JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Characteristics of the initial dengue outbreaks in a region without dengue prior to mid-2009 in a dengue-endemic country

Kalamathy Murugananthan[1,2], Dinesh Coonghe[3], Thirunawukarasu Kumanan[4], Arumugam Murugananthan[5], Gowri Selvaratnam[4], Sivapalan Sivansuthan[6], Gitanjali Sathiadas[7], Natkunam Ketheesan[8], Faizal A Careem[9], Faseeha Noordeen[2]

[1] Department of Microbiology, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka
[2] Diagnostic and Research Virology Laboratory, Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka
[3] Department of Community and Family Medicine, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka
[4] Department of Medicine, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka
[5] Department of Parasitology, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka
[6] Teaching Hospital, Jaffna, Sri Lanka
[7] Department of Paediatrics, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka
[8] Science and Technology, University of New England, Armadale, Australia
[9] Department of Ecosystem and Public Health, University of Calgary, Alberta, Canada

Abstract

Introduction: The present study evaluated the characteristics of the initial dengue outbreaks in the Jaffna peninsula, a region without dengue prior to mid-2009 in dengue-endemic Sri Lanka, a tropical island nation.

Methodology: This is a cross-sectional study conducted using a total of 765 dengue patients' clinical data and samples collected from the Teaching Hospital, Jaffna during the initial dengue outbreaks. Clinical, non-specific, and specific virological laboratory characteristics including the platelet count, NS1 antigen, and anti-DENV IgM/IgG were evaluated as correlates of dengue virus (DENV) infection in the two initial outbreaks of 2009/2010 and 2011/2012 in Northern Sri Lanka.

Results: Firstly, affected age and clinical characteristics were significantly different between the outbreaks ($p < 0.005$). Secondly, NS1 antigen detection in patients with fever days < 5 was statistically significant ($p < 0.005$). Thirdly, platelet count, detection of NS1 antigen, and anti-DENV IgM/IgG profiles were adequate to diagnose 90% of the patients; hepatomegaly and platelet count of < 25,000/mm^3 were identified as predictors of severe disease. Fourthly, secondary DENV infections were detected in the early stages of the illness in many patients. Finally, infecting DENV serotypes were different between the two outbreaks.

Conclusions: Clinical and non-specific laboratory characteristics and the infecting DENV serotypes between the two initial outbreaks in Northern Sri Lanka were significantly different. NS1 antigen, anti-DENV IgM/IgG, and platelet counts were identified 90% of the dengue patients. Hepatomegaly and platelet count of < 25,000/mm^3 were able to predict the disease severity in this study.

Key words: Dengue; initial outbreaks; clinical; laboratory; virological characteristics.

J Infect Dev Ctries 2023; 17(2):260-267. doi:10.3855/jidc.15493

(Received 19 June 2021 – Accepted 11 April 2022)

Introduction

The clinical features of symptomatic dengue virus (DENV) infections vary and most patients experience a self-limiting febrile illness. In less than 5-10% of DENV-infected individuals, the infection causes severe disease, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The DHF is mostly characterized by plasma leakage with or without hemorrhage [1]. Identification of patients who are likely to progress to DF/DSS has been a challenge in many dengue endemic regions. Thus, it is necessary to evaluate the virological and clinical characteristics with non-specific laboratory data such as platelet count, white blood cell count (WBC), packed cell volume (PCV), urine output, and blood pressure during the course of illness for the effective management of patients. Progressive leucopenia followed by a drop in platelet count often leads to plasma leakage in DHF/DSS patients. Although virus culture and identification of DENV is the gold standard for laboratory confirmation of dengue, it is not a feasible diagnostic option due to low sensitivity to the disease progression and the long turnaround time [2]. Conversely, DENV nucleic acid detection by RT-PCR

is highly sensitive in the acute phase of the illness but RT-PCR requires specialized laboratory infrastructure and expertise, which are not widely available in many resource-limited dengue endemic countries [3]. Hence, relatively less expensive laboratory tests with reasonable diagnostic precision and short turnaround time such as NS1 antigen or/and anti-DENV IgM detection would be suitable to confirm DENV infection in patients with clinical suspicion.

During a clinical suspicion, anti-DENV IgM detection is the most used diagnostic test and is carried out after fever days 5 or more. However, it is not suitable for the early diagnosis of DENV infection, especially when patients present during the early viremic phase, prior to fever day 5 [4]. Moreover, there is a possibility of cross reactivity between anti-DENV IgG and other flaviviral antibodies such as antibodies against Japanese encephalitis (JEV), yellow fever and Zika viruses. The latter two infections have not been reported in Sri Lanka, however, the possibility for cross-reactivity with the flaviviral antibodies could not be excluded.

Presence of NS1 antigen, a conserved and secreted glycoprotein, has been used as a diagnostic marker for the early diagnosis of DENV infection in patients with clinical suspicion [3,5,6]. NS1 is a long-lived antigen, which is detectable during the course of dengue disease [7,8], especially in the early viremic phase, when the fever days are ≤ 5 [9]. Differences in NS1 antigenemia have been reported in patients from different geographical areas and in some patients, it is detectable only after fever day 5 [9].

