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Case report

Clinico-genetic characterisation of an encephalitic Dengue virus 4 associated with multi-organ involvement

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ABSTRACT

Neurological manifestations due to Dengue virus (DENV) infection are atypical and uncommon. Genomic information of clinically characterised, neurotrophic DENV in humans is extremely limited albeit their importance in deciphering the pathogenicity is substantial. Here, we report a rare case of fatal DENV-4 infection complicated with encephalitis and multi-organ failure. The clinical presentation was unusual due to its rapid onset of encephalitis despite a very low virus titre. Full genomes of serum and CSF-derived viruses shared 99.99% similarity, indicating the virus dissemination across blood–brain barrier. Even though virus genomes did not reveal any of the neurotrophic substitutions of DENV documented so far, case isolates possessed a combination of 8 novel amino acid alterations, predominantly distributed in non-structural genes of DENV-4.

1. Why this case is important?

Most human Dengue virus (DENV) infections are benign and manifest either sub-clinically or as a self-limiting flu-like illness. However, haemorrhagic manifestations and systemic shock can occasionally lead to fatal complications. Besides, DENV infections seldom present with multi-organ involvement that predominantly affects nervous, cardiac, gastro-intestinal, renal and respiratory systems.[1] While all four serotypes of DENV are known to be neurotrophic,[2–5] DENV-2 and DENV-3 have primarily been associated with central nervous system (CNS) involvement.[2,3] The neuro-pathogenicity of DENV is still controversial and poorly understood, partially because the isolation and genetic characterisation of implicating viruses are grossly deficient. In the present report, we describe a relatively rare case of fatal DENV-4 infection that presented with an unusually early onset of encephalitis and subsequent multi-organ failure. Full-genomes of virus strains isolated from serum and cerebro-spinal fluid (CSF) of the patient were explored to determine unique genetic characteristics and phylogenetic relationship of infecting strains. To the best of our knowledge, this is the first case that presents the whole genome of a clinically characterised, encephalitic DENV-4 strain.

2. Case description

A 28 year old female presented to the emergency department with fever, headache, drowsiness, diarrhoea and vomiting of one day duration. She has had two episodes of generalised tonic–clonic seizures, loss of consciousness and apnoea on the day before admission. There was no recent travel (within 2 weeks) or past medical history of note. On admission, the patient was in a decerebrate position. Physical examination revealed a drowsy, febrile (38.3 °C) patient with low blood pressure (82/63 mmHg) and tachycardia (118/min). No purpuric or petechial rash was noted. Her pupils were fixed at 5 mm bilaterally with no motor response. Spontaneous stiffening of upper limbs was noted during resuscitation. Plantars were absent. The rest of the examination was unremarkable.

Blood analyses showed gradual thrombocytopaenia, elevated haematocrit and prolonged prothrombin/activated partial thromboplastin time, which were suggestive of dengue fever (Table 1). High D-dimer level [>32 mg/L (normal 0.19–0.55)] further supported the coagulopathy. Haematocrit fluctuated by more than 20% on 4th day of illness, indicating plasma leakage. Transaminases were persistently elevated. Serum creatinine was elevated with a

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Table 1
Summary of blood analyses during admission.

Day of admission (Day of illness)[a]	1 (2)	2 (3)	3 (4)	4 (5)	6 (7)
WBC ($\times 10^9$/L)	14.5	19.8	18.2	16.1	17.7
Haemoglobin (g/L)	15.2	15	15.4	14.3	12.4
Haematocrit (%)	43.1	45.1	47.1	40.6	37.4
PLT ($\times 10^9$)	198	218	127	55	72
Serum albumin (g/L)	44	30	26	24	NA
Serum total protein (g/L)	75	60	55	49	NA
Serum ALT (U/L)	116	115	2506	2560	NA
Serum AST (U/L)	187	204	3054	3804	NA
Serum bilirubin (μmol/L)	9.9	8.8	6.8	12.5	NA
PT (s)	NA	16	16.2	14.5	NA
PTT (s)	NA	45.1	39.3	44.5	NA
Serum creatinine (μmol/L)	153	124	162	282	NA
Serum urea (mmol/L)	6.6	6.5	7.7	11.5	NA
Serum sodium (mmol/L)	139	147	154	150	NA
Serum potassium (mmol/L)	3.0	4.5	5.3	3.2	NA

WBC, white blood cells; PLT, platelets; ALT, alanine transferase; AST, aspartate aminotransferase; PT, prothrombin time; PTT, partial thromboplastin time. NA, not available.
[a] Data on sixth illness day is not available.

low potassium level. Serum urea and sodium were within normal limits. Bacterial blood cultures were negative.

