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Emerging and detection of Dengue viral infection in Sri Lanka

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ABSTRACT

Emerging of dengue infection has increased dramatically in Sri Lanka in recent years. Initial infection with a particular serotype is known as primary infection which is usually asymptomatic or mild disease manifestations. Although cross reactive T cells and cross reactive antibodies have been shown to contribute to disease pathogenesis, these mechanisms alone do not explain the immune - pathological mechanisms leading to severe infection. However, this study has suggested that antigen antibody detection and viral RNA detection in host blood contribute to the occurrence of disease. Therefore, it is important to further investigate the quality of dengue specific immune responses and confirmation of dengue RNA content in patients with acute severe dengue and asymptomatic dengue infection.

***Keywords*:** IgG; RT-PCR; IgM; acute infection; Aedes mosquitos

1. INTRODUCTION

Dengue is an arthropod borne viral infection, which is a major public health problem in tropical and subtropical countries including Sri Lanka (WHO). This classic dengue fever is an acute, infectious, self-limited severe flu-like illness which is transmitted by *Aedes* female mosquitoes (Bhatt *et al*., 2013). In 1962 the first dengue case was reported in Sri Lanka and the first outbreak of dengue occurred in 1965–1966 with few DHF case (Sirisena and

Noordeen, 2014; National Dengue Control Unit, Sri Lanka, 2014). Since then Sri Lanka is facing dengue outbreaks and has acquired hyper-endimicity due to co-circulation of all four serotypes of dengue virus. In the year 2015, 29, 777 cases were reported to the epidemiology unit in the island (Epidemiology unit, Ministry of Health, 2015). There are four serotypes have documented in Sri Lanka and it is circulating for last three decades. Thus, the outbreak of four main serotypes could observe through different periods.DENV1 and DENV 2 strains were isolated in 1965 and 1966. DENV3 strain was isolated in 1980s and 1990s and DENV4 serotype observed in 1978 and 2003 in Sri Lanka.

2. CLINICAL MANIFESTATIONS OF DENGUE

Transmission of dengue virus

The transmission of the dengue virus to humans occurs after the bite of an infected mosquito. The ability of the virus to infect the host depends on the capacity of the virus to bind to internalize into and productively infect target cells. Cell systems and the initiation of dengue virus involve the concentration of dengue virus particle through an initial interaction with a low-affinity binding element before transfer to a second receptor protein able to bind and internalize the virus. This mechanism emerge and bring with them the possibility of new strategies to stop dissemination of the virus to identify molecular markers linked to individuals at high risk of developing the severe forms of the disease in the last few years.

Incubation, Infection and symptoms

The incubation period varies from 3 to 7 days and while some infections remain asymptomatic. The majority of individuals will develop classic dengue fever among them (Gubler,1998). Any DENV serotypes can result in a broad spectrum of disease syndromes ranging from an asymptomatic to mild infection or classical DF to potentially fatal DHF and DSS (Izabela *et al.*,2010). Dengue is a rarely fatal but severe dengue is a potentially fatal complication with symptoms including low temperature, severe abdominal pains, rapid breathing, bleeding gums and blood in vomit (Murray *et al.*, 2013; WHO/Impact of dengue; Bhatt *et al*., 2013). DHF and DSS are also characterised by increased vascular permeability, thrombocytopenia and haemorrhaging with haemoconcentration being a key indicator in differentiating it from DF. The symptoms observed often in children. However, children and young adults remain at increased risk for progression to dengue hemorrhagic fever and dengue shock syndrome, particularly during repeat infection with a new DENV serotype. An individual risk factor since DHF / DSS has been found to be more prevalent in white people than in black people.

There are 54 dengue virus strains have been isolated from patients with DHF/DSS. The specific sequence of DEN-l followed by DEN-2 appeared to be associated with the greatest risk for DSS. Secondary infection with DEN-2 which followed primary infections with DEN-I, DEN-3 or DEN-4 was a risk factor for DSS (Lall *et al* 1996). Pre-existent dengue immunity detected by conventional serological techniques was a significant risk factor for the development of DHF. The risk of DSS is only during the second infection and not in the subsequent ones. It is not known if all DEN-2 types are equally prone to cause DSS if preceded by DEN (Lall *et al* 1996).

