



CASE REPORT

An unusual transitory increase of lupus anticoagulant in dengue virus infection complicated with cerebral ischaemia

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KEYWORDS

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Summary Dengue is a common mosquito-transmitted viral disease prevalent at many undeveloped and developing countries. Although, neurological complication and hemostatic disturbance are common in dengue virus infection, cerebral ischaemia is barely mentioned. A 61-year-old woman experienced an acute onset of right hemiparesis in her early course of dengue virus type II infection. Extensive laboratory investigation revealed an increase of lupus anticoagulant activity and a prolonged activated partial thromboplastin time that were normalized in the convalescent stage. Her human leucocyte antigens were A2, A11, B8, Cw7, DR4 and DR9. This patient highlights the co-operation between transient activation of humoral immunity and preferential immunogenetic for coagulopathic thrombosis in specific viral disease.

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Introduction

Dengue is a common mosquito-transmitted viral disease prevalent in Latin American, the Pacific islands and continental Asia. Annually, there are an estimated 50-100 million cases of dengue fever, and 250 000-500 000 cases of dengue haemorrhagic fever throughout the world. Human infection with dengue virus shows different clinical courses, ranging from asymptatology, dengue fever, dengue haemorrhagic fever to dengue shock syndrome. Neurological complications, including

encephalitis, meningitis, encephalopathy, myelitis, polyradiculitis and seizure, are common in advanced stage.¹ Isolated facial palsy or neuropathy may occasionally occur in dengue fever.

Dengue has not been an endemic disease in Taiwan in the past. It was not until after June, 2002 that a serious outbreak of dengue occurred in Taiwan, similar to Latin America in the same year. There were 5388 persons seropositive for dengue fever, and 242 for dengue haemorrhagic fever or shock syndrome. Among this population, 5287 persons with dengue fever and 241 with dengue haemorrhagic fever or shock syndrome resided in Southern Taiwan. A total of 21 persons died. Most of the patients were infected by the dengue II virus,

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while the dengue IV virus was only recovered in a minority of subjects. During that period, a dengue-seropositive woman presented with stroke symptom in her early course of infection. This unusual presentation was associated with a transitory increase of lupus anticoagulant (LAC) activity that has not been described previously.

Case history

A 61-year-old woman caught sorethroat and asthenia when dengue fever was endemic in South Taiwan at 2002. On the following morning, a sudden onset of left side weakness developed while she was watching video at home. She could still elevate her left arm and leg, talk coherently and walk independently except unsteadiness. Chillness, fever and arthralgia developed at noon. She arrived at our emergency service at the evening. She denied having major medical disease, recent craniofacial trauma, surgery, blood transfusion, migraine or illicit drug usage.

On admission, her blood pressure was 136/87 mmHg, pulse rate 78 beats/min, respiratory rate 13 breaths/min and body temperature 37.8 °C. There was no ecchymosis, cutaneous lesion, joint swelling, oral ulcer or lymphadenopathy. Neurological examination revealed mild central facial weakness, hemiparesis and dorsiflexed plantar reflex at left side. Cerebral ischaemia at the right hemisphere was considered. Hematology, biochemistry, serology, lipids profile, hepatitis B and C markers, and urinalysis were normal, except leucopenia (3500/mm³; normal: 5000-10 000/mm³), thrombocytopenia (70 000/mm³; normal: 150 000-350 000/mm³), and an elevation of aspartate aminotransferase (245 IU/ml; normal: <45 IU/l), glutamine aminotransferase (210 IU/ml; normal: <45 IU/l) and C-reactive protein (6.3 µg/dl; normal: <5 µg/dl). Diffuse-weighted, apparent diffusion coefficient and T2-weighted magnetic resonance imaging showed a high intensity at the right corona radiata, compatible with an acute infarct (Fig. 1). Carotid duplex and transcranial Doppler did not reveal abnormal finding. Liver sonography did not show cirrhosis or active lesion. The stroke risk factor survey²⁻⁴ revealed a prolongation of activated partial thromboplastin time (1.40-fold of control) and mixing test (1.36-fold of control after mixing) (aPTT Stago) and a positive LAC activity (1.5-fold increase; normal <1.2-fold increase) (dilute Russell's Viper Venom test, LA Screen/LA Confirm, Gradipore Ltd, Australia). The anti-cardiolipin IgG-antibody, anti-beta₂-glycoprotein I IgG-antibody (Varelisa test-kit,

