Original Research Article

Is the clinicopathological profile of dengue syndrome changing?: a 6 year study of different epidemics at a tertiary care center in India

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ABSTRACT

Background: As an arthropod-borne viral disease, dengue epidemics has created much public health hazards in tropical countries. In the national capital of India, there has been more than six epidemics. Dengue remains a notifiable disease in India. It is important that we understand the changing clinicopathological profile of this viral infection to prepare ourselves better for any impending future epidemic. This study done at a tertiary care center looks into the recent epidemics to understand the changing trends in the disease profile in two phases of three years each. Further, authors also assessed the utility of the tourniquet test in dengue syndrome.
Methods: 260 serologically confirmed patients were recruited in two phases of the study. We evaluated them for clinical, epidemiological and pathological profile of dengue and trends in haematological, biochemical and radiological parameters during the course of the disease and its correlation with the severity of the disease.
Results: Fever, headache, body ache, pharyngeal and conjunctival congestion, rhinitis, rash and diarrhea incidences were similar in both epidemics. Lymphadenopathy was seen in 18(18%) patients compared to 40(25%) in the second epidemic. More number of hemorrhagic manifestations with GI and retinal bleed was seen compared to earlier epidemics, which had higher liver involvement.
Conclusions: Although the presentation of the disease has not shown a drastic change over the last decades, the severity varies depending on the predominant serotype. Patients with evidence of serositis, increased activated lymphocytes has a longer course in disease with poorer outcome. Early rising haematocrit, ALT/AST ratio, LDH and deranged coagulation parameters are important tools in triaging patients for priority of urgent care and hospitalization in a scenario of a future outbreak.

Keywords: Dengue, Hemorrhagic fever, India, Dengue/ Dengue haemorrhagic fever, Dengue virus

INTRODUCTION

Dengue is the most important arthropod-borne viral disease of public health significance. Unfortunately, a vast majority of this disease remains asymptomatic, therefore actual numbers remain under-reported and misclassified. One of the estimate indicates 390 million (95% credible interval 284-528) dengue infections per year, of which only a quarter manifest apparently (with any level of disease).[1] The number of cases reported by different member countries in WHO has increased from 2.2 million in 2010 to over 3.34 million in 2016. Dengue hemorrhagic fever (DHF) has been reported from 60 different countries with 4,50,000 cases of DHF and 100 million cases of dengue fever (DF) annually, caused by all four serotypes.[2] Owing to its potential of rapid transmission, dengue infection is now a leading public health problem in India. Of an estimated 500,000 cases of

DHF/DSS requiring hospitalization each year, roughly 5% die according to WHO statistics. Regional distribution and serotypes of dengue are mentioned elsewhere.[2,3] The last few decades have seen multiple outbreaks and epidemics of DF or DHF from across the country.

In India, Delhi has experienced six outbreaks of dengue fever since 1967 with the last two major epidemics in 1996 and 2003.[2,3] World health organization (WHO) reported a total number of 10,000 laboratory confirmed cases of dengue infection in the epidemic of 1996, and 2185 cases in 2003 outbreak.[3-5] Dengue infection is now a notifiable disease in India, with all the four serotypes of dengue being prevalent all over the country. During 1996 epidemic, Den-2 was the main serotype isolated, while in 2003 outbreak both DEN-2 and DEN-3 were isolated.[5]

Dengue virus infection produces a wide spectrum of signs and symptoms varying from subclinical disease to severe DHF or dengue shock syndrome (DSS), which is on the increase.

Rapid diagnosis of dengue infection is paramount to aid clinician to implement the required therapy and prevent complication like DHF and DSS. Four basic serological tests have been used namely haemagglutination inhibition (HAI), complement fixation (CFT), neutralization tests (NT) and enzyme immune assay (EIA) for detecting IgM and IgG antibodies.[6] The gold standard for diagnosis of recent infection is by detection of virus in patient's sera either by cell culture or PCR. Keeping in view the complications and fatality rates associated with dengue infection, a rapid or accurate method for the diagnosis of dengue fever is important for proper patient management. The RT-PCR following its introduction has been evaluated in many studies and is found to be useful in the rapid diagnosis of dengue viral infection.[7-9]

