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Dengue virus localization in human tissues (case study)

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Abstract

Dengue is an arboviral disease which is present in tropical and subtropical regions of the world. Dengue virus (DENV) can cause benign dengue fever (DF) to the most severe forms, dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). Many studies have been confirmed the dengue viral antigens in various types of naturally infected human tissues but little work has been done and is not certain the localization of viral RNA in human tissues. We studied tissue specimens of two patients who died from clinically diagnosed DHF to confirm serologically or virologically. DENV was detected by dengue specific Reverse transcription polymerase chain reaction (RT-PCR) for the detection of dengue viral RNA. From the RT-PCR, dengue viral RNA was detected in liver, kidney and lungs tissues while the DENV RNA absence in brain, heart, spleen and pancreas tissues. DENV can infect a number of different tissue types.

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Introduction

Dengue fever and dengue haemorrhagic fever are important arboviral disease which present in tropical and subtropical regions of the world. It is a swiftly rising health problem with an estimated 2.5 billion people at risk in more than 100 countries of the world, mainly in countries of South and South-East Asia, Central and South America, the Caribbean and recently in Africa (Guzman *et. al.,* 2010; Shu and Huang 2004, Gubler 1998). Dengue virus (DENV) causes a ranging infections from benign dengue fever (DF), mild flu like undifferentiated fever, to more severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which cause high rate of morbidity and mortality (Gulber 1998, Martina *et. al.*, 2009, Guzman and Kouri 2003, Nimmannitya 1987, World Health Organization 1997). The most important pathophysiological event in DHF/DSS is plasma leakage that causes pleural effusion and ascites which may occasionally leads to death (Martina *et. al.*, 2009). Though the dengue viral antigens have been detected in various human tissues in previous studies, very few published studies that have attempted to detect dengue viral RNA in naturally infected human tissues (Jessie *et. al.*, 2004). There is evidence to suggest that hepatocytes are sites of dengue viral replication, possibly adding to the total viral burden (Smith and Khakpoor, 2009).

As the prevalence of dengue is increasing, in endemic areas it is important to practise early diagnosis methods and efficient patient management. Dengue virus infection has been laboratory diagnosed by detection of specific virus, viral antigen, genomic sequence, and/or antibodies (Gulber 1998, Guzman and Kouri 1996, Guzman and Kouri 2003, World Health Organization 1997).

The rapid detection of the dengue virus genomic sequence by real-time one-step RT-PCR has become a trend. This assay has the advantages of simplicity, rapidity, and a low contamination rate compared to the characteristics of the nested RT-PCR method, which, however, has a sensitivity similar to that of the real-time RT-PCR.

We aimed to investigate the dengue virus localization in human tissues and to detect the dengue NS1 antigen and DENV immunoglobulin M (IgM) and IgG antibodies in the human sera of two patients who died from clinically suspected DHF.

Materials and methods

Blood was obtained by venipuncture (before the death of the patient), from two clinically DHF suspected patients, was centrifuged and serum was separated. Brain, heart, lungs, liver, spleen, pancreas, and kidney tissues were obtained from above two patients who died from clinically suspected DHF. Both serum and tissue samples were stored in -80 ^{o}C freezer until use.

Dengue NS1 antigen and DENV IgM and IgG antibodies detection

Frozen serum was thawed and thoroughly mixed prior to testing. Dengue NS1 antigen strip test (Bio-Rad Laboratories) was done for qualitative detection of Dengue NS1 antigen in human serum according to the manufactures instructions. The qualitative presumptive detection of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies to human serum was detected by using Dengue Duo Cassette (panbio) according to the manufactures instructions.

DENV detection in human tissues

Viral RNA was extracted from Brain, heart, lungs, liver, spleen, pancreas, and kidney tissues and from the serum by using QIAGEN QIAamp viral RNA minikit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The extracted RNA samples were then subjected to a Reverse Transcription Polymerase Chain Reaction (RT-PCR) by using Promega Access RT-PCR System (Promega Corporation, USA). Total volume 45 µL of reaction mixture were added into 0.2 mL thin walled PCR tubes followed by 5 µL of RNA template (10^3–10^6 copies). The reaction mixture was prepared by mixing 1 µM of forward primer and 1 µM of reverse primer with appropriate volume of DEPC-treated distilled water. The tubes were vortexed to dissolve the reaction mixture and followed by a brief Spin in a

centrifuge to bring down the reaction mixture for PCR.

In this assay PCR cycling profile contains of a 45 minutes, 45 °C reverse transcription and a 2 minutes, 94 °C AMV RT inactivation and RNA/cDNA/primer denaturation followed by 40 cycles of PCR at 94°C denaturation for 30 s, 60°C of annealing for 1 min, 68°C extension for 2 min, 1 cycle at 68°C for 7 minutes final extension, and 1 cycle at 4°C soak.

