JAPANESE ENCEPHALITIS VIRUS IS AN IMPORTANT CAUSE OF ENCEPHALITIS AMONG CHILDREN IN PENANG

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Abstract. This study was carried out to determine if Japanese encephalitis virus is an important causative agent of viral encephalitis among pediatric admissions in Penang, Malaysia. 195 children with CNS symptoms and 482 children with non-specific febrile illness admitted into the Pediatric Ward of Penang Hospital during a 16 month period were entered into the study. The presence in serum of cerebrospinal fluid (csf) of Japanese encephalitis virus (JEV) specific IgM was determined by an IgM capture ELISA and cytomegalovirus (CMV) specific IgM was determined using a commercially available kit (Behringwerke AG).

It was determined that 5 of 13 children with a discharge diagnosis of viral encephalitis had JEV specific IgM in csf, indicating that 38.5% of the viral encephalitis cases was due to JEV. One of the non-JEV cases was found to have mumps virus specific IgM in csf, while no etiology was determined for the other cases. It was also determined that 4 of the 195 (2.1%) cases with CNS symptoms had IgM to CMV, suggesting CMV may be an agent of encephalopathy in children in Penang. Other viruses found to be associated with CNS symptoms in children admitted into our study were measles and herpes simplex virus. A viral etiology was confirmed for 13 of the 195 cases (6.7%).

We also screened 482 non-specific febrile cases for IgM to JEV and to dengue viruses and found that 2 (0.4%) had IgM specific for JEV and 9 (1.9%) had IgM specific for dengue virus.

INTRODUCTION

Japanese encephalitis virus (JEV) is a major causative agent of viral encephalitis in Asia, but because a JEV-specific IgM test is not yet easily available to health care institutions in all affected areas, the actual prevalence of the disease is difficult to determine. The availability of a vaccine against JEV infection, however, must give impetus to a serious study of prevalence as well as morbidity and mortality in order to determine a vaccination policy.

In Malaysia, although JE is reported only occasionally to the Ministry of Health over the years, there have been some recorded outbreaks in several parts of the country (Pulau Langkawi in 1979, Penang in 1988, and Serian, Sarawak in 1992). This has highlighted the need to study more carefully the true incidence of JE in this country in order to determine the public health measures which must be designed and implemented for disease control.

The present study was carried out in response to this need and sets out to determine if JEV is an important causative agent of viral encephalitis in Penang and further, to determine what other viral agents may be important in pediatric cases presenting with CNS symptoms. This information has important implications for disease control as well as for patient management, particularly decisions about the administration of therapeutic drugs such as acyclovir and gangcyclovir.

MATERIAL AND METHODS

Patients: A total of 2,219 patients were admitted to the pediatric ward of the Penang Hospital during the study period from 13 December 1990 to 3 March 1992. Of these, 677 children were enrolled into the study. 195 of these children presented with fever and acute onset of central nervous system derangement (fits, drowsi-ness, delirium, coma) while a further 482 cases were febrile with no CNS signs. All these patients were screened for IgM specific for Japanese encephalitis and dengue viruses to determine the seroprevalence of Japanese encephalitis virus infection in hospitalized children with and without CNS signs.

IgM capture ELISA (MACE): The determination of IgM antibodies to dengue and Japanese encephalitis virus was performed essentially as described previously (Innis *et al*, 1989: Cardosa *et al*, 1992) using cell culture derived viral antigens. All sera collected were tested at 1:100 dilution while all cerebrospinal fluids were tested at 1:5 dilution.

Dot enzyme immunoassay (DEIA): IgG antibodies to dengue and Japanese encephalitis virus were detected by a dot enzyme immunoassay (DEIA) as described previously (Cardosa and Tio, 1991). All sera were tested at 1:200 and 1:1,000 dilution. When paired sera were available, the pair was also tested at 1:500 dilution.

IgM antibodies to measles and mumps virus were determined using a commercially available dot enzyme immunoassay kit (Serion, from Imunodiagnostica, Wurzburg) according to the manufacturer's instructions. Sera were tested at 1:100 dilution while csf were tested at 1:5 dilution.

IgM antibodies to cytomegalovirus were determined using a commercially available kit (Behringwerke AG, Marburg, Germany) following the manufacturer's instructions. Sera were tested at a final dilution of 1:42 while csf were tested at a dilution of 1:5.

IgG antibodies to herpes simplex virus (types I and II) were determined by a dot enzyme immunoassay as described previously (Cardosa *et al*, 1991) with antigens purchased from Behringwerke AG. All sera were tested at 1:100 and if positive, and paired sera were available, the pair was tested at further dilutions of 1:200 and 1:400. Demonstration of a seroconversion constituted presumptive evidence of a recent HSV infection. Using DEIA it was not possible to distinguish between HSV I and II.

Interpretation of serological results was carried out according to convention. The detection of specific IgM to a particular virus in serum suggests a current or very recent infection due to that virus. When the IgM is detected in csf, it indicates an intrathecal production of IgM and suggests a CNS infection due to the virus. Only in the case of HSV was it necessary to use the criteria of IgG seroconversion to determine a current or presumptive recent infection, since an IgM test was not available to us.