The clinical profile that was seen in earlier epidemics has changed over the years and various atypical features are encountered in day to day clinical practice. The trends in simple haematological and biochemical parameters are of utmost importance to evaluate the severity of the disease and can be used as a predictor of disease severity. The occurrence of serositis in dengue is well known. However, its connection with disease severity still is an enigma. The RT-PCR following its introduction has been evaluated in many studies and is found to be useful in the rapid diagnosis of dengue viral infection. Its accuracy to correctly detect dengue virus during the first four days of viremia may prove beneficial in regard to the management and prognosis of the dengue infection. There have been many studies on dengue both in India and abroad, but none have studied so many aspects of the clinical profile and haematological parameters in such details. Therefore, this study was planned to study the clinical profile and haematological parameters of dengue syndromes and note any changing patterns in clinical presentation compared to past epidemics.

The utility of simple and quick test like the tourniquet test may aid in the diagnosis of dengue syndromes bedside and help a clinician who may not be privileged with the latest test for diagnosis of dengue. Several studies have shown little correlation between thrombocytopenia and positivity of tourniquet test but none have shown its utility in diagnosis of dengue syndrome. Hence, this study was also designed to validate the use of tourniquet test as a diagnostic test for dengue syndrome.

Aims and objectives

- To study the clinical, epidemiological and pathological profile of dengue syndromes in two phases of three-year epidemics and note any changing patterns between them and as compared to past epidemics.
- To evaluate the trends in haematological, biochemical and radiological parameters during the course of the disease and its correlation with the severity of the disease.
- To establish the utility of the tourniquet test in dengue syndromes.

Informed consent was obtained from each individual & patients were evaluated based on a detailed history as per a pre-defined proforma.

METHODS

The present study was performed in the Department of Internal Medicine Army Hospital (Research and Referral) Delhi Cantt, in two phases of 3 year each. The first phase (PHASE I) included the period from 01 Oct 2009 to 31 Mar 2012 and the second phase (PHASE II) subsequently between 01 April 2016 and 31 January 2019.

The PHASE I included 100 patients of any age group confirmed as having dengue infection (confirmed by serologically positive or RT-PCR) attending the outpatient department or admitted in medical /pediatric ward of Army hospital (R and R) Delhi. The PHASE II (confirmed by serology or RT PCR), included a total 160 patients, when Delhi faced another epidemic of dengue. Our hospital caters for referred patients, who mainly consists of serving Armed Forces personnel and their dependent family members. All good clinical practice guidelines were followed and the hospital ethics committee approved both phases of the study.

Inclusion criteria

- All age groups.
- Only serologically positive or RT-PCR positive cases were taken for the final data analysis (only 30 cases taken for RT-PCR in both studies).
- Case definition criteria for dengue fever: as per WHO guidelines 2009.[10]

Exclusion criteria

- Patients refusing to give consent.
- Patients of fever who have tested negative for dengue infection by serology.

Statistical analysis

The data was presented in terms of descriptive statistics (range, mean, standard deviation) for quantitative variables and frequency, percentage for category variables was presented in each group. For determining the statistical significance, the statistical method for quantitative variables were used as 2 sample student "t" test for normally distributed variables and in cases when quantities variables did not follow a normal distribution, the non- parametric Mann-Whitney statistical test was applied. For categorical variables, the statically test were used as chi-square / Fischer exact. The level of statistical significance has taken as $p <$ than or $= 0.05$. Data was analyzed by using SPSS Statistics version 16.

The study was compared with the study conducted in other studies in India and abroad which have studied the clinical and haematological profiles in dengue syndromes.