RT-PCR reaction products were then analyzed by gel electrophoresis. The 5 µL of each products were loaded in to 2% (W/V) agarose gel (Cambrex, Rockland, ME, USA) contain 1µl of ethidium bromide in 0.5× Tris-Borate-EDTA buffer with a 100-bp ladder as molecular weight marker. Expected size of the amplicon was 180 bp.

Results and discussion

According to the Dengue NS1 antigen strip test, Dengue NS1 antigen was not detected in both human sera and RT-PCR results also proved that the DENV viral materials are not present in both human sera (Table 01). The NS1 antigen strip test is detectable from day 1 to 9 of fever both in primary and secondary infections (Kulkarni *et. al.*, 2011). Here blood samples were collected after day 10 fever and therefore no DENV antigen and/ or detected in sera. Both serum samples were positive for IgM and IgG antibodies (Table 01) as indicative of secondary infection.

Table 1. Diagnostic tests carried out using serum samples.

Diagnostic test	> 10 day fever			
	NS1 Strip test	IgM	IgG	RT-PCR
Case 01	-	+	+	-
Case 02	-	+	+	-

(+) Positive results for the test, (-) Negative results for the test.

In the both cases, tissues from liver and kidney were positive for DENV RNA material and lung tissues of case 01 was positive for DENV RNA material while case 02 lung tissues negative for DENV RNA material (Table 02). Tissues from brain, heart, spleen and pancreas were negative for DENV RNA material (Table 02). Detection of DENV in suspected fever cases has developed in last few decades. It is not certain whether viral RNA can be localized in human tissues. Although previous studies have been detected dengue viral antigens in several human tissues little work has been done to describe the localization of viral RNA. In this study we detected DENV RNA in lungs, liver and kidney tissues similar to Jessie *et. al.*, (2004) however Jessie *et. al.*, (2004) also detected DENV antigens in spleen, and peripheral blood leukocytes which could not report in this study. The presence of DENV RNA in lungs, liver and kidney tissues suggest that viral replication occur in these tissues.

Table 2. DENV RNA detection by RT-PCR in tissues.

Tissue	Brain	Heart	Lungs	Liver	Spleen	Pancreas	Kidney	Blood (serum)
Case 01	-	-	+	+	-	-	+	-
Case 02	-	-	-	+	-	-	+	-

(+)DENV RNA was detected, (-) DENV RNA was not detected.

The limitation of the present study was that no quantitative detection by real-time RT-PCR and/ or enzyme linked immunosorbent assay (ELISA) was not be used and no sera collection of < 10 day fever. Inclusion of quantitative detection of viral RNA could have provided the opportunity to determine the severity of virus localization in each tissue type. Exclusion of < 10 day fever sera bounded the chance

to comment the longevity of DENV viral material in serum.

By identifying the localization of DENV in human tissues is helpful in patient management in hospitals to practise protective treatments. It would reduce either the severity of the disease or the likelihood of patients developing more severe forms of the disease.

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References

Gubler DJ. 1998. Dengue and dengue hemorrhagic fever. Clinical Microbiology Reviews **11,** 480–496.

Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martínez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S, Peeling RW. 2010. Dengue: a continuing global threat. Nature Reviews Microbiology**,** S7- S16. http://dx.doi.org/10.1038/nrmicro2460

Guzman MG, Kouri G. 2003. Dengue: an update. Lancet Infectious Diseases **2**, 33–42. http://dx.doi.org/10.1016/S1473-3099(01)00171-2

Guzman MG, Kouri G. 1996. Advances in dengue diagnosis. Clinical And Diagnostic Laboratory Immunology **3**, 621–627.

Jessie K, Fong MY, Devi, S, Lam SK, Wong KT. 2004. Localization of Dengue Virus in Naturally Infected Human Tissues, by Immunohistochemistry and In Situ Hybridization. The Journal of Infectious Diseases **189,** 1411–1418. http://dx.doi.org/10.1086/383043

Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM, Shetty PC, Jain PA. 2011. Association of platelet count and serological markers of dengue infection- importance of NS1 antigen. Indian Journal of Medical Microbiology **29(4)**, 359-362. http://dx.doi.org/10.4103/0255-0857.90159

Martina BE, Koraka P, Osterhaus AD. 2009. Dengue virus pathogenesis: an integrated view. Clinical Microbiology Reviews **22**, 564-81.

Nimmannitya S. 1987. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian Journal of Tropical Medicine and Public Health **18,** 392–397.

Shu PY, Huang JH. 2004. Current Advances in Dengue Diagnosis. Clinical and diagnostic laboratory immunology **11(4),** 642–650. http://dx.doi.org/10.1128/CDLI.11.4.642-650.2004

Smith DR, Khakpoor A. 2009. Involvement of the liver in dengue infections. Dengue Bulletin **33,** 75-86.

World Health Organization. 1997. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed. World Health Organization, Geneva, Switzerland.