Sri Lanka has been affected by dengue for several decades. Although serologically confirmed dengue has been described since 1960, it was only after 1989, dengue became a public health problem on the island. There was an island-wide outbreak reported between 1965 and 1968 with 51 DHF cases and 15 deaths [10,11]. In 1989, during the first larger dengue outbreak there were 203 cases documented with 20 deaths [11,12]. Subsequently, the number of dengue cases has been on the rise with the occurrence of annual outbreaks. In 2004 and 2009/2010, Sri Lanka experienced explosive dengue outbreaks with 15,457 and 35,008 reported cases and 88 and 346 deaths, respectively [11,13]. Now dengue is a well-established vector-borne viral disease-causing significant morbidity and mortality in many parts of the country. Jaffna peninsula is geographically isolated from the mainland of Sri Lanka. The isolation was confounded by thirty years of political unrest and the travel between the Jaffna peninsula and the mainland of Sri Lanka was restricted. Unrestricted movement of people started between the two regions in mid-2009. Prior to 2009, there were < 50 presumed dengue cases have been reported from Jaffna. However, due to the lack of laboratory services during the conflict, data on molecular or serological tests of these cases are not available [14]. Due to the non-availability of etiological diagnosis in these regions, physicians and the pediatricians rely on non-specific laboratory data such as platelet counts to support their clinical diagnosis. The current study aimed to assess the clinical, non-specific, and specific virological laboratory data including platelet counts, NS1 antigen, DENV nucleic acid, and anti-DENV IgM/IgG as correlates of DENV infections during the two initial outbreaks (2009/2010 and 2011/2012) in northern Sri Lanka. On the other hand, in this study, we describe the clinico-demographic characteristics and infecting DENV serotypes of the two initial dengue outbreaks preceding the current dengue epidemics.

Methodology

Ethical approval

The study was approved by the Ethical Review Committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka (EC No: 2010/EC/13). An informed written consent was obtained from the study participants prior to collect demographic data and blood samples.

Classification of dengue

This cross-sectional study was carried out utilizing clinical laboratory data of patients admitted to medical and pediatric wards of the Teaching Hospital, Jaffna, during the two initial dengue outbreaks in 2009/2010 (outbreak 1) and 2011/2012 (outbreak 2). A total of 765 patients, with clinically suspected dengue satisfying the case definition of Ministry of Health, Sri Lanka and WHO [15,16] were recruited. Demographic and clinical data were obtained from each patient using a pre-tested questionnaire. Non-specific laboratory data and platelet counts were obtained from the patient's clinical notes. Moreover, based on the dengue guidelines [1], the study sample was divided into two disease severity categories, dengue with or without warning signs and severe dengue cases [15].

Diagnosis of dengue

Of the 765 samples, 300 were from the 2009/2010 outbreak and 465 from the 2011/2012 outbreak. WBC and platelet counts had been done during the hospital stay of the patients using an automated hematological

analyzer (Mindray, China), as a routine laboratory procedure for all dengue suspected patients. Serum samples were retrospectively tested for NS1 antigen by ELISA (Pan Bio Diagnostics, Australia), DENV nucleic acid using RT-PCR (Promega, USA), anti-DENV IgM and IgG using ELISA (Pan Bio Diagnostics, Australia). All the tests were performed according to the manufacturer's instructions. DENV RNA was extracted from patients' sera using a viral RNA extraction mini kit (Qiagen, Hilden, Germany, Cat No 5206). Primers (D1 and D2) described by Lanciotti *et al* (1992) were used in RT-PCR to amplify the junction of C/PrM genes of the DENV [17].

Statistical analysis

The data were analyzed using SPSS, Version 21. Mono-variate analysis was performed for all descriptive variables. Student's *t*-test was carried out to evaluate the association between DENV NS1 antigen positivity and the mean platelet counts. Differences in the mean platelet counts between DF and DHF patients were also determined using the Student's *t*-test.

Results

The present study assessed the clinical, non-specific and specific virological laboratory data including platelet counts, NS1 antigen, and anti-DENV IgM/IgG as correlates of symptomatic DENV infection of the two initial dengue outbreaks (2009/10 and 2011/2012) in a region without a past history of epidemic dengue prior to mid-2009. Of the 765 patients, 52.7% and 55.3% of patients were males in outbreaks 1 and 2, respectively. The mean age and the mean duration of fever in the study sample on admission were 26.23 ±

Table 1. Differences in the clinical and non-specific laboratory characteristics of paediatric patients between dengue outbreaks 1 (2009/2010) and 2 (2011/2012) in the Jaffna Peninsula of the Northern Sri Lanka. These were the first two dengue outbreaks in the Northern region while other parts of Sri Lanka have been experiencing regular dengue outbreaks since 1989.