Specimen collection and storage for studies of viral etiology at the Arbovirus Laboratory at Universiti Sains Malaysia was organized on a daily basis. All whole blood and csf specimens were collected daily from the ward. The serum was separated from the clot by centrifugation on the day of collection and both serum and csf were stored at -20°C for serological testing.

Routine laboratory investigations were performed by the Pathology Department of the Penang Hospital and included tests for the exclusion of malaria and rickettsia for children with acute encephalopathy. Etiological diagnosis of bacterial meningitis was also routinely performed by the Penang Hospital Pathology Department.

RESULTS AND DISCUSSION

General observations

Table 1 shows the discharge diagnosis made for the 195 children with CNS symptoms admitted to the Penang Hospital during the study period. Most of these children had simple febrile fits (105 cases; 53.8%). In the sample of 195 children, 13 (6.7%) children were discharged with a clinical diagnosis of viral encephalitis and 21 (10.8%) children with bacterial meningitis. It is evident although bacteria are more important causal agents of CNS infection of children in Penang, viral encephalitis is not trivial.

Viral etiology

Table 2 shows the age, sex and ethnic group of each of the 13 cases with a discharge diagnosis of viral encephalitis and the confirmed viral etiology, if any, is indicated. Five of the thirteen (38.5%) were confirmed to have Japanese encephalitis virus infection by demonstration of the presence of JEV specific IgM. This is consistent with the figures cited for southern Thailand where 40% of encephalitis cases are said to be confirmed JEV infections (Igarashi, 1992). None of the children with simple or complex febrile fits were shown to have JEV specific IgM.

The gender ratio of the JEV and non-JEV cases in this study was 4 males to one female in the confirmed JEVgroup, but the ratio of males to females in the non-JEV group was 1:1. The numbers were too small to make any firm conclusions about whether there are important gender differences in the incidence of JEV, but the possibility that JEV infection occurs more in children likely to spend more time outdoors

Table 1

Discharge diagnosis of patients with CNS symptoms (Dec 1990 - March 1992).

	Discharge diagnosis	No.
I	Simple febrile fits	105
II	Complex febrile fits	10
III	Epilepsy with intercurrent infection and prolonged fits	25
IV	Bacterial meningitis	21
V	Viral encephalitis	13
VI	Encephalopathies of unknown cause	7
VII	Encephalopathies associated with known conditions:	
	a. Electrolyte imbalance	3
	b. Congenital hydrocephalus with blocked V-P shunt	2
	c. Non-accidental injury J/C hemorrhage	2
	d. Septicemia with toxic encephalopathy	2
	e. Acute nephritis with hypertensive encephalopathy	1
	f. SLE with fits	1
	g. Shigellosis	1
	h. Schilder's disease	1
	i. Hypoglycemia following chickenpox	1
	Total	195

Table 2

Cases with a discharge diagnosis of viral encephalitis.

Case	Age	Sex	Ethnic group	Date admitted	Etiology
1	7	M	Chinese	17/12/90	none
2	3/12	M	Malay	17/12/90	none
8	9	M	Malay	16/2/91	JEV
17	2	F	Malay	15/3/91	none
21	7	F	Indian	18/3/91	Mumps
23	3	F	Indian	19/3/91	none
56	10	M	Malay	15/5/91	JEV
94	5	F	Indian	22/7/91	JEV
123	11	M	Malay	24/9/91	none
146	1	M	Malay	10/10/91	none
165	2	M	Malay	4/11/91	JEV
193	1	M	Chinese	10/12/91	JEV
226	10/12	F	Malay	3/3/92	none

must be considered.

A summary of the serological results (for virus infection) obtained for all patients entered into the study is shown in Table 3. This shows that 6.7% of the cases with CNS symptoms had a confirmed viral etiology, with 2.6% being due to JEV and 2.1% being due to CMV. Of the non-specific febrile cases studied, 0.4% were due to JEV and 2.5% due to dengue virus infection.

Table 3

Summary of virus etiology of all cases studied.

	With CNS symptoms	Non specific febrile cases
No. screened:	195	482
Virology:		
JEV	5 (2.6%)	2 (0.4%)
CMV	4 (2.1%)	0
HSV*	2	1
Mumps	1	0
Measles	1	0
Dengue	0	9 (1.9%)
Total virology positive:	13 (6.7%)	12 (2.5%)

* since only an IgG test was available, this was performed only when paired specimens were available.

A viral etiology was confirmed for 13 of 195 or 6.7% cases presenting with CNS signs. In the group of non-specific febrile admissions with no CNS signs, we determined that 2 of 482 or 0.4% could be attributed to JEV infection while 9 (1.9%) had dengue infection. This observation is important because it supports the idea that JEV infection does not necessarily cause CNS signs and symptoms and that estimation of prevalence of JEV infection may be a difficult task.

In conclusion, it is clear that JEV infection is a major cause of viral encephalitis in children in Penang, while cytomegalovirus is an important agent causing encephalopathy in children in Penang.

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