Development of Effective Control Strategies for Stereotype and Genotype Monitoring

Agnes Ani Prameela[1], Dr. Rupesh Sharma[2]

[1]Research Scholar, OPJS University, Churu, Rajasthan
[2]Assistant Professor, OPJS University, Churu, Rajasthan

Abstract

As far as arthropod-borne viral infections go, dengue fever is the most common in India. Because all four dengue virus serotypes have been found in Delhi, India's capital, the city is considered to be dengue virus endemic. Blood samples taken from suspected patients in New Delhi, India, during the post-monsoon season of 2015 were tested for the presence of dengue viruses and are described in this article. RT-PCR found dengue virus in 29% of the samples examined. The research found DENV-2, DENV-1, DENV-3, and DENV-4 in 66.66%, 22.22%, and 16.75% of the samples tested positive for each of the four DENV serotypes. The DENV2 serotype was found to predominate in the investigation. In addition, 22.22 percent of the samples tested positive for both DENV-1 and DENV-2 and 5.55 percent of samples tested positive for both DENV-2 and DENV-4 for co-infection. According to our findings, the age bracket of 21-30 years old had the highest percentage of dengue-positive patients. DNA sequencing of individual serotypes will also provide information on the circulating genotypes in this area. Studying illness severity in connection to various serotypes and concurrent infections will also add to our understanding of how this virus spreads. Serotype and genotype monitoring will aid in the development of effective control strategies for DENV.

Keywords: *Dengue; Dengue Hemorrhagic Fever; Dengue Virus, Severe Dengue, Signs and Symptoms.*

1. INTRODUCTION

Infected Aedes mosquitoes cause dengue fever, the most widespread mosquito-borne illness. It is believed that the Flavivirus genus contains all four forms of dengue virus that cause human illness (dengue type 1, 2, 3, and 4). Symptomatic dengue virus infection has been categorized into three distinct diseases: dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, according to the WHO 1997 classification (DSS). All dengue patients are categorized into three severity levels according to the revised WHO classification of 2009: dengue without warning signs (abdominal pain, persistent vomiting and fluid accumulation), dengue with warning signs (abdominal ailment and persistent vomiting as well as fluid accumulation and mucosal bleeding), and severe dengue. There are over 100 nations where dengue fever is endemic, with the majority of occurrences occurring in the Americas; Southeast Asia; and the Western Pacific areas of the World Health Organization. Dengue fever is the most common reason for hospitalization in India, affecting almost every state. There was a distinct urban distribution of dengue fever a few decades ago, but currently the disease may be seen in peri-urban and rural regions as well as metropolitan ones. As part of the National Vector Borne Disease Control Program (NVBDCP), the Integrated Disease Surveillance Program (IDSP), and the Department of Health Research's network of 52 Virus Research and Diagnostic Laboratories (VRDLs), an extensive network of more than 600 sentinel hospitals in India monitors for the presence of dengue fever. An estimated 33 million cases were reported in the United States in 2010. At least 10,000 cases of dengue were confirmed in the NVBDCP's labs last year. As a result, the true extent of the dengue epidemic in India may be significantly underestimated.

Dengue fever is a major drain on the country's economy and puts a strain on the health care system. Dengue virus transmission is mostly prevented and controlled in India via a combination of case identification, case treatment, and vector control. There is a new dengue vaccine on the market, and many more are in the works. Decisions on the appropriate use of current and developing preventive and control techniques need information on the burden of dengue illness, its prevalence, incidence, and geographic distribution. A systematic review and meta-analysis were undertaken to assess the disease burden for dengue fever in India using this information. Dengue virus serotype distribution was also examined, as well as the percentage of secondary infections calculated.