RESULTS

Demographic profile

The 100 cases of dengue patients studied ranged in the age from 7 to 78 years in the PHASE I compared to 160 cases in the PHASE II. There was no significant association between the various age groups and the occurrence of dengue syndrome (p-value = 0.417) in the first epidemic.
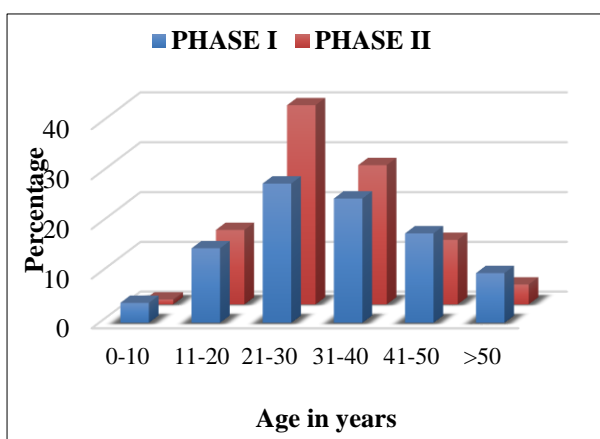

Figure 1: Age distribution in both epidemics.

Males were more affected in both epidemics. Preponderance was mostly due to the patient being drawn from the Armed Forces only. Age and sex distribution for both epidemics are mentioned in (Figure 1 and 2).

Clinical profile of the patients

The most common clinical manifestation was fever seen in all 100 (100%) patients along with headache and body ache, observed in all patients in both epidemics. The mean duration of fever was 6.5 days during PHASE I (2009 to 2012) while it was 6.2 days in the PHASE II (2016-2019) period (Table 1).
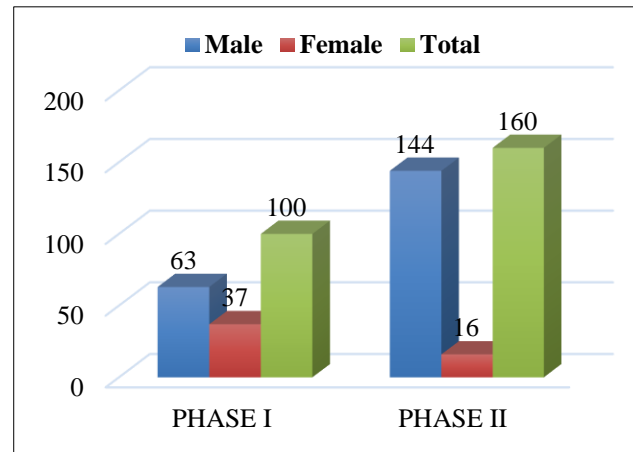

Figure 2: Sex distribution in both epidemics.

Table 1: Clinical features.

Clinical manifestation	2009/2012 (n=100)	2016/2019 (n=160)
Fever	100(100%)	160(100%)
Headache	100(100%)	160(100%)
Body ache	100(100%)	160(100%)
Pharyngeal congestion	80(80%)	144(90%)
Rhinitis	55(55%)	96(60%)
Skin Rash	28(28%)	44(28%)
Diarrhoea	16(16%)	21(12.8%)
Conjunctival congestion	30(30%)	50(31.5%)
Lymphadenopathy	18(18%)	40(25%)

In older studies of hemorrhagic fever reported from Thailand, headache, body ache, rhinitis and skin rash incidence were much lower.[11,12]

Bleeding manifestations seen in DSS and DHF Grade II was significantly higher (p value <0.001) as compared to DF in both phases of the study.

Fifteen patients had UGI haemorrhage of whom two had recurrent haemorrhage in the PHASE II. Seven out of fifteen patients had documented erosive gastritis, as seen on upper GI endoscopy. In addition, the occurrence of Retinal bleed was significantly higher (7.8%) in this phase of dengue. The trend of the other sites of bleed remained the same in the two phases (Table 2).

Table 2: Bleeding manifestation in dengue cases.

Bleeding manifestations	No. of cases (2009/12) (n=61)	No. of cases (2016/19) (n=64)
Cutaneous	19(31.15%)	18(28.12%)
Epistaxis	17(27.9%)	21(32.81%)
Bleeding gums	16(26.23%)	17(26.56%)
Haematemesis	05(0.08%)	15(23.43%)
Haematuria	02(0.03%)	01(1.56%)
Menorrhagia	01(0.02%)	02(3.12%)
Retinal hemorrhage	01(0.02%)	5(7.81%)

Haematological profile of the patients

Haemoglobin level

The mean hemoglobin was 12.38gm/dl in the first phase of this study compared to 12.46gm/dl in the second phase with similar nadir values.

