Neurology Asia 2009; 14 : 53 – 58

Ten year clinical and serological outcomes of Nipah virus infection

SR Siva, HT Chong, CT Tan

Division of Neurology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Background and Objective: Nipah virus is an emerging zoonotic virus which caused fatal outbreak among Malaysian pig-farmers in 1998-1999. The Nipah virus outbreak represented one of the bat-derived paramyxoviruses that have emerged during the last decade to cause severe human and animal disease. Long-term neurological assessments and serological pattern descriptions are limited. We assessed persistent symptoms, neurological and functional outcome of 36 Nipah virus infection survivors after 10 years of the outbreak in Malaysia. Their serological pattern of Nipah virus for both IgM and IgG were studied. *Methods:* During September 2008 and March 2009, we administered a questionnaire on persistent symptoms and functional disability for all the Nipah virus infection survivors and Nipah infection contacts. Blood were collected for serological test for Nipah virus IgM and IgG. *Results:* A total of 70 subjects were included in the study, 39 of whom had virus Nipah infection in the past. Among the Nipah virus infection survivors, 31 (79%) were male; mean age was 46 $\pm$ 1.8 years. Sixteen Nipah infection survivors (41%) were asymptomatic. The most common persistent clinical features were fatigue (12, 31%), daytime somnolence (10, 26%) and focal neurological deficits (8, 21%). Five out of 13 (38%) Nipah encephalitis survivors had significant disability on the modified Rankin scale. Serologically, all subjects were tested negative on the Nipah IgM serology test. IgG were positive for 39 subjects in which 3 had asymptomatic infection during the outbreak.
Conclusion: Persistent fatigue and daytime somnolence were common disabling symptoms after 10 years of Nipah virus infection, seen in those with previous encephalitis as well as non-encephalitic infection. Serologically all patients had negative Nipah IgM but positive IgG after 10 years of illness.

INTRODUCTION

Between 1998 and 1999, an outbreak of viral encephalitis involving more than 260 reported cases with over 100 fatalities occurred in West Malaysia especially among pig farm workers in the states of Perak and Negeri Sembilan.[1-5] Although Japanese encephalitis virus was initially implicated as the main causative agent for this outbreak, epidemiological and laboratory investigations subsequently discovered Nipah virus as the aetiologic pathogen.[5] Named after the village of Kampung Baru Sungai Nipah in Negri Sembilan where it was first isolated, the Nipah virus is a member of the *Paramyxoiridae* family, related to but distinct from Hendra virus which is also associated with a zoonotic virus.[6]

The main presenting features were fever, headache, dizziness, and vomiting. In the initial outbreak, 55% - 61% had a reduced level of consciousness and prominent brain-stem dysfunction. Distinctive clinical signs included segmental myoclonus, areflexia and hypotonia, hypertension, and tachycardia and thus suggested the involvement of the brain stem and the upper cervical spinal cord among other areas of central nervous system.[2, 7]

Serology plays an important role in the diagnosis of Nipah virus infection. In one Malaysian study at least 76% of Nipah patients have positive serology for Nipah virus during the acute illness.[2, 8] However, the long term clinical and serological outcome of Nipah infection is poorly studied.[9] This study aims to look at persistence of serology and to analyze the Nipah survivors in terms of their persistent clinical symptoms, clinical state and neurological outcome 10 years after the initial outbreak.

METHODS

Serologically confirmed survivors of the Nipah virus outbreak from 1998 to 1999, as defined by the detection of serum IgG or IgM anti-Nipah virus had been previously identified by the Divisions of Neurology, Department of Medicine and the

Address correspondence to: Dr HT Chong, c/o Neurology Laboratory, University Malaya Medical Centre 59100 Kuala Lumpur, Malaysia. E-mail: chongvictor@gmail.com

Department of Social and Prevention Medicine, University of Malaya, Kuala Lumpur. These survivors had been periodically contacted over the ensuing years by a team from the Division of Neurology and Department of Social and Prevention Medicine, University of Malaya. These patients and their family members who were available and willing to participate in this study were recruited. All subjects with positive serology were defined as having Nipah virus infection. Those with evidence of cerebral involvement were classified as Nipah encephalitis. Those with febrile illness without cerebral involvement were classified as non-encephalitic Nipah virus infection. Those with seroconversion without symptom were classified as asymptomatic Nipah infection. The diagnosis of encephalitis was based on clinical, cerebrospinal fluid findings or characteristic findings in magnetic resonance imaging (MRI). Those with negative serology served as controls in the analysis of persistence of symptoms.