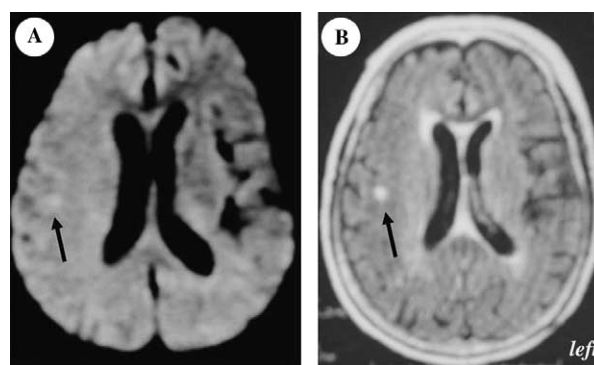


Figure 1 The magnetic resonance imaging showed a diffuse-weighted (A, arrow) and FLAIR-weighted (B, arrow) lesion compatible with an acute infarct at the right corona radiata.

Pharmacia&Upjohn, Germany), thrombin time, factor II activity, protein C, protein S, anti-thrombin III, homocysteine, cryoglobulin and cryofibrinogen were within reference range. The anti-nuclear factor, antibodies to extractable nuclear antigens, ds-DNA, Coombs' test, Venereal Disease Research Laboratory and *Treponema pallidum* haemagglutination test were negative. Human leucocyte antigens were A2, A11, B8, Cw7, DR4 and DR9.

The serum capture IgM/IgG ratio, measured by the modified capture ELISA,⁵ was 5.0 for dengue II virus. The NS1 serology-specific antibody to dengue virus type II was absent.⁶ The one-step SYBR Green I-based RT-PCR assay⁷ confirmed the dengue virus II in this patient. Three weeks later, the serum capture IgM/IgG ratio was turned to 1.7 and NS1 serology-specific antibody to dengue virus type II increased to 1.5. These findings supported a primary dengue virus type II infection basing on (1) the capture IgM/IgG ratio was ≥ 1.2 in acute phase; (2) a negative NS1-specific IgG antibody response in sera during acute phase but a positive serotype specificity in convalescent stage or >9 days after illness onset. In addition, the IgM antibody to Japanese encephalitis virus, adenovirus, enterovirus 70, parvovirus B19, cytomegalovirus, Epstein-Barr virus, varicella zoster virus and herpes simplex virus type I and II, and *Mycoplasma pneumoniae* were absent, and the IgG antibody to these pathogens did not increase in the convalescent stage. The HIV test and hepatitis virology markers were negative. Dengue fever with cerebral ischaemia was established.

During hospitalization, a favorable improvement was ensued. Her activated partial thromboplastin time, mixing test, LAC activity, platelet count, leucocyte count and hepatic enzymes were normal at 1, 3, 6 and 12 months after onset, respectively.

Discussion

Hemostatic disturbance displays an important cause for cerebral ischaemia in infection.⁸ Dengue virus modifies hemostasis in two separate but inter-related ways: A direct action on the cells involved in hemostasis such as the platelets and endothelial cells, and an immunological and inflammatory reaction.^{9,10} These actions are steered by dengue fever itself, or indirectly by cytokines and chemokines released from mononuclear phagocytotic cells activated by the dengue virus. There are two polar results; the first and most common is consumptive coagulopathy, thrombocytopenia, thrombocytic dysfunction, and an inhibition of plasminogen activator inhibitor 1 or an overexpression of tissue plasminogen activator leading to haemorrhage, and the second one is a generation of soluble adhesive molecules, anti-platelet antibodies and anti-endothelial cell antibodies rendering procoagulation. Generally, consumptive coagulopathy and thrombocytopenia¹¹ are predominant in dengue virus infection and, in addition to fibrinolysis,¹² are pronounced in advanced stages of the disease,¹³ and that explains the high prevalence of haemorrhagic diathesis in complicated dengue virus infection.

LAC prolongs the phospholipid-dependent clotting reactions by forming a bivalent antigen-antibody complex with an increased affinity for phospholipids which competes with coagulation factors for the same catalytic surface. A generation of prothrombotic lupus anticoagulant has been reported in association with a few microbials to date that include HIV,^{14,15} hepatitis C virus,^{14,15} hepatitis B virus, Epstein-Barr virus, varicella zoster virus, parvovirus B19,¹⁶ *T. pallidum*, *Mycobacterium leprosy*, *M. pneumoniae*, streptococcal viridans, *Coxiella burnetii* and *Bartonella henselae* (Table 1). An abnormal increase of anti-phospholipid antibodies has been mentioned in flaviviral infection: LAC and anti-cardiolipin antibody in hepatitis C and B virus, and anti-beta₂-glycoprotein I antibody in Japanese encephalitis virus.¹⁷ A concomitant prolongation of aPTT and a diluted Russell's Viper Venom test without a clotting factor

deficiency supported a perturbation of phospholipids/cofactor in this patient.