Haematocrit

All patients showed a rise of haematocrit out of proportion to the hemoglobin; however, the haematocrit was raised significantly in 28% patients in the first epidemic compared to 32% in the second epidemic.

The mean haematocrit values were significantly higher in DHF patients compared to DF patients (p value <0.001). This rise was not seen on the first two days of fever; however, the haematocrit started to rise on day 3-4, peaked on day 5-6 and normalized by day 10-12 in both first and second phases.

Total leucocyte count

The mean TLC in the PHASE I of our study was 5337 (range 700-28,300/cu mm) which was similar in the PHASE II.

The occurrence of lymphocytosis was seen in 6% of patients while leucopenia was seen in 40% of patients. This was most marked between days 3-6 of fever. In the PHASE II of our study, 6% had lymphocytosis and turk reaction cells were seen in 15% cases. Inverse relationship of PCV with platelet count, a hallmark of Dengue syndrome was observed in both phases of the study.

Table 3: Coagulation profile in both phases.

Parameter	Phase 1 (n=100)	Phase 2 (n=160)
Elevated PTT	2	17
Decreased fibrinogen	6	6

Coagulation profile

PT and PTT

Elevated PTT was observed in significantly higher number of patients in the second epidemic as shown below (Table 3).

Platelet count

The mean platelet count in the first part of the study was 73,400/µL (range 14,000- 1, 70,000/ µL). This was similar in the second epidemic with the mean value being 71,700/µL (range 14,000- 1, 70,000/ µL). The difference between DF and DHF/DSS was statistically significant in both epidemics (p value <0.001) on application of N-Par test (Mann-Whitney test). The hemorrhagic manifestation was characteristically seen when the platelet count was below 20,000/ µL which suggested that there was strong correlation between the bleeding manifestation and the degree of thrombocytopenia (p value <0.001).

Biochemical profile of dengue patients

In the 2009 -12 Phase, 40% of the patients had deranged liver function in the form of either elevated serum bilirubin or transaminases (AST or ALT) or both. Eight patients had clinically detectable jaundice. The mean level of AST and ALT were found to be elevated in DHF/DSS in both epidemics. However, in the second epidemic only five patients suffered from elevated bilirubin levels.

No patient with DF had hypoproteinemia in the first epidemic; but 10 patients (16%) suffered in the second epidemic.

Serum lactate dehydrogenase (LDH) done in all patients from the both phases of the study with a normal range between (250-400).There were 22 patients in the first epidemic and 38 in the second epidemic who had LDH levels elevated significantly (more than 40% of the upper limit). The mean LDH levels were significantly elevated in DHF/DSS as compared to DF (p value <0.001).

Activated lymphocytes

We did peripheral blood smear in all patients for the detection of the percentage of activated lymphocytes. The levels of activated lymphocytes were similar in both epidemics.

Serositis is an important feature of capillary leak. The first epidemic saw 12% patients having Rt sided pleural effusion while 18% had bilateral pleural effusion. Between 2016 and 2019 17% patients had Rt sided pleural effusion; only 3% patients had bilateral pleural effusion. Ascites was seen in 12% patients and 5% had pericardial effusion in the first epidemic (Table 4).

The occurrence of serositis (pleural effusion and ascites) was significantly high in DHF/DSS as compared to DF in both epidemics using frequency table -chi square test (p value <0.001).

Table 4: Incidence of serositis in two epidemics.

Parameters		2009-12	2016-2019
Pleural effusion	Only Rt side PE 12%	17%	
	Bilateral PE 18%	03%	
Ascites		12%	11%
Pericardial effusion		05%	04%

Authors did the tourniquet test in all 260 patients from both epidemics. No significant correlation could be established between thrombocytopenia, bleeding manifestation and positive tourniquet test (p value =0.94) in both phases. In both epidemics, more than 60% of patients had IgM positive.

DISCUSSION

Dengue is now emerging as the most important medically important arboviral infection, with 40% of global population living at risk of this infection. Outbreaks are reported from almost all parts of tropics and subtropics with higher morbidity and mortality.[13] After the 1996 outbreak in Delhi, these were the two major epidemics that occurred in the same region.[14] In both phases our study, 23% patients were in Grade I, 54% in Grade II and 23% patients in Grade III/IV DHF.