The previous Nipah survivors were scattered among four villages namely; Bukit Pelanduk, Kampung Sungai Chua, Kampung India and Tanah Merah Site C, all located in the district of Port Dickson, some 90 km south of Kuala Lumpur. Between November 2008 and March 2009 the survivors were invited to participate in the study through home visits with the assistance of the local government clinic's paramedic staff. As for normal subjects, the household members of Nipah survivors were invited to participate in the study. The household members consisted mainly of the spouses and children of the Nipah survivor's patients. The control subjects were asymptomatic during the 1998-1999 outbreaks and were serologically negative for IgM or IgG antibody.

Informed consents were obtained. A questionnaire was developed based on the previous records of Nipah encephalitis patients regarding persistent or new symptoms and their clinical state. The survivors were interviewed and examined. They were interviewed on their persistence symptoms and a detailed neurological examination done by the investigator. The patient's current clinical state was established according to Modified Rankin Scale (see appendix). Blood of patients were taken into an EDTA bottle for serological test of Nipah IgM and IgG and their random glucose were done with a glucometer (One touch Horizon brand). The EDTA bottles were stored in a polyfoam box with ice packs to keep the temperature at –4^{O}C. The blood was then transported within 24 hours to the Medical Microbiology laboratory in University of Malaya for spinning and for storage under -80^{O}C. The specimens were later sent to Institute Medical Research (IMR) for further analysis of Nipah antibodies. The test also repeated at Medical Microbiology laboratory of University of Malaya. Data were entered into Microsoft Excel. Descriptive and comparative statistical data were analysed with a standard statistical software package (Stata version 10.1). Means, standard deviations, medians and ranges were presented for continuous variables. Nominal non-parametric variables were analyzed with Chi square test or Fisher exact test (if n<5). Ordinal variables were analysed using Kendall correlation. P value < 0.05 is considered as statistically significant.

RESULTS

Demographic characteristics

A total of 70 patients and their family members consented and participated in the study; among them 39 (56%) were found to have positive serology. Seven patients were not previously admitted to the centres which studied the outbreak (University Malaya Medical Centre or General Hospital of Seremban). Their previous admission records were thus unavailable for comparison. Among those with positive serology, 31 (79%) were male, the mean age was 46 $\pm$ 1.8 years, and there were 20 (51%) Indians, 18 (46%) Chinese and 1 (2.6%) Malay. Among the 31 subjects with negative serology, 25 (81%) were female, their mean age was 49 $\pm$ 2.1 years, and there were 16 (51%) Chinese, 14 (45%) Indians and 1 (3.2%) Malay.

Clinical symptoms

All 70 subjects completed the questionnaire, and the 39 Nipah virus infection patients underwent detailed follow-up neurological examinations. Table 1 lists the main persistent symptoms among 39 Nipah virus infection survivors who had Nipah encephalitis, non-encephalitis Nipah infection, or asymptomatic Nipah infection compared to 34 seronegative control subjects. Sixteen (41%) Nipah infection patients were asymptomatic. The commonest and significant persistent symptoms and signs were fatigue or lethargy, daytime somnolence and the presence of focal neurological deficits. Other symptoms included headache, giddiness, seizure and memory impairment (Table 1). Persistent neurological

Table 1: Clinical features of Nipah virus infection survivors

Clinical Features	Nipah infection		Control N=31 (%)
	Encephalitis N=12 (%)	Non-encephalitis N=27 (%)	
Fatigue / lethargy	2 (17)	10 (37)*	3 (10)
Daytime somnolence	3 (25)	7 (26)*	1 (3)
Focal neurological deficits	5 (42)**	3 (11)	0 (0)
Headache	1 (8)	5 (19)	2 (6)
Dizziness / giddiness	0 (0)	3 (11)	1 (3)
Epileptic seizures	2 (17)	1 (4)	0 (0)
Memory Impairment	1 (8)	1 (4)	0 (0)
Irritability / emotional outburst	1 (8)	1 (4)	0 (0)
Speech difficulty	1 (8)	0 (0)	0 (0)
Dry Cough	0 (0)	1 (4)	0 (0)
Weight gain	0 (0)	1 (4)	0 (0)
Numbness	1 (8)	0 (0)	0 (0)
Abnormal movement	1 (8)	0 (0)	0 (0)
Reduced consciousness	1 (8)	0 (0)	0 (0)

NB. * $P < 0.05$ when compared to control, ** $P < 0.05$ when compared to either control or non-encephalitis group.

symptoms were not uncommon and predominantly seen in Nipah encephalitis survivors only. All asymptomatic Nipah infection survivors had no neurological deficit. Significant neurological sequelae were only found in survivors of Nipah encephalitic. One of the Nipah encephalitis survivors were in vegetative state and 3 patients had remote symptomatic epilepsy, 2 of whom were poorly adherent to treatment because of logistic difficulties. A complete description of all the 8 Nipah encephalitis survivors was described in Table 2.