2. LITERATURE REVIEW

Jeyanthi Suppiah et. al, (2018): From 2014 to 2016, Malaysia suffered an unprecedented epidemic of dengue fever, which resulted in a dramatic rise in the number of cases and deaths. Several factors might contribute to the onset of dengue fever. This research is focused on viral variables such as dengue serotype and genotype. Dengue genotypes were not studied in any of the research that looked at how the dengue serotypes were linked to the disease's clinical symptoms. There are two Malaysian tertiary institutions where the current research will be conducted, which will look at dengue serotype and genotype-specific clinical features between 2014 and mid-2017. Taqman Real-Time RT-PCR, sequencing, and phylogenetic analysis were used to serotype and genotype 120 retrospective dengue blood specimens. Statistical analysis of dengue serotype and genotype data was performed on 101 out of 120 clinically matched individuals in order to create a descriptive relationship between genetic components and clinical outcomes in dengue-infected patients. DENV 1 genotype I was determined to be the most common dengue serotype and genotype throughout the research period. Patients infected with DENV 1 and DENV 3 were more likely to have non-severe clinical symptoms. A considerable number of people with DENV 2 infection had severe dengue (p 0.001), but those with DENV 1 infection exhibited no significant warning signals. There was a statistically significant correlation between DENV 2 infection and symptoms such as prolonged vomiting (p 0.010), epigastric discomfort (p 0.018) and plasma leaking (p 0.004). Furthermore, DENV 3 infection was associated with increased rates of myalgia and arthralgia ($p = 0.015$ and $p = 0.014$). Patients with severe dengue were found to have a high prevalence of DENV 2 Cosmopolitan genotype. Myalgia and DENV 3 genotype I was discovered to be related. In a similar vein, individuals with arthralgia were considerably more likely to have DENV 3 genotype III than non-arthralgia controls.

Creuza Rachel Vicente et. al, (2016): To produce bleeding, dengue is caused by an RNA virus belonging to the Flaviviridae family, which has four serotypes (DENV-1 to DENV-4). Because dengue is a mosquito-borne disease, this research sought to determine how serotype affects the result. Dengue patients with serotyping findings from Vitória, Esprito Santo, Brazil, between 2009 and 2013 were included in this cross-sectional investigation. The Information System for Notifiable Diseases was used to get data. A variety of statistical tests, including the chi-square test, Fisher exact test, Mann–Whitney U test, and logistic regression, were used in conjunction with data on gender and age to look for links between various serotypes and the severity of dengue. There were 485 confirmed dengue cases in the sample, with a median age of 26 years and a female preponderance of 46.4%. More than two-thirds of the samples were caused by DENV-1, which is followed by DENV-4 (16.1%), which is followed by DENV-2 (6.4%), and DENV-3 (0.02%). Most severe dengue cases were caused by DENV-2, with 32.3 percent, followed by DENV-4, with 6.4 percent of cases, DENV-1 with 4.5 percent of those and none of the other three. Serotype 2 of dengue is seven times more likely to cause severe dengue than the other serotypes. In comparison to DENV-1 and DENV-4, cases of DENV-2 showed a greater percentage of severe dengue, according to this research. An essential way to minimize an increase in severe dengue outbreak outcomes by forecasting the health care assistance required for early diagnosis and treatment is to identify serotypes circulating in the area early.

Chee-Fu Yung et. al, (2015): There are few studies on the effects of dengue on adults and the severity of the illness. From April 2005 to December 2011, we collected data on adult febrile patients who had no other known diagnosis but dengue. In this study, the World Health Organization's (WHO) 1997 and 2009 definitions for dengue hemorrhagic fever (DHF) and severe dengue were used to describe outcomes (SD). Only 22.0 percent of the 469 confirmed cases of dengue were found to have the infecting serotype DENV-1, which was followed by 57.1 percent of the cases of DENV-2, 17.1 percent of the cases of DENV-3 and 3.8 percent of DENV-4. Those with DENV-1 infection had a higher likelihood of having red eyes, while patients with DENV-2 infection had joint discomfort and a decreased platelet count. Adjusted Relative Risk [aRR] = 1.74) and SD (aRR = 2.1) were linked with DENV-1 and DENV-2, respectively, after controlling for possible confounders. Genetic testing revealed mainly two strains: DENV-1 genotype 1, and DENV-2 cosmopolitan 2. Among Singapore's adult dengue patients, infecting dengue serotype and potentially genotype may have a significant effect in illness severity.

Laurent Thomas et. al, (2014): Each of Martinique's four recent dengue outbreaks was defined by the preponderance of one or two serotypes. We examined the correlation between dengue serotype and illness severity in a retrospective database study. A total of 715 patients, ranging in age from 14 to 91, were assessed in the adult emergency department between 2005 and 2010 for dengue fever, with a male/female ratio of 0.87. In this study, DENV-4 infections were more likely to present with a milder clinical picture than in previous studies. At the critical stage of dengue sickness, secondary infections of DENV-2 were most often seen, with evidence of plasma leakage. Females were more vulnerable to DENV-1 infections, which often resulted in intermediate-severity illness without plasma leakage. Serotypes vary in virulence, independent of the host's immunological

condition, based on these findings. There was a higher risk of plasma leakage in patients with subsequent DENV-2 infections, however.