Like previous outbreaks, this epidemic also began following the monsoon season, when the climatic factors (temperature and humidity) became conducive for Aedes spp. breeding. The post-monsoon dengue outbreak is a regular feature of dengue activity in Indian subcontinent.[7,15]

The number of serologically positive cases were only 8% during the monsoon season of in both phases of our study and no dengue serology positive cases were seen during the pre-monsoon period. The reason for this trend of dengue infection was optimum temperature with high relative humidity and abundant stocks of freshwater reservoirs generated due to rain. The clinical presentation and profile was significantly more severe in the earlier epidemics (1990-2003) which could be attributable to more pathogenic serotype and late presentation of the patient to the care -giver. This is also attributable due to poor understanding and awareness of the disease as compared to the recent epidemics. It was also evident from the severity of symptoms, increased incidence of serositis and prolonged period of recovery with greater morbidity post recovery. A large number of patients (39%) presented with DHF (including DSS) in our study, which is the severe form of the disease in terms of morbidity and mortality. These findings are in accordance to the previous studies from Delhi, which also recorded

an escalation in number DHF cases (52-60%) during the 1996 epidemic.[16,17] The mean age of distribution in our study was 33.29 years reflecting that majority of patients infected were adults. This is in accordance to previous epidemics and studies from Singapore and Indonesia.[18,19] This shift in the affected age group is attributable to changes in locations where disease transmission takes place.[20,21]

Tourniquet test did not correlate well with other bleeding manifestations and currently WHO no longer recommends tourniquet test as essential for the diagnosis of DHF.[10] No deaths occurred in patients with platelet counts lesser than 50,000/ µL. However, a significant correlation was present between the proportions of bleeding manifestation among the patients with mild, moderate and severe thrombocytopenia.

Haematocrit is was shown as an important predicting factor for severity of infection; however, documenting hemoconcentration may not be a good indicator for diagnosis and monitoring of fluid unless pre-illness haematocrit is known, particularly because there is a high prevalence of anemia in the Indian population. Pre-illness haematocrit if known helps for accurate comparison. This will help the clinician in early diagnosis of DHF/DSS and prognosticating the outcome. The trends in the hemoglobin, TLC, DLC platelet count show no change in comparison to the previous epidemics. However, leucopenia seen early in the disease points towards dengue fever as one of the diagnosis in a patient who presents with fever.

In this study, 40% of the patients had significant deranged liver function (either elevated serum bilirubin or raised serum transaminases or both).

LDH activity determines as an index of liver damage.[22] Our study gave valuable information regarding LDH as an important marker for the severity of disease. There were concomitant deranged liver function in 33(68%) out of 48 patients who had elevated LDH in our study. The activated lymphocyte count was more than 40% in all patients. These parameters were invariably high in DSS and majority of cases of DHF. Patients with high activated lymphocyte count had evidence of capillary leak, early bleeding manifestation and a complicated course of illness which included an ICU stay.

Occurrence of polyserositis in dengue fever is an important indicator of severity of the disease. Such a patient needs to be evaluated thoroughly, treated meticulously and monitored closely to prevent mortality. The presence of capillary leak syndrome (raised haematocrit, thrombocytopenia and serositis) seen exclusively in DHF/DSS is a marker of the severe form of disease and warrants ICU care with fluid replacement and life support measure as it is associated with high mortality.

RT-PCR is a sensitive and specific method for the detection of dengue virus in the early phase but it may not prove to be helpful after the antibodies appear in the blood. For the diagnosis of samples taken after five days, serology remains the mainstay.

CONCLUSION

Dengue continues to be a major vector born disease in India and the country faces an epidemic almost every year. Over the past two decades, the presentation of the disease has not shown a drastic change. However, the severity may vary depending on the predominant serotype. We found that the current trend of the disease has evolved leading to more number of hemorrhagic manifestations with GI and retinal bleed as compared to the earlier epidemics, which had higher liver involvement. In addition, hypoproteinaemia are now been seen even in DF. The early rising haematocrit, ALT/AST ratio, LDH and deranged coagulation parameters are important tools that could be used to assess the severity and aid in triage of patients for priority of urgent care and hospitalization in a scenario of an outbreak. Though we have now become wiser regarding the evolution of the disease and how to manage it effectively, a lot remains to be learnt in order to reduce the mortality every year in this viral disease.