Functional index

All of the non-encephalitic Nipah infection survivors (n=24) and asymptomatic Nipah infection (n=3) patients, as well as the controls (n=31), had no or minimal functional impairment as measured by the modified Rankin scale. Among the survivors of Nipah encephalitis, 5 (of 13, 38%) had moderate to severe disability, significantly worse than the others (Table 3).

Serology

Among the 39 subjects with positive serology, all were positive for IgG, and none for IgM. The details of the Nipah serology status during the initial outbreak for 26 subjects with Nipah virus infection could be traced. Twelve subjects had positive IgM, while 14 had positive IgG. In the current study, all of the 26 subjects had positive IgG only, and none had positive IgM.

DISCUSSION

Acute Nipah encephalitis is associated with high mortality, 32% to 41% in Malaysia[2,7], and 73% in Bangladesh and India.[10] Of the survivors, persistently neurological disabilities were reported in 15% to 19% of Malaysian patients within 6 months after the acute illness.[2,7]

Medium term neurological and functional outcome in Nipah virus infection in Bangladesh were described by Sejvar *et al.*[11] In their study, about 22 previously identified Nipah virus

Table 2: Persistent neurological deficits among the Nipah encephalitis survivors

Patients	Neurological signs
1	Aged 40, completely bed bound, open eyes spontaneously with no verbal communications, only moving left lower limb with contractures of all other limbs, required long term urinary catheterisation, on PEG feeding and bilateral strabismus.
2	Aged 40, in a vegetative state with tetraparesis, contractures all 4 limbs, no verbal communications, required long term urinary catheterisation and PEG feeding.
3	Aged 54 with right 3rd and 8th nerve palsy with intermittent tinnitus on right ear.
4	Aged 58 with dysmetria and dysdiadochokinesia on right upper limb
5	Aged 47 with right horizontal gaze nystagmus, dysarthria, broad based gait with tendency to fall towards right side
6	Aged 58 with a MMSE score of 18/30 with impairment in registration, attention, calculations and recall.
7	Aged 47 with MMSE score of 12/30 with impairments in terms of orientation, recall and language.
8	Aged 42 with a MMSE score 15/30 did poorly in recall, attention and calculations.

- MMSE – Mini Mental State Examination
- PEG- Percutaneous-entero-gastrotomy

infection survivors were subjected to systematic questioning based on a questionnaire on persistent symptoms and functional difficulties, neurological evaluations and brain magnetic resonance imaging (MRI). The median interval between acute illness and first follow up was 14 months and subsequently reassessment was done after 24 months. Data from the study suggested neurological sequelae, including encephalopathy, cranial nerve palsies, and dystonia were frequent in survivors of acute Nipah virus infection. In their study, patients with encephalitis were reported to have neurological dysfunction years after acute infection and new neurological deficits may develop months or years after acute Nipah virus illness, a finding shown earlier among the patients in the Malaysian outbreak.[12,13]

The most significant finding in this long term study was that close to a third (31%) of the patients with encephalitis (17%) and non-encephalitic Nipah infection (37%) had persistent fatigue and lethargy; and daytime somnolence was seen in

Table 3: Modified Rankin Scale Disability of the study subjects

Disability	Nipah encephalitis** (n=13)	Non-encephalitic Nipah virus infection* (n=23)	Controls (n=34)
Asymptomatic	3	13	28
No significant disability	4	14	3
Slight disability	3	0	0
Moderate disability	1	0	0
Moderately severe disability	0	0	0
Severe disability	1	0	0

NB. * P<0.001 when compared to the control, **P< 0.02 when compared to either the control or non-encephalitis group.

about a quarter; 25% of those with encephalitis and 26% in those with non-encephalitis Nipah infection. This finding is consistent with previous report from Singapore, where 8 out of 9 survivors of acute Nipah encephalitis developed major psychiatric features assigned to the encephalitis; 5 had major depression, 2 became depressed about one year after the acute illness; 2 each had chronic fatigue syndrome and personality changes. On the other hand, 5 out of the 9 patients did not have residual neurological deficits.[14] In the medium term outcome study of the 22 Nipah infection patients from Bangladesh, all but one patient had disabling fatigue, with a medium duration of 5 months. Three patients continued to have profound fatigue more than 2 years after the acute illness, which prevent them from resuming daily roles and activities. Behavioral abnormalities were reported by caregivers of over 50% of subjects under 16, with mood problems of >6 months being another common symptom.[11] Previous MRI studies among Singapore Nipah encephalitis patients have shown that the MRI lesion can persists.[9,15] It has also been shown that patients with asymptomatic Nipah virus infection may have abnormal cerebral MRI which may be persistent.[9,16] One of the shortcoming of this study is that the patients who were relatively well could have relocated in search of employment. This could have the effect of overestimating the proportion of patients with residual symptoms and signs.