3. METHODOLOGY

Participants in this research were clinically suspected dengue patients that were sent to the VRDL at the Bangalore Medical College and Research Institute (BMCRI), Bengaluru, India from January to December 2017. Within 30 minutes after collection, blood samples were transferred to the laboratory in a marked v acutainer tube (SST advanced; Cat no. BD 367954; Beckton Dickinson, USA). It was kept at 4°C for up to 48 hours after collection. They were kept in numerous vials at -80°C for a long time and only thawed once for testing purposes.

A subset of the sample (n=331) was submitted to further serological and Real-time PCR tests to ensure its viability. In order to get a complete picture of a patient's medical history—including the length of time they had been unwell and the severity of their symptoms—details such as the patient's demographics, as well as their medical history—were gathered for all instances. A regression model was built using clinical indications and symptoms.

Reverse transcriptase PCR was used to amplify the isolated RNA. In accordance with the previously mentioned procedure, we carried out standard PCR testing. To see the primer sequences, please refer to Table 1. We used the Master Cycler Nexus gradient apparatus for the reverse transcription PCR testing (Eppendorf; US). Cyclists faced the following challenges. At 95°C for 10 minutes, the first denaturation was carried out. Denaturation at 95°C for 35 cycles followed by annealing at 60°C, then extension at 72°C were performed. The remaining 5 minutes of the extension were spent at 72°C. In a 2 percent agarose gel, the PCR products could be seen.

Table 1: Primers Used for Conventional PCR and Sequencing

Sl. No	Oligo Name	Sequence (5′ to 3′)
1	5A	AGTTGTTAGTCTACGTGGACCGACA
2	5B	CCCCGTAACACTTTGATCGCTCCATT
3	5C	CGCCACAAGGGCCATGAACAG
4	5D	GCACATGTTGATTCCAGAGGCTGTC
5	5E	GTTTCCAATCCCATTCCTGAATGTGGTGT

Commercial equipment was used for the PCR gel purification and sequencing (Eurofins; Bengaluru). Sanger sequencing was used to analyses the PCR product. Finch TV (V 1.4) software was used to do a quality assessment and trimming of the sequence. As a result of the BLAST query, it was determined that the sequences were identical. In order to receive a GenBank entry number, we submitted all sequences that exceeded 200 bp after trimming.

4. ANALYSIS

The State Level VRDL, BMCRI received a total of 6126 clinically suspected dengue samples for Dengue IgM testing in 2017. The ELISA results of 1986 samples (32.4% of the total) indicated the presence of Dengue IgM antibodies (NIV kit). The NIV ELISA kit indicated that 105 of the 331 samples chosen as a subgroup by stratified random selection from the community were positive for Dengue IgM antibodies. There was no statistically significant difference in age, gender, or sample positivity between the whole population and the sampling subgroup (X2 >0.05). Table 2 provides a clinical and demographic description of the patients examined in this investigation. Dengue infection could not be predicted using a logistic regression model.

Table 1: Demographic and Clinical summary

Parameter	Statistical Summary
Age	27.88 ± 15.19 years
Gender	133 Female / 198 Male
Duration of Illness	5.17 ± 4.28
Fever	98.4 %
Nausea	14.5%
Vomiting	26.5%
Rash	0.04%
Abdominal Pain	18.7%
Myalgia	51.5%
Arthralgia	41.9 %
Headache	55.5%
Retro orbital pain	0.07%
Bleeding	0.012%
Previous history of infection	0.04%

It was only in 53,5 percent of instances that the IgM ELISA and the IgM fast test were in accord. The correlation between the NS1 ELISA and the NS1 Rapid test, on the other hand, was shown to be greater (89.2 percent). Concurrence rates between genders and ages were determined to be statistically insignificant. In 48.94% of the cases, a fast test for IgG revealed a positive result (Data summarized in Figure 1).