Clinical and non-specific laboratory characteristics	Outbreak 1 (2009/2010) n = 78	Outbreak 2 (2011/2012) n = 102	*p* value
Headache	73.1 (57)	66.7 (68)	0.355
Retro orbital pain	24.4 (19)	47.1 (48)	0.002*
Myalgia	38.5 (30)	73.5 (75)	< 0.001*
Arthralgia	16.7 (13)	28.4 (29)	0.064
Dyspnoea	5.1 (4)	2.9 (3)	0.452
Flushed extremities	41 (32)	32.4 (33)	0.230
Pallor	3.8 (3)	41.2 (42)	< 0.001*
Rash	17.9 (14)	10.8 (11)	0.168
Splenomegaly	3.8 (3)	2.9 (3)	0.737
Hepatomegaly	35.9 (28)	23.5 (24)	0.070
Haemorrhagic manifestations	16.7 (13)	21.6 (22)	0.410
Pleural effusion	3.8 (3)	7.8 (8)	0.261
Platelet count < 25,000/mL	7.7 (6)	4 (3.9)	0.27
Platelet count 25,000 – 100,000/mL	48.7 (38)	48 (49)	0.93
Platelet count > 100,000/mL	43.6 (34)	48 (49)	0.55
Severity	43.6 (16)	26.5 (27)	0.353

N: Number of patients; *: Statistically significant; WBC: White blood cell.

Table 2. Differences in the clinical and non-specific laboratory characteristics of adult patients between dengue outbreaks 1 (2009/2010) and 2 (2011/2012) in the Jaffna Peninsula, the Northern Sri Lanka. These were the first two dengue outbreaks in the Northern region while other parts of Sri Lanka have been experiencing regular dengue outbreaks since 1989.

Clinical and non-specific laboratory characteristics	Outbreak 1 (2009/2010) n = 222	Outbreak 2 (2011/2012) n = 363	*p* value
Headache	88.2 (195)	74.4 (270)	< 0.001*
Retro orbital pain	39.4 (87)	41.6 (151)	0.595
Myalgia	72.9 (161)	65.0 (236)	0.049*
Arthralgia	56.1 (124)	38.0 (138)	< 0.001*
Dyspnoea	4.1 (9)	4.4 (16)	0.846
Flushed extremities	60.2 (133)	30.0 (109)	< 0.001*
Pallor	3.6 (8)	43.0 (156)	< 0.001*
Rash	22.6 (50)	11.3 (41)	< 0.001*
Splenomegaly	4.5 (10)	5.8 (21)	0.510
Hepatomegaly	24.0 (53)	31.1 (113)	0.063
Haemorrhagic manifestations	24.4 (54)	19.8 (72)	0.190
Pleural effusions	4.1 (9)	17.1 (62)	< 0.001*
Platelet count < 25,000/mL	5 (11)	8.8 (32)	0.085
Platelet count 25,000 – 100,000/mL	57.9 (128)	63.1(229)	0.214
Platelet count >100,000/mL	37.1(32)	28.1(102)	< 0.001*
Severe dengue	28.5 (63)	35.5(129)	0.079

N: Number of patients; *: Statistically significant; WBC: White blood cell.

Table 3. Differences in the virological profiles between dengue outbreaks 1 (2009/2010) and 2 (2011/2012) in the Jaffna Peninsula of the Northern Sri Lanka.

Laboratory tests	Outbreak 1 (2009/2010)	Outbreak 2 (2011/2012)	*p* value	Outbreaks 1 and 2
	n = 300	n = 465		n = 765
NS1 antigen by ELISA	124 (41.33%)	149 (32.04%)	0.009*	273(35.68%)
DENV nucleic acid by RT-PCR	64 (21.33%)	141 (30.32%)	0.006*	205 (26.79%)
NS1 antigen + DENV nucleic acid	38 (12.66%)	93(20%)	< 0.001*	131 (17.12%)
Anti-DENV IgM by ELISA	148 (49.33%)	235(50.53%)	0.745	383 (50.06%)
Anti-DENV IgG by ELISA	203 (67.66%)	257(55.26%)	0.001*	460 (60.13%)

N: Number of patients; *: Statistically significant ($p < 0.05$).

15.6 years and 5.5 ± 1.6 days, respectively. The mean age of the patients affected in the 2009/2010 and 2011/2012 outbreaks were 25.31 ± 15.4 and 26.83 ± 15.8 years, respectively. The common clinical features of adult and pediatric patients noted in 2009/2010 and 2011/2012 outbreaks were fever (100%), headache, myalgia, flushed extremities, retro-orbital pain, and arthralgia (Tables 1 and 2). The number of adult and pediatric patients with different ranges of platelet counts and the number of patients with severe dengue in outbreaks 1 and 2 are given in Tables 1 and 2.