Cerebrospinal fluid (CSF) analysis revealed an elevated opening pressure (28 cmH_2O) with a zero white cell count, 1380/mm^3 of red blood corpuscles, elevated glucose (5.7 mol/L) and low protein (9.27 mg/dL) concentrations. No organisms, acid fast bacilli and encapsulated blastoconidia were seen. CSF antigen (cryptococcal), antibody (Herpes Simplex (HSV), Measles and Mumps viruses) and polymerase chain reaction (PCR) (cytomegalovirus, HSV, Varicella zoster, Japanese encephalitis viruses and *Toxoplasma gondii*) assays excluded respective infections. CSF aerobic and anaerobic cultures gave no growth.

Serum indirect and capture IgG ELISAs for DENV (Pan Bio®, Alere™, Australia) were positive on the 2nd and 6th day of illness respectively while capture IgM ELISA (Pan Bio®, Alere™, Australia) on both serum and CSF remained negative (Table 2). Positive PCR and dengue NS1 ELISA (Biorad Laboratories, France) on 2nd (serum) and 3rd (serum and CSF) day of illness confirmed DENV infection. DENV was isolated from serum and CSF through inoculation of vero cells (ATCC: CCL-81). Serotyping of virus isolates revealed DENV-4.[6] Full genome sequencing of serum and CSF-derived viruses was performed using an in-house protocol (Supplementary Tables 1 and 2).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2012.12.021.

Lung and brain imaging excluded active focal lung lesions, pleural effusion, acute intracranial haemorrhages, territorial infarcts, hydrocephalus and shift of the midline structures. MRI brain revealed severe ischaemic-encephalopathy, possible meningeal inflammation and bilateral mastoiditis. Severe global hypokinesia was evident in 2D echocardiography (10% left ventricular ejection fraction, severe ischaemic mitral regurgitation and mild to moderate tricuspid regurgitation).

We concluded the diagnosis as severe dengue with encephalitis, cardiomyopathy, transaminitis and coagulopathy associated with a secondary DENV-4 infection. Despite inotropic support for hypotension, intravenous hydrocortisone for septic shock, a short course of phenytoin for status epilepticus and intravenous acyclovir, ceftriaxone and ampicillin as empiric cover for meningoencephalitis, the patient died on 8th day of illness.

3. Other similar and contrasting cases in literature

Subsequent to the earliest evidence suggestive of CNS manifestations associated with dengue fever in 18th century,[7] a substantial number of dengue-related neurological cases has been described, especially in recent years.[8–30] DENV-4 has also been detected in brain tissues of a fatal dengue haemorrhagic fever case in Mexico.[5] However, a very few such descriptions have reported successful isolation of infecting viruses and their genomes. None has compared complete virus genomes from serum and CSF of the same individual as in the case presented here.

4. Discussion

Severe dengue predominantly present in the late-acute to early-convalescent phase of secondary DENV infections.[31–33] Severe complications are known to be associated with high DENV titres.[31] In this context, the case presented here is remarkable due to early onset of complications amidst a very low serum virus titre (3rd

Table 2
Summary of molecular, antigen-based and serological diagnostic assay results.

Test assays	Sample 1 (1st DOA – 2nd day of illness)	Sample 2 (2nd DOA – 3rd day of illness)		Sample 3 (5th DOA – 6th day of illness)
	Serum	Serum	CSF	Serum
DENV Real-time PCR	Positive (Cp: 25.26)	Positive (Cp: 26.10)	Positive (Cp > 35)	Negative
DENV serotyping	DENV-4	DENV-4	DENV-4	Negative
Platelia™ Dengue NS1 AG ELISA (Pos ≥ 1)	Positive (13.9)	Positive (10.1)	Positive (11.1)	Negative (0.10)
Panbio® Dengue IgM Capture ELISA (Pos > 11)	Negative (1.22)	Negative (2.12)	Negative (1.54)	Negative (6.52)
Panbio® Dengue IgG Capture ELISA (Pos > 22)	Negative (6.99)	Negative (8.12)	Negative (5.62)	Positive (69.18)
Panbio® Dengue IgG Indirect ELISA (Pos > 11)	Positive (28.97)	Positive (28.54)	Not performed	Positive (40.18)
DENV Isolation (confirmed by IFA)	Not performed	Positive for DENV-4	Positive for DENV-4	Not performed

Bracketed values in column 1 indicate positive cut-off values recommended by manufacturers of each assay. Test values for each assay on serum and CSF collected on different days of admission have been shown in brackets in respective columns. CSF, cerebro-spinal fluid; Cp, crossing point value; DENV, Dengue virus; DOA, day of admission; IFA, immune-fluorescent assay; PCR, polymerase chain reaction.