The seronegativity of IgM implied that there was unlikely any recent relapse among the subjects, which was consistent with the history and clinical features, as previous study showed that IgM was positive in 10 out of 22 relapse/ late onset patients. Nipah virus IgG was tested positive in all 36 patients with previous history of infection. Sustained positive titres indicated a long term serologic memory that resulted from antigen-independent polyclonal activation and differentiation of memory B cells.[17] This findings showed that the persistence of Nipah IgG is a reliable marker of previous exposure or infection, consistent with previous serology study.[18]

ACKNOWLEDGEMENTS

We acknowledge the willing assistance of the study patients and their family members, whose participation made this study possible. We also extend our gratitude to the staff of the Department of Social and Preventive Medicine, University of Malaya, and Bukit Pelanduk Health Centre, in particular, Ms Rajesh, Ms Suguna, Mr Maran, Mr Sundaram, and Ms Atiqa, for all their assistance in the study. We also acknowledge the help of Mr Ravindran Thayan, Research Officer, and other staffs of the Virology Unit, Institute Medical Research, Ministry of Health, Malaysia, who performed the serology tests, and the help of the Microbiology Department, University of Malaya. This study was funded by the Malaysian Ministry of Science, Technology and Innovation IRPA grant, number 06-02-03-0198.

REFERENCES

1. Chua KB, Bellini WJ, Rota PA, *et al*. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 2000; 288(5470):1432-5.
2. Goh KJ, Tan CT, Chew NK, *et al*. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000; 342(17):1229-35.
3. Outbreak of Hendra-like virus--Malaysia and Singapore, 1998-1999. *Can Commun Dis Rep* 1999; 25(12):108-12.
4. Update: outbreak of Nipah virus--Malaysia and Singapore, 1999. *MMWR Morb Mortal Wkly Rep* 1999; 48(16):335-7.
5. Chua KB, Goh KJ, Wong KT, *et al*. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999; 354(9186): 1257-9.
6. Paton NI, Leo YS, Zaki SR, *et al*. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* 1999; 354(9186):1253-6.
7. Chong HT, Kunjapan SR, Thayaparan T, *et al*. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Neurol J Southeast Asia* 2000; 5:61-7.
8. Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. *Microbes Infect* 2001; 3(4):289-95.
9. Lim CC, Lee WL, Leo YS, *et al*. Late clinical and magnetic resonance imaging follow up of Nipah virus infection. *J Neurol Neurosurg Psychiatry* 2003; 74(1):131-3.
10. Chong HT, Jahangir Hossain M, Tan CT. Differences in epidemiologic and clinical features of Nipah virus encephalitis between the Malaysian and Bangladesh outbreaks. *Neurol Asia* 2008; 13:23-6.
11. Sejvar JJ, Hossain J, Saha SK, *et al*. Long-term neurological and functional outcome in Nipah virus infection. *Ann Neurol* 2007; 62(3):235-42.
12. Tan CT, Goh KJ, Wong KT, *et al*. Relapsed and late-onset Nipah encephalitis. *Ann Neurol* 2002; 51(6):703-8.
13. Chong HT, Tan CT. Relapsed and late-onset Nipah encephalitis, a report of three cases. *Neurol J Southeast Asia* 2003; 8:109-12.
14. Ng BY, Lim CC, Yeoh A, Lee WL. Neuropsychiatric sequelae of Nipah virus encephalitis. *J Neuropsychiatry Clin Neurosci* 2004; 16(4):500-4.
15. Lim TCC, Lee KE, Lee WL, *et al*. Nipah virus encpehalitis: Serial MR studies of an emerging disease. *Neuroradiology* 2002; 222:219-26.
16. Tan KS, Ahmad Sarji S, Tan CT, *et al*. Patients with asymptomatic Nipah virus infection may have

abnormal cerebral MR imaging. *Neurol J Southeast Asia* 2000; 5:69-73.
17. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 2002; 298(5601):2199-202.
18. Ramasundrum V, Tan CT, Chua KB, *et al*. Kinetics of IgG and IgG seroconversion in Nipah virus infection. *Neurol J Southeast Asia* 2000; 5:23-8.