The study sample (n = 765) was categorized as those with fever for < 5 days (1); those with fever for 5-7 days (2) and those with fever for > 7 days (3) to determine the association between the presence of NS1 antigen and anti-DENV IgM/IgG and fever days (< 5, 5-7, > 7) in DENV infection. More than half of the patients (388/765) presented with ≤ 5 days of fever and of whom 204 (52.6%) were positive for NS1 antigen and 200 (51.5%) were positive for DENV nucleic acid. Five patients were positive for DENV nucleic acid between fever days 5 and 7. Detecting NS1 antigen in patients with fever days < 5 was significant ($p = 0.001$) when compared to those with fever days > 5. Of the 765 dengue suspected patients, 50.1% (383/765) were positive only for anti-DENV IgM; 19.1% (n = 146) were positive for both NS1 antigen and anti-DENV IgM and 7.7% (n = 59) were positive for NS1 antigen alone. Only 22.2% (85/383) were positive for anti-DENV IgM when the fever days were < 5. Most patients (47.8%, 183/383) had anti-DENV IgM when the fever days were between 5 and 7; 10.4% (40/383) had anti-DENV IgM when fever days were > 7.

Of the 765 patients, 205 were positive for DENV RNA by RT-PCR. Of these 205 patients, 64 were from the 2009/2010 outbreak and the rest were from the 2011/2012 outbreak (Table 3). DENV-1, DENV-2, DENV-3 and DENV-4 were detected in 12 (18.7%), 19 (29.6%), 25 (39%) and 1 (1.6%) of patients, respectively in the 2009/2010 outbreak. In the 2009/2010 outbreak, 7 patients (10.9%) had co-infection with DENV-2 and DENV-3 (Table 4). In contrast, in the 2011/2012 outbreak, DENV-1 was found to be the dominant serotype (56%) and DENV-4 was not identified in any of the patients. Ten patients (7.1%) had co-infection with either DENV-1 and DENV-2 or DENV-1 and DENV-3 (Table 4). In the 2009/2010 outbreak, DENV-2 and DENV-3 were responsible for 95.2% of all DHF cases in that outbreak. In the 2011/2012 outbreak, DENV-1 was responsible for 86.8% of all DHF cases in that outbreak. In the whole study sample (n = 765), 35 patients had severe dengue with low platelet count of < 25,000/mm^3 (n = 53) and hepatomegaly ($p < 0.001$). However, those with severe dengue did not have a significant association with splenomegaly ($p = 0.815$) (Table 5).

Discussion

Dengue was not a public health problem in the Jaffna District until 2009 due to the isolation of the population from the rest of the island since 1980 due to travel restrictions during the internal conflict. The end of the conflict in mid-2009 allowed free movement of people from the other parts of the country to Jaffna and vice versa and this contributed to a marked increase in the number of reported dengue cases from the Jaffna peninsula with larger outbreaks in 2009-2010 and

Table 4. Differences in the distribution of DENV serotypes between dengue outbreaks 1 (2009/2010) and 2 (2011/2012) in the Jaffna Peninsula of the Northern Sri Lanka.

Outbreak	DENV-1	DENV-2	DENV-3	DENV-4	Mixed infections	Total
Outbreak 1 (2009/2010)	18.8% (n = 12)	29.7% (n = 19)	39% (n = 25)	1.6% (n = 1)	10.9% (n = 7)	64
Outbreak 2 (2011/2012)	56% (n = 79)	17% (n = 24)	19.9% (n = 28)	ND	7.1% (n = 10)	141

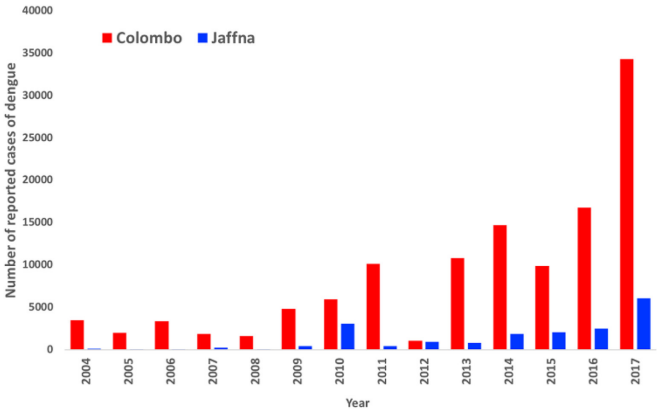
DENV: Dengue virus; n: Number of patients; ND: Not detected.

2011/2012 (Figure 1) compared to the previous years [11,14]. To a lesser extent, factors such as clinicians' capacity to identify suspected dengue cases, the lack of the surveillance system, and diagnostic capacities/capabilities during the conflict era may have also contributed to the low case numbers reported prior to 2009.

In the 2009/2010 and 2011/2012 dengue outbreaks (n = 765), males were predominantly affected than females and this finding is similar to most other studies conducted elsewhere and in Sri Lanka [12,18-21]. The reason for high number of males being affected might be attributed to the fact that a significant proportion of males engage in agriculture and livestock production in Jaffna. They often work with an exposed torso increasing the risk of acquiring DENV infection through mosquito bites [11]. However, a few studies have also reported a higher ratio of females with dengue [22,23]. In both outbreaks, the mean age of the affected patients was almost similar (26.2 years) and such findings have been reported by previous studies as well [12,24]. In both outbreaks, all clinically dengue suspected patients presented with fever and other associated symptoms. The percentage of patients with headache, retro-orbital pain, arthralgia, and flushed extremities, pallor, rash/hemorrhages, and effusion were significantly different between the two outbreaks (Table 1). The virological laboratory characteristics were also significantly different between the two outbreaks (Table 3). These differences might be due to the clinical evolution of the dengue disease and infecting DENV serotypes in a previously non-epidemic region. Moreover, the main reason for detecting virological parameters such as NS1 antigen and DENV nucleic acid in a significantly large number of patients may be due to the low mean duration of fever on admission, especially during the 2011/2012 outbreak.

