

Laboratory confirmation of dengue and chikungunya co-infection

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Introduction

Dengue fever and chikungunya are arboviral diseases transmitted by *Aedes* mosquitoes. Unlike dengue, chikungunya typically consists of a self-limiting and non-fatal acute illness characterised by fever, rash and incapacitating arthralgia [1].

Case report

In November 2006, a 70-year old man presented with a history of high fever, severe headache and arthralgia of knees, and small joints of his hands and feet of 3 days' duration. On examination, the patient had mild swelling of the small joints of hands. There was no skin rash, bleeding into skin or mucosal membranes. His blood analysis showed a leucopenia (WBC 3.04×10^3 per μl) with a relative lymphocytosis (51%). The total platelet count was 115×10^3 per μl . The haemoglobin was 14.3 g/dl with a packed cell volume of 43.8%. The first-hour erythrocyte sedimentation rate, serum alanine transaminase level and urine analysis were normal.

As it was difficult to differentiate between dengue fever and chikungunya, polymerase chain reaction (PCR) was used to confirm the aetiology. Viral RNA was extracted from serum samples using QiAmp Viral RNA Kits (Qiagen Inc., USA). Reverse transcription-PCR (RT-PCR) protocols were used to amplify dengue [2,3] and chikungunya [4] viruses respectively. RT-PCR confirmed the presence of both dengue and chikungunya viruses in the patient's serum (figure 1).

Comment

This is the first case report of chikungunya and dengue co-infection confirmed by molecular assays in Sri Lanka. As the clinical and biochemical manifestations of this case were suggestive of both dengue fever and chikungunya, early aetiological confirmation was important as delayed diagnosis of dengue could result in fatal complications.

Laboratory confirmation of dengue and chikungunya can be made by several methods. One of the widely used

methods to confirm a recent infection is detection of disease specific IgM antibodies. However, this method cannot be used for early diagnosis, as IgM does not appear in the viraemic phase (first 5 days of infection) in both dengue and chikungunya. The options available for early diagnosis of these infections are either virus isolation or RT-PCR. Of these, the more convenient and least time-consuming method is the latter.

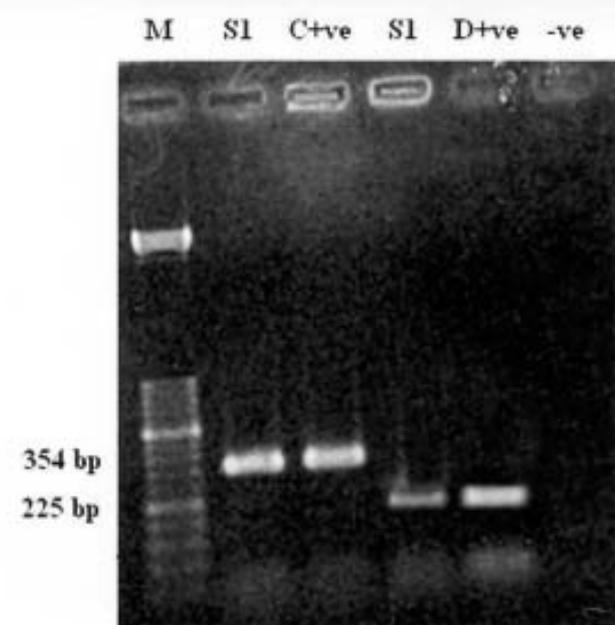


Figure 1. Photograph showing the amplified products in a 2% agarose gel stained with ethidium bromide. Amplified products of the positive controls for dengue (225 bp) and chikungunya (354 bp) were run in parallel with the respective products of the clinical sample. bp = base pairs, M = 50 bp DNA marker, S1 = clinical sample, C+ve = chikungunya positive control, D+ve = dengue positive control and -ve = negative control.

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Correspondence: HACH, e-mail <chandith@lycos.com> Competing interests: none declared. Received 20 December 2007 and revised version accepted 23 May 2008.

References

1. Pialoux G, Alex-Gauzere B, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infectious Diseases* 2007; **7**: 319-27.
 2. Chow VTK, Seah CLK Chan YC. Use of NS3 consensus primers for the polymerase chain reaction amplification and sequencing of dengue viruses and other flaviviruses. *Archives of Virology* 1993; **133**: 157-70.
 3. Sudiro TM, Ishiko H, Green S, Vaughan DW, Nisalak A, Kaqlayanarooj S, Rothman AL, Raengsakularach B, Janus J, Kurnae I, Ennis FA. Rapid diagnosis of dengue viraemia by Reverse Transcription-polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 1997; **56**: 424-9.
 4. Hesebe F, Parquet MC, Pandey BD, Mathenge EGM, Morita K, Balasubramaniam V, Saat Z, Yusop A, Sinniah M, Natkunam S Igarashi A. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. *Journal of Medical Virology* 2002; **67**: 370-4.
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