within normal limits. A smear revealed no organisms and culture of the fluid was negative. Chest X-ray was clear. A clinical diagnosis of meningitis was made, and the child was treated with Inj. Kemicetine and sedatives.

In spite of treatment, the condition of the child continued to deteriorate. She developed convulsions, became progressively more drowsy, and died 36 hours after admission.

At autopsy, there was patchy consolidation in both lungs. The brain was oedematous, the sulci being partially obliterated. There was no meningeal exudate.

Histological examination confirms the presence of bronchopneumonia. In the brain, a mild round cell infiltration is seen in the leptomeninges. There is evidence of cerebral oedema. Many small vessels in various parts of the brain show a cuffing of small round cells. This finding is most prominent in sections of the cerebral cortex, cerebellum and pons. No cellular inclusions are detected.

A virus (designated as GH-8/59) was isolated from the brain by inoculation of suckling mice. It was identified as a herpes simplex virus in a neutralization test using herpes simplex (Z strain) antiserum (Table 1). The virus, after mouse adaptation, produced herpes-like pocks on the chorio-allantoic membrane of chick embryonated eggs.

CASE 2

Clinical History

The patient, a 5-month-old Chinese male infant, was admitted on April 2, 1962 to the Paediatric Unit of the Singapore General Hospital with a history of high fever for 4 days, and cough which was unproductive of sputum. He also had twitchings of hands and legs during this period, but there were no generalised convulsions or clenching of teeth. There was no history of any fall or injury. However, 3 days prior to

the development of symptoms, the patient had been vaccinated against small pox.

The child was the ninth pregnancy of the mother, having been delivered normally at full term, with a birth weight of 9 lb. 2 oz. His milestones were normal. The other siblings in the family were well.

On examination, the child was febrile (103°F) and drowsy. The fontanelle was not tense. Pupils were reactive. Muscle tone and motor power were good, reflexes were normal, and there was no apparent loss of sensation. The neck was not rigid, while Kernig's sign was equivocal. The pharynx was injected. No abnormalities were detected in the other systems.

Laboratory Findings on Admission

Haematological examination showed haemoglobin, 12·6 gm. %; total white, 7,600, with a differential count of neutrophils, 50%; lymphocytes, 40%; eosinophils, 0%; and monocytes, 10%.

The cerebrospinal fluid was clear and was of normal pressure. It contained cells, 35 per c. mm.; chlorides, 660 mg. %; globulin +; glucose, 65 mg. %; and total protein, 120 mg. %. A smear showed few lymphocytes, polymorphs and red blood cells, but no organisms.

Chest X-ray did not reveal any abnormality.

Progress and Treatment

The patient maintained a swinging temperature despite injections of kemicetin and antipyretics. The blood picture 2 days after admission showed a total white count of 14,000, with neutrophils, 76%, lymphocytes, 18%, eosinophils, 2%, and monocytes, 4%. Examination of the cerebrospinal fluid 4 days after admission revealed a cell count of 32 per c. mm., chlorides, 540 mg. %, globulin +, glucose, < 20 mg. %, and total protein, 180 mg. %; a smear showed numerous lymphocytes, few polymorphs and red blood cells, but no organisms.

The patient's condition did not improve, and he developed spasticity of limbs and neck rigidity. He was given luminal, and started on prednisolone. He became deeply comatose on the fifth day and died on the sixth day.

Autopsy Findings

At autopsy, the significant findings were confined to the central nervous system.

The brain weighed 650 gm. the superficial vessels were congested, and small, localized areas of subpial haemorrhage were seen over the left frontal region and on the medial surface of the right cerebral hemisphere. The brain tissue generally was soft and pulpy. Within the brain substance, punctate haemorrhages were seen scattered in both the grey and the white matter.

The spinal cord appeared normal.

Histology

The leptomeninges overlying the cerebral cortex are oedematous and show an inflammatory reaction with preponderance of round cells (Fig. 1). The blood vessels within the brain substance are congested, and most of them are surrounded by a cuffing of round cells of varying thickness (Fig. 2). Numerous haemorrhages are seen. There are foci of neuronal degeneration with neuronophagia, as well as focal areas of necrosis.