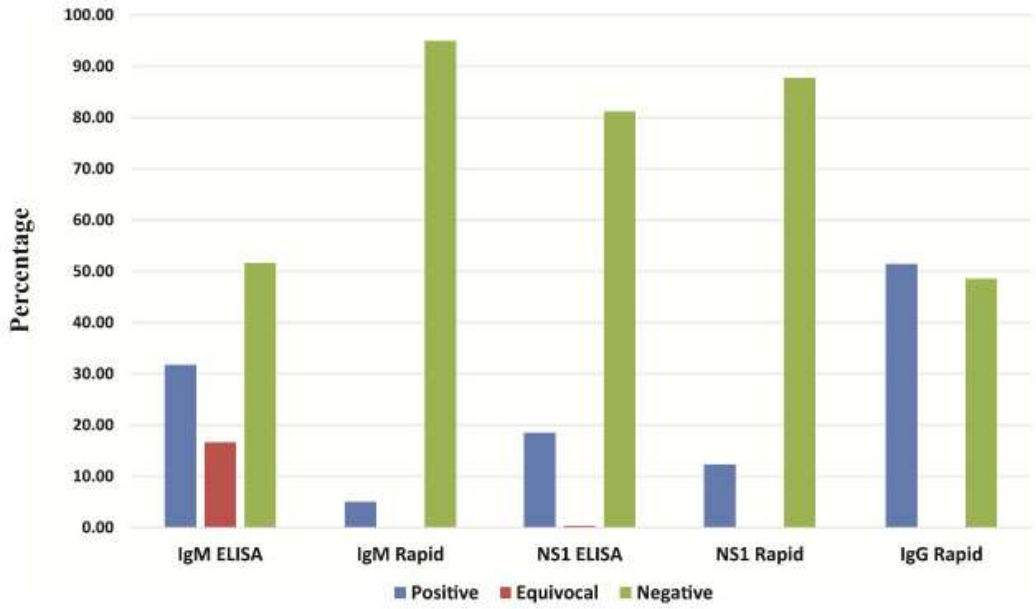


Figure 1: Results of various serological tests (by percentage)

When the fever started, the NS1 ELISA was positive 4.5 days later (95 percent CI; 3.87-5.12) Similar to the NS1 findings, real-time PCR was shown to be positive after 4.48 days (95 percent confidence interval, 3.77-5.18). At 5.39 days, the IgM ELISA was positive (95 percent CI; 4.69-6.08).

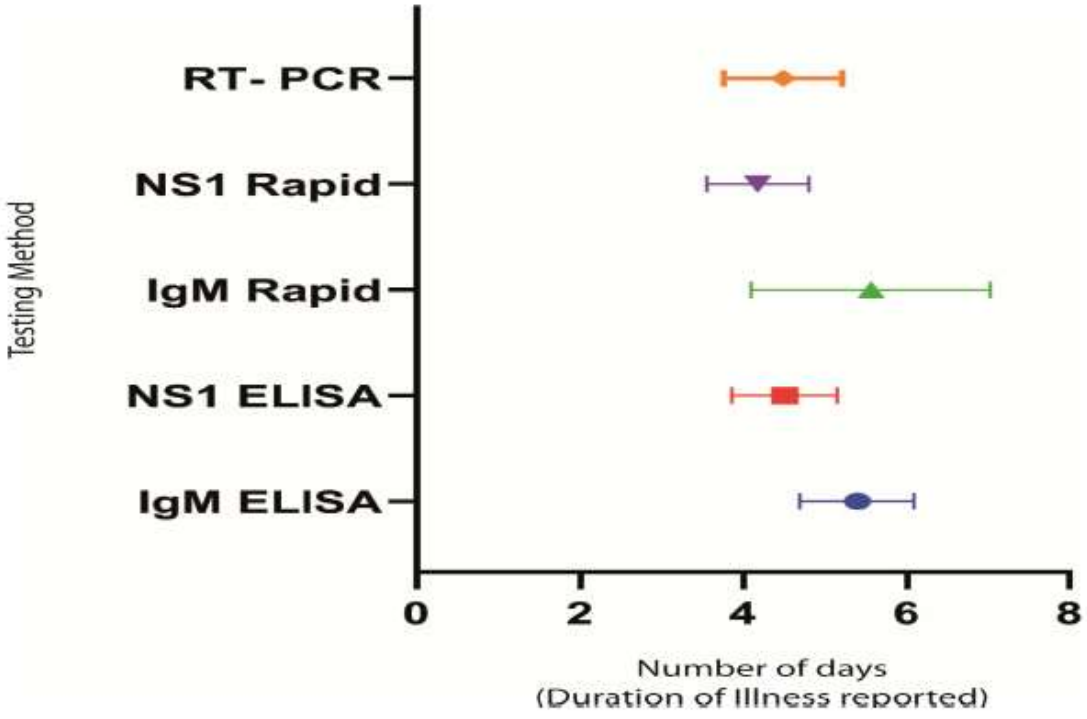
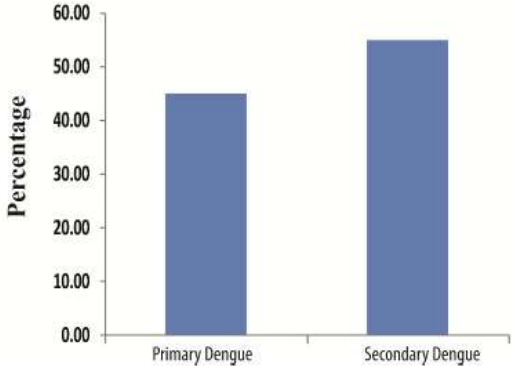
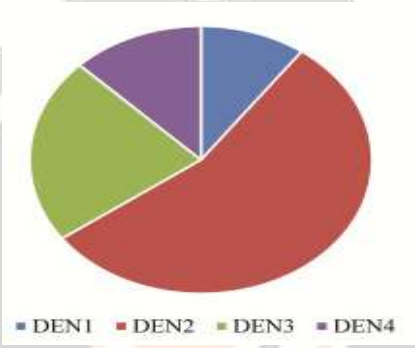