Chronic diseases such as bronchial asthma, diabetes mellitus and anaemia are additional risk factors (Lall *et al* 1996). Enhanced DEN-2 activity has been observed in leukocytes from asthmatic patients compared to healthy persons, supporting the fact that bronchial asthma is a risk factor for DHF/DSS as seen on analysis of epidemiological data. A genetic risk for DHF has been suggested which may be an important factor. Maternal dengue antibodies play a dual role by first protecting and later increasing the risk of DHFI/ DSS by DEN-2. In infants who developed DHF/DSS, there was a strong correlation between the mother's DEN-2 neutralizing antibody titres and infant's age at the time of onset of severe illness (Lall *et al* 1996). While access to care, appropriate interventions, host genetic factors and previous exposure to DENV are all known to affect the outcome of the infection. Although DENV-1 and DENV-2 are the main circulating serotypes and all four dengue virus are also detected. Infections with different serotypes may cause nearly identical clinical syndrome, but some differences in clinical manifestations have been reported. It is unclear whether dengue serotypes differ in their propensity to cause severe disease. These four viral types are called as four serotypes because each has different interactions with the antibodies in human blood serum. The four dengue viruses are similar and share approximately 65% of their genomes. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms.

3. MATERIAL AND METHODS

Sample collection

The blood samples (n = 100) collected from DF/DHF suspected patients (based on the clinical assessment by physicians) from the adult wards of the General Hospital, Kandy, Sri Lanka. The below table shows the lab number of the patient, age of the patient, sex, fever, headache, retro orbital pain, myalgiya, arthralgiya, rash, petichiae reaction, ecchymoses or purpura, mucoal bleeding, GIT bleeding, cannula site bleeding, pleural effusion, ascitis, liver enlargement, shock, WBC and PLT. The detection of the virus has been experimented by the IgM, IgG content and RT- PCR confirmation method.

Table 1. Clinical manifestation of individual patient (Lab number shows the patients' details).

Lab number	Fever days	Age	Sex	Fever	H/O fever	Headache	Retro orbital pain	Myelgia	Arthralgia	Rash	Petichiae	Ecchymoses or purpura	Mucosal Bleeding	GIT Bleeding	Cannula site Bleeting	Plueral Effusion	Ascitis	Liver Enlagement	SHOCK	WBC	PLT
KD1	4	22	M	√	X	√	X	√	√	X	X	X	X	√	X	X	X	x	√	2.1	96
KD2	3	31	M	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	3.6	119
KD3	5	50	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	7.3	38
KD4	5	43	M	√	X	√	X	X	√	X	X	X	X	X	X	X	X	X	X	4.6	119

KD5	5	47	F	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	4.0	98
KD6	5	15	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	4.8	200
KD7	4	20	M	√	X	√	√	√	√	X	X	X	X	X	X	X	X	√	X	2.6	106
KD8	5	34	M	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	4.3	180
KD9	4	24	M	√	X	√	√	√	X	√	√	X	X	X	X	√	X	√	X	4.8	160
KD10	4	30	F	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	5.1	120
KD11	5	24	F	√	X	X	X	X	X	X	√	X	X	X	X	X	X	X	X	4.1	260
KD12	5	27	M	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	3.5	96
KD13	5	23	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	1.9	71
KD14	3	20	M	√	X	√	√	√	X	X	X	X	X	X	X	X	X	√	X	2.2	48
KD15	5	33	M	√	X	X	X	√	X	X	X	X	√	√	X	X	X	X	X	2.8	23
KD16	4	29	F	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	6.9	184
KD17	5	37	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	1.9	69
KD18	5	48	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	4.0	210
KD19	5	24	F	√	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	3.2	102
KD20	5	32	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	8.8	348
KD21	5	23	M	√	X	√	√	√	X	X	X	X	X	X	X	X	X	√	X	4.2	71
KD22	5	24	M	√	X	√	√	√	X	X	X	X	X	X	X	X	X	√	X	3.3	143
KD23	3	17	M	√	X	√	√	√	X	X	X	X	X	X	X	X	X	√	√	6.2	158
KD24	5	53	F	√	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	2.1	101
KD25	4	17	M	√	X	√	√	√	X	X	√	X	X	X	X	X	X	X	X	3.6	110
KD26	3	30	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	2.3	142
KD27	5	55	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	√	X	5.8	32
KD28	5	17	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	5.6	157
KD29	3	47	M	√	x	√	X	√	X	X	X	X	X	X	X	X	X	X	X	3.2	138
KD30	4	12	M	v	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	3.8	59
KD31	4	56	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	3.6	110
KD32	4	31	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	5.0	152
KD33	4	37	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	3.0	168
KD34	3	28	M	√	X	√	X	√	X	√	X	X	X	X	X	X	X	X	X	4.9	170
KD35	2	32	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	5.8	186