In the present study, the majority of the patients were positive for NS1 antigen when the fever day was < 5, suggesting that NS1 antigen detection is better done at an early stage in dengue-endemic regions and similar finding has been reported by others as well [3,7]. On the other hand, NS1 antigen has been shown to be positive

Figure 1. A comparison of reported dengue cases from 2004 to 2017 in all government healthcare facilities of Colombo and Jaffna Districts of Sri Lanka (Adapted from the Epidemiology Unit, Ministry of Health, Sri Lanka).



This study describes the first two outbreaks 2009/2010 and 2011/2012 in the Jaffna Peninsula, the Northern Sri Lanka. However, the mainland of Sri Lanka, predominantly the Colombo District, has been experiencing regular dengue outbreaks since 1989.

for up to fever days 6 to 7 [25] and 9 [8]. The evolution of dengue disease in different populations is believed to be the reason for the differences in NS1 antigenemia during the course of fever [25,26].

Of the 765 patients, 21.6% (n = 165) were positive for anti-DENV IgM only indicating a primary DENV in these patients. However, 19% (n = 146) of the patients were positive for NS1 antigen and anti-DENV IgM;7.7% (n = 59) were positive for NS1 alone. Thus, in total, ~30% of the study population (293%) had primary DENV infection due to the presence of NS1 antigen or anti DENV IgM or both [27].

Of the 765, over 30% of the cases were positive for anti-DENV IgG alone indicating past exposure or secondary DENV infections. The anti-DENV IgM disappears relatively faster than that in a primary infection [28]. Less than 30% of the study sample was positive for both anti-DENV IgM and IgG indicating another subset of secondary DENV infections [2], however, some of these patients might have had a primary DENV infection in which both anti-DENV IgM and IgG were detectable at the time of sample collection [29]. Overall, 60% were positive for anti-

Table 5. Clinical and laboratory characteristics of patients with severe dengue.

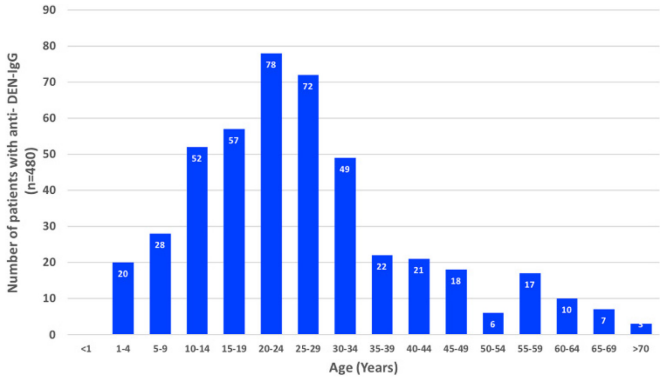
Presence of splenomegaly, hepatomegaly and platelet count of < 25,000/mm^3	Severity (%)	*p* value
Splenomegaly (n = 37)	4.3 (n = 37)	0.825
Hepatomegaly (n = 218)	27 (n = 218)	< 0.001*
Platelet count < 25,000/mm^3 (n = 53)	45.8 (n = 35)	< 0.001*
Platelet count 25,000 – 100,000/mL (n = 445)	19.2 (147)	0.10
Platelet count > 100,000/mL (n = 267)	6.9% (53)	< 0.001

N: Number of patients; *: Statistically significant ($p < 0.05$).

DENV IgG with or without anti-DENV IgM indicating that the majority of the patients would have had a secondary infection or past exposure to DENV. High rate of secondary infection might be due to the circulation of multiple DENV serotypes. Furthermore, we noted a prevalence of 34% (157/460) anti-DENV IgG in those that were < 19 years of age (Figure 2), as reported in some other dengue-endemic countries during different stages of the evolution of dengue [30,31,32]. Detection of anti-DENV IgG is not useful in the detection of acute DENV infection and the presence of these antibodies can be attributed to past DENV infections. There is a possibility of cross-reactivity between anti-DENV IgG and other flaviviral antibodies such as antibodies against Japanese encephalitis (JEV), yellow fever, and Zika viruses. The latter two infections have not been reported in Sri Lanka, however, the possibility for cross-reactivity with flaviviral antibodies could not be excluded.