The modern concept for anti-phospholipid antibody generation is an activation of the humoral reaction by a neoepitopic complex formed by a binding of the domain antigen of cofactor protein with anionic/neutral phospholipids that are translocated from the inner to the outer leaflet during apoptosis. A molecular mimicry promises a microbial peptide sequence to masquerade the domain antigen of cofactor protein for phospholipid binding. For example, in beta₂-glycoprotein I, a molecular mimicry is seen between domain V and the GDKV peptide (G²⁶⁴DKVSFFCKNKEKCC) in cytomegalovirus, and hexapeptide T¹³³LRVYK in domain III and T²⁶⁸⁷LRVLE in the structural protein of Japanese encephalitis virus. Immunization of either GDKV or and T¹³³LRVYK is able to generate anti-beta₂-glycoprotein I antibody. Annexin V, and especially beta₂-glycoprotein I and prothrombin, are cofactor proteins for LAC generation. In previous studies, an administration of TIFI, a 19 amino acid peptide (TIFILFCCSKEKRKKKQAAAT) derived from cytomegalovirus, induces LAC and anti-beta₂-glycoprotein I antibody generation.¹⁸ The Swiss protein database was used to compare the sequence of TIFI, dengue virus, prothrombin and beta₂-glycoprotein I. A high homology is seen among K¹⁰EKRKKK in TIFI and E¹³²KTKK in non-structural protein 2 in dengue virus type II, K⁴⁶⁷LKKPVA, E⁴⁹EVRK and R⁶⁰⁸LKK in prothrombin, and ³⁰¹KNKEKK and ²⁸³QEKFK in beta₂-glycoprotein I (Table 2). This result may support a molecular mimicry between prothrombin or beta₂-glycoprotein I and dengue virus type II for LAC generation.

HLA is important for antigenic presentation in anti-phospholipid antibody generation. The DR53 is the modulator for anti-beta₂-glycoprotein antibody generation in the anti-phospholipid antibody syndrome.¹⁹ Regard to LAC, a relatively high frequency of DR4 or DQ1 is found in the primary anti-phospholipid antibody syndrome and DR7 or Bw44 in the chlorpromazine-related subjects.²⁰ In this patient, DR4 was found. This finding strengthens the predisposing proviso of a specific human

Table 1 Microbials that associate with lupus anticoagulant positivity

Bacterium	Streptococcal viridans, <i>Coxiella burnetii</i>	<i>Bartonella henselae</i>
Virus	HIV, hepatitis B virus, hepatitis C virus, Epstein-Barr virus	Varicella zoster virus, adenovirus, parvovirus B19, cytomegalovirus
Spirochete	<i>Treponema pallidum</i>	
Others	<i>Mycobacterium leprosy</i>	<i>Mycoplasma pneumoniae</i>

Table 2 The homology of sequences among TIFI, dengue virus, prothrombin and beta₂-glycoprotein I

TIFI sequence	T	I	F	I	L	F	C	C	S	K	E	K	R	K	K	K	Q	A	A	T
Dengue virus NS2										132	E	K	T	K	K		121	A	A	T
Prothrombin											467	K	L	K	K	P	V	A		
Beta ₂ -glycoprotein I									49	E	E	V	R	K						
												608	R	L	K	K				
									301	K	N	K	E	K	K					
									283	Q	E	K	F	K						

leucocyte antigen genotype for LAC generation in infection.

A mimetic microbial sequence, and a perfect cooperation among antigen presenting macrophages, specific HLA, autoreactive T and B cells, and downstream messenger cytokines all influence LAC generation. These intricate cascades may explain why a great variation of LAC generation occurs in response to infection and other stimuli. Nevertheless, an abnormally increase of LAC in this patient further strengthens the critical role of anti-phospholipid antibodies in antibody-mediated thrombosis in infection. A transitory increase renders difficulty for confirmative diagnosis. An elucidation of the interaction between the microbial structure and immunobiology may provide valuable pharmacological information and a preventive strategy in dealing with infection-related cerebral ischaemia.

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