Figure 2: Mean and Standard deviation of tests showing positivity by number of days after onset of fever. The Mean with 95% Confidence interval is shown.

Primary or secondary infection was determined based on the IgG/IgM ratio. In addition, 43.96 percent of the cases were found to be primary dengue serology, with the remainder being secondary.



(A)



(B)

Figure 3: A) Distribution of primary and secondary dengue cases. B) Distribution of Dengue Serotypes as assessed by RT PCR testing.

More than a third of the 112 samples examined by real-time RT-PCR were positive. RT-PCR identified all four serotypes, with the DEN-2 subtype being the most prevalent (Fig 3B). Only 13 of the samples tested by conventional PCR were positive. BLAST analysis showed that all of the sequences were from Dengue virus after analyzing them. More over half of the 13 samples tested were DEN-2 serotypes, with three each of DEN-1 (den-1), DEN-3 (den-3), and DEN-4 (den-4).

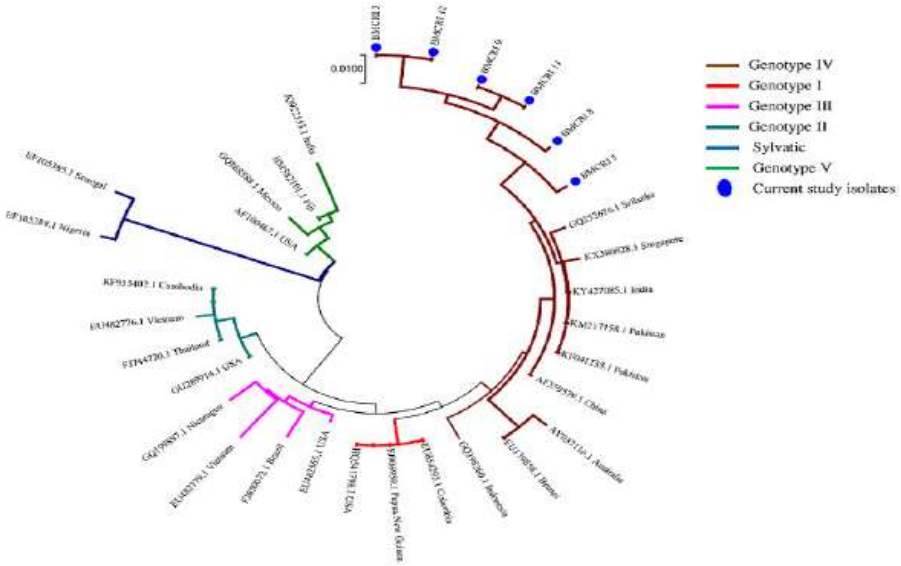


Figure 4: Phylogenetic analysis

5. CONCLUSION

Multiple serological and molecular analyses of dengue probable cases show that no one test can be utilized as a diagnostic marker on its own. In the early stages of infection, the clinical signs are not distinct enough to offer a lead. The number of days from the beginning of fever is an important factor in determining the most appropriate diagnostic test. Genotype IV, the most frequent dengue serotype in this area, was shown to be present in the DEN-2 serotype. Dengue serotypes in a particular location should be tracked using molecular diagnostics.