KD36	5	37	F	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	3.5	113
KD37	5	23	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	4.2	111
KD38	5	20	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	2.6	140.0
KD39	5	19	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	2.5	78.0
KD40	5	33	M	√	X	√	X	X	X	X	X	X	√	√	X	X	X	√	X	3.3	54
KD41	5	34	F	√	X	√	X	X	√	X	X	X	X	X	X	X	X	X	X	2.5	122
KD42	3	24	M	√	X	√	√	X	X	X	X	X	X	X	X	X	X	X	X	9.6	216
KD43	3	24	M	√	X	x	X	√	√	X	X	X	X	X	X	X	X	X	X	2.2	114
KD44	5	19	M	√	X	√	√	√	X	X	√	X	√	√	X	X	X	√	X	2.9	160
KD45	5	24	M	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	3.7	120
KD46	5	29	F	√	X	√	√	√	√	X	X	X	X	X	X	X	X	X	X	4.8	78
KD47	5	15	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	2.5	148
KD48	5	40	F	√	X	X	X	X	√	X	X	X	X	X	X	X	X	X	X	2.8	58
KD49	5	36	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	2.6	125
KD50	4	18	F	√	X	X	X	X	X	X	√	X	X	X	X	X	X	X	X	3.3	217
KD51	5	28	F	√	X	X	X	X	√	X	X	X	X	X	X	X	X	X	X	2.8	160
KD52	5	35	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	√	X	3.5	173
KD53	5	23	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	3.2	169
KD54	5	19	F	√	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	3.4	78
KD55	5	26	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	3.1	86
KD56	4	22	F	√	X	X	X	X	√	X	X	X	X	X	X	X	X	X	X	5.0	200
KD57	5	19	M	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	6.6	206
KD58	5	25	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	2.8	103
KD59	4	21	M	√	X	X	X	√	√	X	X	X	X	√	X	X	X	X	X	5.9	196
KD60	5	58	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	3.0	78
KD61	5	15	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	√	X	5.4	53
KD62	5	17	M	√	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	1.9	135
KD63	5	56	M	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	3.5	81
KD64	5	19	F	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	4.1	201
KD65	5	49	F	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	4.9	87
KD66	5	39	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	5.6	45

KD67	5	33	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	132
KD68	5	16	F	√	X	√	X	√	X	X	X	X	X	X	X	X	X	√	X	93
KD69	5	42	M	√	X	X	X	√	√	X	√	X	X	X	X	X	X	√	√	33
KD70	4	22	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	125
KD71	3	48	F	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	158
KD72	5	19	F	√	X	√	X	√	X	√	X	X	X	X	X	X	X	√	X	173
KD73	5	55	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	32
KD74	5	23	M	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	48
KD75	5	21	M	√	X	√	X	√	√	X	X	X	X	X	X	X	X	√	X	93
KD76	5	45	M	√	X	√	X	√	√	X	X	X	X	X	X	X	X	X	X	148
KD77	4	29	M	√	X	√	√	√	√	X	X	X	X	X	X	X	X	X	X	174
KD78	4	18	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	162
KD79	5	62	M	√	X	X	√	X	X	X	X	X	X	X	X	X	X	√	√	52
KD80	4	24	F	√	X	√	X	X	X	X	X	X	√	X	X	X	X	X	X	40
KD81	5	19	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	118
KD82	5	17	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	45
KD83	5	28	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	50
KD84	5	19	F	√	X	√	X	X	X	X	X	X	√	X	X	X	X	X	X	80
KD85	5	22	M	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	106
KD86	4	13	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	48
KD87	5	14	M	√	X	√	X	√	X	X	X	X	X	X	X	X	X	X	X	97
KD88	5	12	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	√	X	101
KD89	5	25	F	√	X	√	√	X	X	X	X	X	X	X	X	X	X	X	X	179
KD89	5	40	F	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	61
KD90	5	17	F	√	X	√	X	√	√	X	X	X	X	X	X	X	X	√	X	119
KD91	5	13	F	√	X	√	X	X	X	X	X	X	X	X	X	X	X	X	X	136
KD92	4	50	M	√	X	√	√	√	√	X	X	X	X	X	X	X	X	√	X	133
KD93	3	34	F	√	X	X	X	√	√	X	X	X	X	X	X	X	X	X	X	111
KD94	5	43	F	√	X	√	X	X	X	√	X	X	X	X	X	X	X	X	X	70
KD95	5	20	M	√	X	√	X	X	X	X	X	X	X	√	X	X	X	√	X	98
KD96	3	25	M	√	X	X	X	√	X	X	X	X	X	X	X	X	X	X	X	132