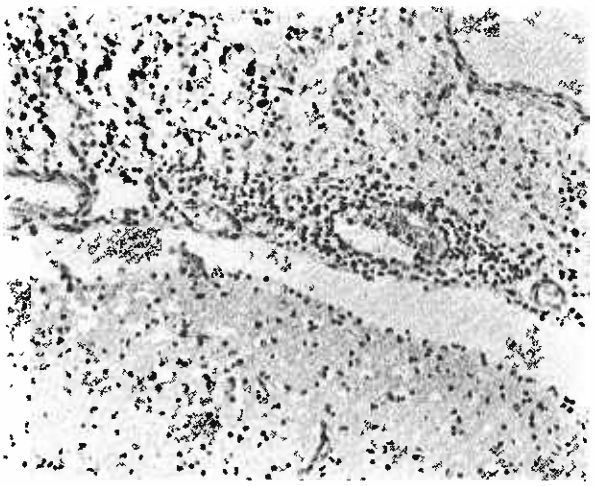

Fig. 1. The leptomeninges of the celebral coxtex showing oedema and infiltration with small round cells. Haematoxylin and eosin. X150.

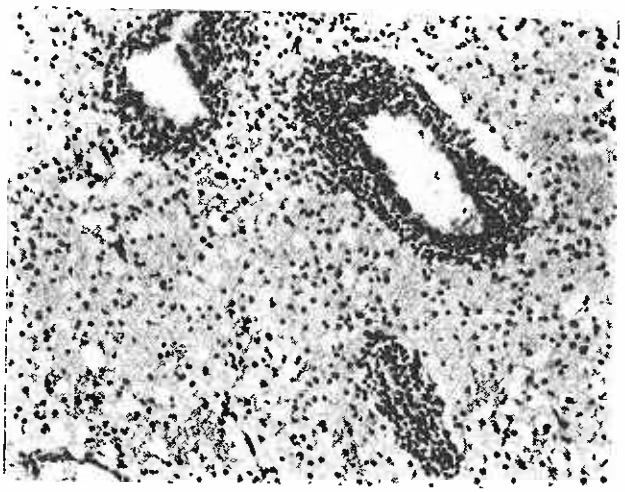

Fig. 2. Blood vessels within the brain substance showing perivascular cuffing of round cells. Haematoxylin and eosin. X150.

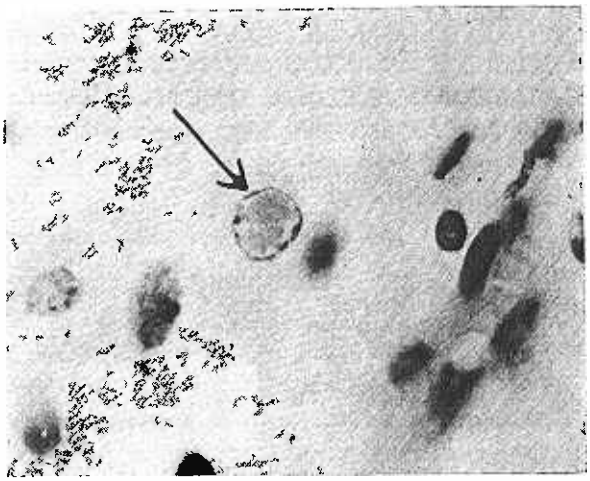

Fig. 3. Arrow shows nucleus of astrocyte containing homogeneous, eosinophilic inclusion (Cowdry type A) almost filling the nucleus. Haematoxylin and eosin. X 1180.

Within some oligodendrocytes and astrocytes, intranuclear inclusions (Type A homogeneous) are detected. These appear as homogeneous, eosinophilic masses almost filling the nucleus, and displacing the nucleoli and chromatin to the periphery (Fig. 3). The nucleus itself is enlarged. These inclusions are most readily detected in sections taken from the internal capsules.