Only 9.6% of the patients were positive for NS1 antigen, anti-DENV IgM, and IgG among which 40% of the patients had severe dengue indicating a possible re-infection in this subgroup. NS1 antigen and anti-DENV IgM were detectable early due to the current infection and the anti-DENV IgG would have resulted from a past infection [27]. Of the patients tested, 18.6% (n = 142) were positive for NS1 antigen and anti-DENV IgG, while 28.4% (n = 218) were positive for anti-DENV IgM and IgG. These patients may have had secondary DENV infection and therefore have a greater risk of developing severe dengue [33,34]. The number of DHF cases was high in both primary and secondary infections, suggesting the risk of DHF in both [11]. In this subset also, anti-DENV IgG resulting from cross-reactivity to other flaviviral infections cannot be ignored and can only be excluded through viral neutralization at least in a subset of samples. Hepatomegaly and a low platelet count of < 25,000/mm^3 were significantly associated with severe dengue disease based on the current study findings and these are in agreement with the study carried out by Agrawal *et al.* [35]. However, in contrast to the current study, Agrawal *et al.* [35] also show a significant association between splenomegaly and severe dengue disease.

Figure 2. Age-specific anti-DENV IgG seroprevalence in the study sample (n = 765).



In the current study, 45.3% (n = 347) of the patients had laboratory-confirmed DENV infection as detected by NS1 antigen and/or DENV nucleic acid detection. Combining DENV NS1 antigen and nucleic acid detection is useful in diagnosing DENV infections in the acute stage of illness [6,4,36-38]. The region where this study was conducted is also endemic for typhoid [39], typhus [40], and leptospirosis [41], which are clinically indistinguishable from dengue at the early stage. A total of 81 patients who were negative for NS1 antigen, DENV nucleic acid, and anti-DENV IgM/IgG in this cohort may have had infections, which were at early stages clinically indistinguishable from dengue.

Based on the current WHO recommendations [1], patients with platelet count < 1,00,000/mL and clinical symptoms suggestive of dengue require hospital admission. Based on the guidelines, 11.4% of the patients (n = 87) were positive for NS1 antigen with platelet count > 1,00,000/mL and they did not qualify for admission to the hospital (Table 2). If the platelet count of < 1,00,000/mL or the presence of anti-DENV IgM were considered for admission, 79.6% (n = 609) of the patients would qualify for hospital admission. NS1 antigen, anti-DENV IgM and platelet count < 1,00,000/mL together would increase the detection of patients who would require hospital admission. In the present study, > 90% of the patients admitted to the hospital on clinical suspicion for dengue had either positivity for NS1 antigen or anti-DENV IgM or both with platelet count < 1,00,000/mL. RT-PCR for the identification of DENV infection in the viremic phase is of value. However, in resource limited settings utilizing the platelet counts with NS1 antigen and anti-DENV IgM/IgG will improve the laboratory diagnosis of dengue. Additionally, adapting a diagnostic algorithm [35,42] will help to differentiate at the onset of infection other febrile infections that clinically mimic dengue.

In the 2009/2010 outbreak, DENV-2 and DENV-3 were the predominant cause of infection with a few cases of DENV-1 infection. In contrast, in the Western Province DENV-1 had been the predominant cause of infection in the same period in 2009/2010 [13]. Dengue cases started to increase in Jaffna in mid-2009 and this period correlated with the opening of major highways

connecting the Jaffna peninsula to the rest of the country after 30 years. DENV infections could have been introduced to the Jaffna peninsula from other parts of Sri Lanka where DENV-2 and DENV-3 were prevalent. However, we are unable to confirm this as phylogenetic data are not available from all the dengue-endemic regions of Sri Lanka. Prior to 2007, DENV-3 was the predominant type followed by DENV-1 and DENV-2 in the Western Province of Sri Lanka [25]. Moreover, DENV-2 or DENV-3 infection may have caused severe dengue in the Jaffna peninsula as most of the people in the region might not have had prior immunity against DENV-2 or DENV-3. DENV-1 was found to be the predominant serotype responsible for the infection and severe clinical presentation in the 2011/2012 outbreak. This finding is in agreement with other studies conducted in other parts of Sri Lanka during the same period [43]. When considering the initial dengue outbreaks in the Jaffna peninsula, there was a shift in the DENV serotypes between the 2009/2010 and 2011/2012 outbreaks (Table 4).

In the 2009/2010 outbreak, mixed infections were caused by both DENV-2 and DENV-3, correlating with the predominant serotypes circulated in the population during 2009 and 2010. Similarly, the most predominant DENV serotype circulated during the 2011/2012 outbreak was DENV-1 and thus co-infections were detected between DENV-1 and DENV-2 or DENV-1 and DENV-3. The presence of DENV co-infections in both outbreaks reflects the hyper-endemicity of DENV in the Jaffna peninsula. DENV co-infections might contribute to the dominance and establishment of certain DENV serotypes in a region with or without future clinical implications.

Conclusions

Significant differences were noted in the clinico-laboratory characteristics between the 2009/2010 and 2011/2011 dengue outbreaks. Hepatomegaly and a low platelet count of < 25,000/mm^3 were able to predict severe dengue in the study sample. This will assist in the early detection of patients who are likely to progress to severe disease. NS1 antigen, anti-DENV IgM/IgG detection, and platelet count improved the diagnosis of DENV infection in patients suspected of having dengue.