KD97	5	23	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	√	X	2.2	50
KD98	3	30	M	√	X	X	X	X	X	X	X	X	X	X	X	√	X	X	X	5.5	93
KD99	5	23	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	√	X	2.2	50
KD100	3	30	M	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	5.5	93

4. RESULTS AND CONCLUSIONS

IgM and IgG detection methods have been used to detect the antibody level and the PCR detection method used as the confirmation of viral cDNA content.

Table 2. Clinical diagnostic methods (Lab number shows the patients' details).

Lab number	IgM ELIZA	IgG ELIZA	PCR
KD1	1	1	
KD2	0	0	1
KD3	1	1	
KD4	1	1	
KD5	1	1	
KD6	1	1	
KD7	1	1	1
KD8	1	1	
KD9	1	1	
KD10	1	1	
KD11	1	1	
KD12	1	1	
KD13	1	0	
KD14	1	1	1
KD15	1	1	
KD16	1	1	
KD17	1	1	
KD18	1	1	
KD19	1	1	
KD20	1	1	

KD21	1	1	
KD22	1	1	
KD23	1	1	
KD24	1	1	
KD25	1	1	
KD26	0	1	1
KD27	1	1	
KD28	1	1	
KD29	0	0	1
KD30	1	1	
KD31	1	1	
KD32	0	0	1
KD33	1	1	
KD34	1	1	
KD35	0	1	1
KD36	1	1	1
KD37	1	1	
KD38	1	1	
KD39	0	0	1
KD40	1	1	
KD41	1	1	1
KD42	0	1	1
KD43	0	1	1
KD44	1	1	
KD45	1	1	
KD46	1	1	
KD47	0	1	1
KD48	1	1	
KD49	1	1	
KD50	0	0	1
KD51	1	1	
KD52	1	1	
KD53	1	1	

KD54	1	1	
KD55	1	1	
KD56	1	1	
KD57	1	1	
KD58	1	1	
KD59	0	1	1
KD60	1	1	
KD61	1	1	
KD62	1	1	
KD63	1	1	
KD64	1	1	
KD65	1	1	
KD66	1	1	
KD67	1	1	
KD68	1	1	
KD69	0	1	1
KD70	0	1	1
KD71	1	1	1
KD72	1	1	
KD73	1	1	
KD74	0	0	1
KD75	1	1	
KD76	1	1	
KD77	1	1	
KD78	1	1	1
KD79	1	1	
KD80	1	1	
KD81	1	1	
KD82	1	1	
KD83	1	1	
KD84	1	1	
KD85	1	1	
KD86	1	1	

KD87	1	1	
KD88	1	1	
KD89	0	1	1
KD89	1	1	
KD90	1	1	
KD91	1	1	
KD92	1	1	
KD93	0	1	1
KD94	1	1	
KD95	1	1	
KD96	1	1	
KD97	1	1	
KD98	1	1	
KD99	1	1	1
KD100	0	1	1

5. DISCUSSION

Dengue is currently the most important vector borne viral pathogen affecting humans and is emerging as a major threat in Sri Lanka. Dengue in Sri Lanka can be observed in early 1960s with its high morbidity and mortality and it has been confirmed in 1962 according to scientific confirmations. There are 48 *Aedes* species belonging to 11 subgenera have been reported till today. Exploring the relationships between virological features of clinically apparent dengue virus (DENV) infection with patient immune status would help expand our understanding in choosing the correct diagnostic marker for the infection in relation to the time of presentation in the hospitalised patients. As well, neither vaccines nor specific therapies are available although both areas are currently the focus of intense research efforts Data from this particular cohort of patients (General Hospital, Kandy), enrolled early with undifferentiated fever (based on the clinical assessment by physicians) will be used to develop a practical diagnostic algorithm.