The above histological changes are seen throughout the brain, except the cerebellum which appears normal. No pathological changes are seen in the spinal cord.

Histological examination of the other organs of the body reveals no significant abnormal findings.

Virology

The brain and cord specimens obtained at autopsy were made into a 20% suspension in 10% rabbit serum-saline. The tissue suspension was inoculated into suckling mice intracerebrally and subcutaneously, and also into embryonated eggs by the chorio-allantoic route. An agent (designated as GH-3/62) was isolated in mice and was identified as herpes simplex virus in a neutralization test in mice with specific herpes simplex antiserum (Table I). In eggs, herpes-like pocks were seen on the chorio-allantoic membrane.

DISCUSSION

The diagnosis of herpes simplex encephalitis depends on laboratory tests, the three main criteria used being (1) isolation of the virus from the brain; (2) demonstration of the characteristic intranuclear inclusion bodies of the Cowdry type A in brain cells; and (3) demonstration of a significant rise in specific antibodies between acute and convalescent phase sera. The majority of confirmed cases reported in the literature have been diagnosed on the presence of one or two of the above criteria (Miller et al., 1966). In the two cases reported here, the virus was isolated from both cases and intranuclear inclusion bodies were detected in one. The patients' sera were not tested for specific antibodies.

Clinically, the patients present with the usual manifestations of viral encephalitis, with non-specific symptoms such as headache, fever, nuchal rigidity, evidence of raised intracranial pressure, delirium and coma (Blackwood et al., 1966; Rawls et al., 1966). Focal features, however, may be present, such as focal seizures, hemiparesis, aphasia, and focal electro-encephalographic and angiographic abnormalities (Miller et al., 1966; Rawls et al., 1966). These features may mimic those of a cerebral abscess, and are believed to be due to the local destructiveness of the virus.

TABLE I

IDENTIFICATION OF VIRUS ISOLATES BY NEUTRALIZATION TEST IN ADULT MICE

Virus strain	Virus titre (log LD_{50})		Log neutralization index
	NMS*	IMS**	
Herpes simplex			
(Z strain)	5·4	2·5	2·9
GH-8/59	5·7	3·2	2·5
GH-3/62	6·0	3·8	2·2

* Normal mouse serum

** Immune mouse serum prepared against herpes simplex (Z strain) virus.

The findings in the cerebrospinal fluid are nonspecific, with a mononuclear pleocytosis and increase in protein content. Presence of numerous erythrocytes and xanthochromia have been regarded as suggestive (Miller et al., 1966).

Most of the reported cases died within 2 to 3 weeks from the onset of the illness, although several cases have been reported to have recovered and to be alive 3 years later (MacCallum et al.,. 1964; Pierce et al., 1964; Leider et al., 1965). Our two cases succumbed after a clinical history of 7 and 10 days, respectively.

Post-mortem examination of the brain reveals the changes which are generally met with in viral encephalitis. Microscopic changes include perivascular cuffing with small round cells, focal necrosis, microglial proliferation, and focal demyelination. However, certain characteristic features may be found in the brain of herpes simplex encephalitis: (a) the massive destructiveness of the lesion, which may result in widespread necrosis of the brain; various necropsy reports suggest that the destructive process shows predilection for the temporal lobes, insula, cingulate gyrus, and the orbital surface of the frontal lobes; (b) the presence of groups of erythrocytes forming "lakes of blood" with little surrounding cellular reaction (Miller et al., 1966); and (c) the presence of type A Cowdry intranuclear inclusions in neurones or glial cells (Haymaker et al., 1958). In our second case, the destructiveness of the virus was reflected in a generalised softening of the whole brain, with microscopic evidence of widespread degeneration and necrosis. Blood lakes were not a prominent feature. The characteristic intranuclear inclusions were present within numerous astrocytes and oligodendrocytes.