Acknowledgements

The authors thank Mr. S Tharmasena of the Department of Microbiology, Faculty of Medicine, the University of Jaffna for assisting in the laboratory work for the study.

References

1. World Health Organization (2011) Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever. Available: https://apps.who.int/iris/handle/10665/204894. Accessed: 05 January 2022.
2. Shu PY, Huang JH (2004) Current advances in dengue diagnosis. Clin Immunol 4: 642-650.
3. Pok KY, Lai YL, Sng J, Ng LC (2010) Evaluation of non-structural 1 antigen assays for the diagnosis and surveillance of dengue in Singapore. Vect Zoon Dis 10: 1010-1016.
4. Dussart P, Petit L, Labeau B, Bremand L, Leduc A, Moua D, Matheus S, Baril L (2008) Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum. PLOS Negl Trop Dis 20: 2.
5. Guzman MG, Kour G (2004) Dengue diagnosis advances and challenges. Int J Infect Dis 8: 69-80.
6. Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, Mohamad M, Chua KB (2007) Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J Virol Methods 140: 75-79.
7. Hu D, Di B, Ding X, Wang Y, Chen Y, Pan Y, Wen K, Wang M, Che X (2011) Kinetics of non-structural protein 1, IgM and IgG antibodies in dengue type 1 primary infection. Virol J 8: 471.
8. Sirisena N, Noordeen F, Fernando L (2017) NS 1 lasts longer than the dengue virus nucleic acid in clinically suspected patients with dengue fever and dengue haemorrhagic fever. VirusDisease 28: 341-344.
9. Guzman MG, Jaenisch T, Gaczkowski R, Hang VT, Sekaran SD, Kroeger A, Vazquez S, Ruiz D, Martinez E, Mercado JC, Balmaseda A, Harris E, Dimano E, Leano PA, Yoksan S, Villegas E, Benduzu H, Villalobos I, Farrar J, Simmons CP (2010) Multi-country evaluation of the sensitivity and specificity of two commercially available NS1 ELISA assays for dengue diagnosis. PLOS Negl Trop Dis 4: 811.
10. Vitarana T, Jayakuru WS (1997) Historical account to dengue haemorrhagic fever in Sri Lanka. WHO/SEARO. Dengue Bull 21: 117-118.
11. Sirisena PDNN, Noordeen F (2014) Evolution of dengue in Sri Lanka- Changes in the virus, vector and climate. Int J Infect Dis 19: 6-12.
12. Messer WB, Kanakaratne N, Thevanesam V, Ranawaka G, Shahani A, de Silva A (2012). Clinical features of hospitalized dengue patients in Sri Lanka from 2004 to 2006. Sri Lankan J Infect Dis 1: 9-18.
13. Tissera HA, Ooi EE, Gubler DJ, Tan Y, Logendra B, Wahala WM, de Silva AM, Abeysinghe N, Palihawadana P, Gunasena S, Tam CC, Amarasinghe A, Letson GW, Margolis HS, De Silva AD (2011) New dengue virus type 1 genotype in Colombo, Sri Lanka. Emerg Infect Dis 17: 2053-2055.
14. Murugananthan K, Kandasamy M, Rajeshkannan N, Noordeem F (2014) Demographic and clinical features of suspected dengue and dengue haemorrhagic fever in the Northern Province of Sri Lanka, a region afflicted by an internal conflict for more than 30 years—a retrospective analysis. Int J Infect Dis 27: 32-36.
15. World Health Organization (2009) Dengue guidelines for diagnosis, treatment, prevention and control. Available: http://www.who.int/iris/handle/10665/44188. Accessed: 10 June 2021.