Anti-DENV IgM/ IgG tests and DENV RT-PCR tests are able to detect clinically apparent DENV infection from days 1 to 8. Acute sera were shown to be positive for DENV infection based on anti-DENV IgM / IgG and RT-PCR tests based on the stage the patient is in the evolving DENV infection. The detection of DENV infection in the laboratory is carried out using the rapid ICT assays in many dengue endemic developing countries. The rapid dengue assay is designed to detect both IgM and IgG simultaneously. In addition, RT-PCR detection method should be regulated nationally to prevent the introduction of unregulated assays to the market. Validating widely used rapid assays for anti-dengue virus IgM detection would increase the reliability of the results and help support the clinical diagnosis. Validating

of RT- PCR method and designing new primers are usually done by comparing the accuracy indices such as sensitivity and specificity with a known reference standard or a gold standard method. Demographic and societal changes, decreasing resources for vector-borne infectious disease prevention and control and changes in public health policy have all contributed to increased epidemic dengue activity. Coincident with this has been a change in public health policy that placed emphasis on emergency response to epidemics by using high-technology mosquito control methods rather than on preventing those epidemics by using larval source reduction through environmental hygiene the only method that has been shown to be effective. Currently DF and DHF cases are reported from every parts of Sri Lanka. The peak point of dengue virus spread in is at the monsoon period. *A. albopictus* is most efficient vector to spread DENV in Sri Lanka. The risk is strongly distributed by rainfall, temperature, a high household density, deficiencies in the water supply, garbage and the urbanization (lower income residents) throughout the world. Although most dengue infections are asymptomatic, patients can present with a wide spectrum of clinical symptoms ranging from mild febrile illness through to severe manifestations of bleeding and organ impairment due to a systemic vascular leak syndrome. Clinical diagnosis of dengue and identification of which patients are likely to develop severe disease remain challenging.

6. CONCLUSION

According to KD 39 and KD 74 both IgM and IgG tests were negative but PCR test results were positive. Apparently IgM test was negative and IgG test was positive and PCR detection was positive. It might be due to the initial infection of the virus and immune cells have secreted antibody. Therefore RT-PCR can be considered as the confirmationary detection method comparing with IgM test and IgG test. Simultaneously RT-PCR detection method helps to distinguish the viral cDNA content in the patients' blood samples. The prevalence of the dengue infection is 24% among the collected 100 samples.

References

[1] Sirisena, PDNN, Noordeen F. Evolution of dengue in Sri Lanka – changes in the vector, virus and climate. *International Journal of Infectious Diseases.* 2014, 6-12.

[2] Thamarasi Senaratne, Faseeha Noordeen. Diagnosis of dengue in Sri Lanka: improvements to the existing state of the art in the island. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 2014, 108: 685-691.

[3] Thomas Jaenisch, Dong Thi Hoai Tam, Nguyen Tan Thanh Kieu, Tran Van Ngoc, Nguyen Tran Nam, Nguyen Van Kinh, Ngoun chanpheaktra. Clinical Evaluation Of Dengue And Identification Of Risk Factors For Severe Disease: Protocol for a multicentre study in 8 countries. 2016, 16: 120.

[4] Anoop M., Aneesh Issac, Thomas Mathew, Sairu Philip, Nabeel Abdul Kareem. Genetic Characterization Of Dengue Virus Serotypes Causing Concurrent Infection In An Outbreak In Ernakulam, Kerala, South India, 2007, 48, 849-857.

[5] Chee-Fu Yung, Kim-Sung Lee, Tun-Linn Thein, Li-Kiang Tan, Victor C. Gan, Joshua G. X. Wong, David C. Lye, Lee-Ching Ng, Yee-Sin Leo. Dengue Serotype-Specific Differences in Clinical Manifestation, Laboratory Parameters and Risk of Severe Disease in Adults, Singapore; 2015, 92(5): 999-1005.

[6] Samirbhatt, Peter w.Gething, Oliver j.Brady, Jane P.messina, Andreww. Farlow, Catherinel. Moyes, John M. Drake, John S. Brownstein, Anne G. Hoen. The global distribution and burden of dengue. 10.1038/12060:504- 507.

[7] Sergio I. de la Cruz-Hernández, Hilario Flores-Aguilar, Silvia González-Mateos, Irma López-Martinez, Celia Alpuche-Aranda, Juan E. Ludert, Rosa M. del Angel. Determination of Viremia and Concentration of Circulating Nonstructural Protein 1 in Patients Infected with Dengue Virus in Mexico, 2013, 88(3): 446-454.