6. REFERENCE

1. Jeyanthi Suppiah et. al, (2018)," Clinical manifestations of dengue in relation to dengue serotype and genotype in Malaysia: A retrospective observational study" https://doi.org/10.1371/journal.pntd.0006817
2. Creuza Rachel Vicente et. al," Serotype influences on dengue severity: a cross-sectional study on 485 confirmed dengue cases in Vitória, Brazil" Vicente et al. BMC Infectious Diseases (2016) 16:320 DOI 10.1186/s12879-016-1668-y
3. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, Lye DC, Ng LC, Leo YS. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. Am J Trop Med Hyg. 2015 May;92(5):999-1005. Doi: 10.4269/ajtmh.14-0628. Epub 2015 Mar 30. PMID: 25825386; PMCID: PMC4426593.
4. Thomas L, Najioullah F, Besnier F, Valentino R, Césaire R, Rosine J, Cabié A; Working Group on Dengue. Clinical presentation of dengue by serotype and year of epidemic in Martinique. Am J Trop Med Hyg. 2014 Jul;91(1):138-45. Doi: 10.4269/ajtmh.13-0595. Epub 2014 May 27. Erratum in: Am J Trop Med Hyg. 2014 Oct;91(4):862. Césaire, Jacques Rosine Raymond [corrected to Césaire, Raymond]; [corrected to Rosine, Jacques]. PMID: 24865681; PMCID: PMC4080553.
5. Simmons CP, Farrar JJ, Nguyen vV, Wills B. Dengue. N Engl J Med. 2012; 366:1423–1432.
6. Abchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, Jiwariyavej V, Dulyachai W, Pengsaa K, Wartel TA, Moureau A, Saville M, Bouckenooghe A, Viviani S, Tornieporth NG, Lang J. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomized, controlled phase 2b trial. Lancet. 2012; 380:1559–1567
7. Villar LA, Rivera-Medina DM, Arredondo-García JL, Boaz M, Starr-Spires L, Thakur M, Zambrano B, Miranda MC, Rivas E, Dayan GH. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9–16 years old: a randomized, controlled, Phase II trial in Latin America. Pediatr Infect Dis J. 2013; 32:1102–1109.
8. Thomas L, Moravie V, Besnier F, Valentino R, Kaidomar S, Villain Coquet L, Najioullah F, Lengellé F, Césaire R, Cabié A. Working Group on Dengue Clinical presentation of dengue among patients admitted to the adult emergency department of a tertiary care hospital in Martinique: implications for triage, management, and reporting. Ann Emerg Med. 2012; 59:42–50.
9. Najioullah F, Combet E, Paturel L, Martial J, Koulmann L, Thomas L, Hatchuel Y, Cabié A, Cesaire R. Prospective evaluation of nonstructural 1 enzyme-linked immunosorbent assay and rapid immunochromatographic tests to detect dengue virus in patients with acute febrile illness. Diagn Microbiol Infect Dis. 2011; 69:172–178.
10. Srikiatkhachorn A, Rothman AL, Gibbons RV, Sittisombut N, Malasit P, Ennis FA, Mannitya S, Kalayanarooj S. Dengue–how best to classify it. Clin Infect Dis. 2011; 53:563–567.
11. Halstead SB. Dengue: the syndromic basis to pathogenesis research. Inutility of the 2009 WHO case definition. Am J Trop Med Hyg. 2013; 88:212–215.
12. Farrar JJ, Hien TT, Horstick O, Hung NT, Jaenisch T, Junghanns T, Kroeger A, Laksono IS, Lum L, Martinez E, Simmons CP, Tami A, Tomashek KM, Wills BA. Dogma in classifying dengue disease. Am J Trop Med Hyg. 2013; 89:198–201.
13. Halsey ES, Marks MA, Gotuzzo E, Fiestas V, Suarez L, Vargas J, Aguayo N, Madrid C, Vimos C, Kochel TJ, Laguna-Torres VA. Correlation of serotype-specific dengue virus infection with clinical manifestations. PLoS Negl Trop Dis. 2012;6: e1638.
14. St John AL, Abraham SN, Gubler DJ. Barriers to preclinical investigations of anti-dengue immunity and dengue pathogenesis. Nat Rev Microbiol. 2013; 11:420–426.