16. Ministry of Health, Sri Lanka (2012) Guidelines on management of dengue fever and dengue haemorrhagic fever in adults. National guidelines. Available: http://www.epid.gov.lk/web/images/pdf/Publication/guidelines_for_the_management_of_df_and_dhf_in_adults.pdf. Accessed: 10 June 2021.
17. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam SV (1992) Rapid detection and typing of dengue viruses from clinical samples by reverse transcriptase-polymerase chain reaction. J Clin Microbiol 30: 545-551.
18. Gupta E, Dar L, Kapoor G, Broor S (2006) The changing epidemiology of dengue in Delhi, India. Virol J 3: 92.
19. Bharaj P, Chahar HS, Pandey A, Diddi K, Dar L, Guleria R, Kabra SK, Broor S (2008) Concurrent infections by all four dengue virus serotypes during an outbreak of dengue in 2006 in Delhi, India. Virol J 5: 1.
20. Anker M, Arima Y (2011) Male–female differences in the number of reported incidence of dengue fever cases in six Asian countries. Western Pac Surveill Response J 2: 17.
21. Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S (2011) Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J Infect Dis Immun 3: 85-89.
22. Bosmasang E, Suzara-Masage EC (2008) Clinical and laboratory features of the dengue virus serotypes among infected adults in Cardinal Santos Medical Center. Philippine J Microbiol & Infect Dis 37: 5-14.
23. Kularatne SA, Gawarammana IB, Kumarasiri PR (2005) Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults: a descriptive study in Sri Lanka. Southeast Asian J Trop Med Public Health 36: 686-692.
24. Kanakaratne N, Wahala WM, Messer WB, Tissera HA, Shahani A, Abeysinghe N, de Silva AM, Gunasekera M (2009) Severe dengue epidemics in Sri Lanka, 2003–2006. Emerg Infect Dis 15: 192.
25. Huhtamo E, Hasu E, Uzcategui NY, Erra EO, Nikkari S, Kantele A, Vapalahti O, Piiparinen H (2010) Early diagnosis of dengue in travelers: Comparison of a novel real-time RT-PCR, NS1 antigen detection and serology. J Clin Virol. 47: 49-53.
26. da Costa VG, Marques-Silva AC, Moreli ML (2014) A meta-analysis of the diagnostic accuracy of two commercial NS1 antigen ELISA tests for early dengue virus detection. PLOS ONE 9: e94655.
27. Arya SC, Agarwal N, Parikh SC (2011) Simultaneous detection of dengue NS1 antigen, IgM plus IgG and platelet enumeration during an Outbreak. SQU Med J 11: 470-476.
28. Centers for Disease Control and Prevention Diagnosis of dengue (2019) Available: https://www.cdc.gov/dengue/healthcare-providers/diagnosis.html. Accessed: 10 June 2021.
29. Sa-Ngasang A, Anantapreecha S, A-Nuegoonpipat A, Chanama S, Wibulwattanakij S, Pattanakul K, Sawanpanyalert P, Kurane I (2006) Specific IgM and IgG responses in primary and secondary dengue virus infections determined by enzyme-linked immunosorbent assay. Epidemiol Infect 134: 820-825.
30. Goh KT, Yamazaki S (1987) Serological survey on dengue virus infection in Singapore. Trans R Soc Trop Med Hyg 81: 687-689.
31. Graham RR, Juffrie M, Tan R, Hayes CG, I Laksono, Ma'roef C, Erlin, Sutaryo, Porter KR, Halstead SB (1999) A prospective sero-epidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. Studies in 1995-1996. Am J Trop Med Hyg 61: 412-419.
32. Tuntaprasart W, Barbazan P, Nitatpattana N, Rongsriyam Y, Yoksan S, Gonzalez JP (2003) Sero-epidemiological survey among school children during the 2000-2001 dengue outbreak of Ratchaburi Province, Thailand. Southeast Asian J Trop 34: 564-568.
33. Gurugama P, Garg P, Perera J, Wijewickrama A, Seneviratne SL (2010) Dengue viral infections. Indian J Dermatol 55: 68.
34. Senaratne T, Wimalaratne H, Alahakoon DG, Carr MC, Noordeen F (2016) Characterization of dengue virus infections in a sample of patients suggests unique clinical, immunological and virological profiles that impact on the diagnosis of dengue and dengue hemorrhagic fever. J Med Virol 88: 1703-1710.
35. Agrawal VK, Prusty BSK, Reddy CS, Reddy KM, Agrawal RK, Bandaru SS (2018) Clinical profile and predictors of Severe Dengue disease: A study from South India. Caspian J Intern Med. 9: 334-340.
36. Dussart P, Labeau B, Lagathu G (2006) Evaluation of an enzyme for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol 13: 1185-1189.
37. Wang SM, Sekaran SD (2010) Evaluation of a commercial SD dengue virus NS1 antigen capture enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. J Clin Microbiol 27: 93-97.
38. Detta S, Wattal C (2010) Dengue NS1antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiol 28: 107-110.
39. Mahagamage MGYL, Pavithrani S, Pathmalal M (2019) Water quality and microbial contamination status of groundwater in Jaffna Peninsula, Sri Lanka. J Water and Development 40: 3-12.
40. Anandaselvam PJ, Ketheesan N, Murugananthan K (2014) Emerging scrub typhus infection in the northern region of Sri Lanka. BMC Research Notes 7:719.
41. Sanjeewani RMSS (2017) Temporal and spatial trends of leptospirosis cases in Sri Lanka. Int J Innovative Research and Development 6: 131-135.
42. Senaratne TN, Noordeen F (2014) Diagnosis of dengue in Sri Lanka: Improvements to the existing state of the art in the island. Trans R Soc Trop Med Hyg 108: 685-691.
43. Ocwieja KE, Fernando AN, Sherrill-Mix S, Sundararaman SA, Tennekoon RN, Tippalagama R, Krishnananthasivam S, Premawansa G, Premawansa S, De Silva AD (2014) Phylogeographic and molecular epidemiology of an epidemic strain of dengue virus type 1 in Sri Lanka. The Am J Trop Med Hyg 91: 225-234.

Corresponding author

Prof Faseeha Noordeen
Department of Microbiology, Faculty of Medicine,
University of Peradeniya, Peradeniya 20400, Sri Lanka
Tel: +94 81 2396530; +94 77 2293301
E mail: faseeha.noordeen12@gmail.com;
faseeha.noordeen@med.ac.lk

Conflict of interests: No conflict of interests is declared.