[8] Duane J. Gubler. Dengue and Dengue Hemorrhagic Fever. 1998; 480-496.

[9] Maria G. Guzman, Scott B. Halstead, Harvey Artsob, Philippe Buchy, Jeremy Farrar, Duane J. Gubler, Elizabeth Hunsperger, Axel Kroeger, Harold S. Margolis, Eric Martínez, Michael B. Nathan, Jose Luis Pelegrino, Cameron Simmons, Sutee Yoksan, Rosanna W. Peeling. Dengue: a continuing global threat. S7-S16; 10.1038/nrmicro2460.

[10] Diana C Quintero-Gil, Marta Ospina, Jorge E Osorio-Benitez, Marlén Martinez-Gutierrez. Differential replication of dengue virus serotypes 2 and 3 in coinfections of C6/36 cells and *Aedes aegypti* mosquitoes. 2014, 8(7): 876-884.

[11] Michael Worobey, Andrew Rambaut, Edward C. Holmes. Widespread intra-serotype recombination in natural populations of dengue virus. *Proc Natl Acad Sci U S A* 96(13) (1999) 7352-7357.

[12] Sofia Lizeth Alcaraz-Estrada, Martha Yocupicio-Monroy, Rosa María del Ange. Insights into dengue virus genome replication. 2010 ; 575–592 ;10.

[13] Izabela Agaata, Jolanda Smit. Dengue virus life cycle: Viral and host factors modulating infectivity. (2010) 67: 2773-2786.

[14] Shamala D. S. Laboratory Diagnosis of Dengue: A Review. 2015. vol 14.

[15] Ana P. P. Vilela, Leandra B. Figueiredo, João R. dos Santos, Álvaro E. Eiras, Cláudio A. Bonjardim, Paulo C. P. Ferreira, Erna G. Kroon. Dengue Virus 3 Genotype I in Aedes aegypti Mosquitoes and Eggs, Brazil 2005-2006. 2010; vol. 16.

[16] Ananda Amarasinghe, Joel, N. Kuritsky, G. William Letson, Harold S. Margolis. Dengue Virus Infection in Africa. (2011); vol. 17.

[17] Johnson K. M. Viral haemorrhagic fevers. In: Cecil's Textbook of Medicine. Philadelphia: W.B. Saunders, Igaku-Shoin / Saunders International. 1982: 1690.

[18] Niranjan Kanesa-Thasan, Gwong-Jen J. Chang, Bonnie L. Smoak, Alan Magill, M. Jeanne Burrous, Charles H. Hoke, J.R. Molecular and Epidemiologic Analysis of Dengue Virus Isolates from Somalia. (1998); vol. 14.

[19] Justin R. Anderson, Rebeca Rico-Hesse. Aedes Aegypti Vectorial Capacity Is Determined By The Infecting Genotype Of Dengue Virus.(2006); 75(5): 886–892.

[20] Kim Knowlton, Gina Solomon, Miriam Rotkin-Ellman. Mosquito-Borne Dengue Fever Threat Spreading in the Americas. (2009); vol. 14.

[21] Eili Huhtamo, Nathalie Y. Uzcátegui, Heli Siikamäki, Auli Saarinen, Heli Piiparinen, Antti Vaheri, Olli Vapalah. Molecular Epidemiology of Dengue Virus Strains from Finnish Travelers. (2008); vo. l14.

[22] Marion C. Lanteri, Michael P. Busch. Dengue in the context of "safe blood" and global epidemiology: to screen or not to screen. 2012 August; 52(8): 1634-1639.

[23] Hywel L. Cooper, Fethi Louafi, Peter, S. Friedmann. Dengue Hemorrhagic Fever Transmitted by Blood Transfusion. (2007); 127: 1145-1153.

[24] Bruno Pozzetto, Meriam Memmi, Olivier Garraud. Is transfusion-transmitted dengue fever a potential public health threat. (2015); 113-123.

[25] D. Teo, LC. Ng, S. Lam. Is dengue a threat to the blood supply. (2009); 66-77.

[26] Alcaraz-Estrada S. L., Yocupicio-Monroy M., Angel R. M. D. (2010). Insights into dengue virus genome replication, *Future Virol*. 5(5), 575-592.

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