REFERENCES

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7.
2. Guzman MG, Kouri G. Advances in dengue diagnosis. Clini Diagnostic Lab Immunol. 1996;3(6):621-7.
3. Vaughn DW. Serological investigation of a febrile outbreak in Delhi, India, using a rapid immunochromatographic test. J Clin Microbiol. 1998;36(9):2795-6.
4. Tripathi BK, Gupta B, Sinha RS, Prasad S, Sharma DK. Experience in adult population in dengue outbreak in Delhi. J Assoc Physici India. 1998;46(3):273-6.
5. Kumar M, Pasha ST, Mittal V, Rawat DS, Arya C, Agarwal N, et al. Unusual Emergence of Guate 98-like Molecular Subtype of DEN-3 during 2003 Dengue Outbreak in Delhi. Dengue Bull. 2004;8:457-61.
6. Vijayakumar TS, Chandy S, Sathish N, Abraham M, Abraham P, Sridharan G. Is dengue emerging as a major public health problem? Indian J Med Res. 2005;121(2):100-7.
7. Thein S, Aung MM, Shwe TN, Aye M, Zaw A, Aye K, et al. Risk factors in dengue shock syndrome. Am J Trop Med Hygiene. 1997;56(5):566-72.
8. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis. 2000;181(1):2-9.
9. Guzman MG, Kouri G, Valdes L, Bravo J, Alvarez M, Vazques S, et al. Epidemiologic studies on Dengue in Santiago de Cuba, 1997. Am J Epidemiol. 2000;152(9):793-9; discussion 804.
10. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: World Health Organization. Geneva, Switzerland; 2009. Available at: https://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf. Accessed 10 October 2019.
11. Halstead SB, Nimmannitya S, Margiotta MR. Dengue d chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. Am j trop med hygiene. 1969;18(6):972-83.
12. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hygiene. 1969;18(6):954-71.
13. Ahmed S, Arif F, Yahya Y, Rehman A, Abbas K, Ashraf S, et al. Dengue fever outbreak in Karachi 2006--a study of profile and outcome of children under 15 years of age. JPMA J Pak Med Assoc. 2008;58(1):4-8.
14. Sharma SN, Raina VK, Kumar A. Dengue/DHF: an emerging disease in India. J Commun Dis. 2000;32(3):175-9.
15. Morens DM, Rigau-Perez JG, Lopez-Correa RH, Moore CG, Ruiz-Tiben EE, Sather GE, et al. Dengue in Puerto Rico, 1977: public health response to characterize and control an epidemic of multiple serotypes. Am J Trop Med Hygiene. 1986;35(1):197-211.
16. Anuradha S, Singh NP, Rizvi SN, Agarwal SK, Gur R, Mathur MD. The 1996 outbreak of dengue hemorrhagic fever in Delhi, India. South Asian J Trop Med Public Health. 1998;29(3):503-6.
17. Aggarwal A, Chandra J, Aneja S, Patwari AK, Dutta AK. An epidemic of dengue hemorrhagic fever and dengue shock syndrome in children in Delhi. Ind Pediatr. 1998;35(8):727-32.
18. George R, Lam SK. Dengue virus infection--the Malaysian experience. Ann Acad Med, Singapore. 1997;26(6):815-9.

19. Goh KT. Dengue--a re-emerging infectious disease in Singapore. Ann Acad Med, Singapore. 1997;26(5):664-70.
20. Singh NP, Jhamb R, Agarwal SK, Gaiha M, Dewan R, Daga MK, et al. The 2003 outbreak of Dengue fever in Delhi, India. Southt Asian J Trop Med Public Health. 2005;36(5):1174-8.
21. Sumarmo. Dengue haemorrhagic fever in Indonesia. South Asian J Trop Med Public Health. 1987;18(3):269-74.
22. Kurane I, Takasaki T. Dengue fever and dengue haemorrhagic fever: challenges of controlling an enemy still at large. Rev Med Virol. 2001;11(5):301-11.