Herpes simplex virus is one of the agents commonly encountered in the aetiological studies of central nervous system disease. Studies in other countries have shown that this virus was responsible for 6 to 9 per cent of viral encephalitis cases (Lennette et al., 1959; Meyer et al., 1960; Klemola et al., 1965), but some workers believe that this form of encephalitis is more common than is reported (Kent and Nicholson, 1964; Blackwood et al., 1966). The cases reported in the literature show a wide age distribution. Haymaker et al. (1958) reviewed 42 cases ranging in age from infancy to 77 years. The incidence of herpes simplex encephalitis in Singapore is not known. Although only two cases, both fatal, have been confirmed, we believe that more cases would be uncovered if routine virological studies are performed, and the brain tissue carefully examined for the characteristic intranuclear inclusion bodies. It may be pointed out that in the second case reported here, a diagnosis of postvaccinal encephalitis (because of the smallpox vaccination prior to the illness) would have been suspected had not a herpes simplex virus been isolated and the intranuclear inclusion bodies seen in the brain tissue.

SUMMARY

Two confirmed fatal cases of herpes simplex encephalitis in children are reported. The virus was isolated from the brain of both cases. Histological examination of brain tissue showed the presence of intranuclear inclusions of the Cowdry type A in one case. The clinical and pathological features of the disease are briefly discussed. This is the first report of herpes simplex encephalitis in Singapore.

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REFERENCES

1. Blackwood, W., Dudgeon, J.A., Newns, G.H. and Phillips, B.M. (1966): "Case of encephalitis due to herpes simplex", Br. med. J. 1, 1519.
2. Chan Y. C. (1965): Unpublished data.
3. Haymaker, W., Smith, M.G., van Bogaert, L. and de Chenar, C. (1958): "Pathology of viral disease in man characterized by nuclear inclusions", In *Viral Encephalitis*, ed. by W. S. Fields and R. J. Blattner, p. 95. Springfield, Ill., Charles C. Thomas, Publisher.
4. Institute for Medical Research (1957): Annual Report, p. 109, Kuala Lumpur, Federation of Malaya.
5. Kent, T.H. and Nicholson, D.P. (1964): "Herpes simplex encephalitis", Am. J. Dis. Child. 108, 644.
6. Klemola, E., Kaariainen, L., Ollila, O., Pettersson, T., Jansson, E., Haapanen, L., Lapinleimu, K. and Forsell, P. (1965): "Studies on viral encephalitis", Acta med. scand, 177, 707.
7. Lee, L.H. (1961): Personal communication.
8. Leider, W., Magoffin, R.L., Lennette, E.H. and Leonards, L.N.R. (1965): "Herpes simplex virus encephalitis. Its possible association with reactivated latent infection", New Engl. J. Med. 273, 341.
9. Lennette, E.H., Magoffin, R.L., Schmidt, N.J. and Hollister, A.C., Jr. (1959): "Viral disease of the central nervous system. Influence of poliomyelitis vaccination on etiology", J. Am. med. Ass. 171, 1456.

10. MacCallum, F.O., Potter, J.M. and Edwards, D.H. (1964): "Early diagnosis of herpes simplex encephalitis by brain biopsy", Lancet 2, 332.
11. Meyer, H.M., Jr., Johnson, R.T., Crawford, I.P., Dascomb, H.E. and Rogers, N.G. (1960): "Central nervous system syndromes of 'viral' etiology. A study of 713 cases", Am. J. Med. 29, 334.
12. Miller, J.K., Hesser, F. and Tompkins, V.N. (1966): "Herpes simplex encephalitis. Report of 20 cases", Ann. intern. Med. 64, 92.
13. Pierce, N.F., Portnoy, B., Leeds, N.E., Morrison, R.L. and Wehrle, P.F. (1964): "Encephalitis associated with herpes simplex infection presenting as a temporal lobe mass: Report of 2 cases with survival", Neurology, Minneap. 14, 708.
14. Rawls, W.E., Dyck, P.J., Klass, D.W. Greer, H.D., III and Hermann, E.C., Jr. (1966): "Encephalitis associated with herpes simplex virus", Ann. intern. Med. 64, 104.