SEROLOGICAL AND MOLECULAR DIAGNOSIS OF EMERGING DENGUE INFECTION AT A TERTIARY CARE HOSPITAL IN KANPUR, INDIA

Deepika S[1], Sujatha R*, Nidhi Pal[3], Anil K[4], Deepak S[5]

1. PhD scholar, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)
2. Prof and Head, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)
3. Tutor, Department of Microbiology, Govt Institute of Medical Sciences, Greater noida
4. Assistant Professor, Central Research Laboratory, Rama Medical College Hospital and Research Center Kanpur (India)
5. Tutor, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)

*Abstract :*Dengue is a worldly common mosquito-borne disease. Dengue fever presents clinical characteristics similar to other febrile illness. Thus laboratory diagnosis is important for adequate management of the disease. **Material and Methods:** Clinical data and blood samples were collected from suspected patients with dengue who attended Rama Hospital. Serologic methods; ELISA test were performed to detect NS1, IgM and IgG and real-time PCR were performed in recent acute dengue illness serum samples for molecular typing. **Result:** Of 400 samples, 284 were diagnosed positive. 96(24%) were indicate sero-positivity with NS1 antigen by ELISA. IgM and IgG were diagnosed in 111(27.75%) and 150(37.5%) respectively. The major clinical presentation was a febrile illness, myalagia, low blood pressure, low hemoglobin count, thrombocytopenia and elevated liver enzymes. Majority of Dengue positive cases were reported in post monsoon season. In recent acute dengue virus infection cases, 06 and 04 cases were positive for DEN-2 and 04 DEN-3 respectively. The median of Ct value of DEN 2 and DEN 3 were 20.4 and 22.7 respectively**.** No DEN-1 and DEN-4 were found in this study. **Conclusion:** According to our study only DEN 2 and DEN in Kanpur during the study period. But the epidemiology of dengue virus and its prevalent serotypes are changing. Therefore continuous detection of prevalence of different serotypes is necessary for monitoring the severity of different serotypes and its management.

Keywords: Dengue fever, DEN-1, DEN-2, RT-PCR.

I. INTRODUCTION

Dengue is a worldly common mosquito-borne disease in tropical and subtropical areas[1]. Viral transmission occurs most frequently through the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Four viral

serotypes were identified, DENV-1, DENV-2, DENV-3 and DENV-4[2]. Dengue clinical features vary from febrile illness to the severe dengue hemorrhagic fever (DHF) that can lead to shock and death[3,4].

Thus, precise and early diagnosis is extremely relevant for adequate management of the disease. There are many diagnostic methods to detect an acute dengue infection, including serological methods, Real time PCR, viral genome sequencing, and viral isolation[4,5,6]. Of these serological methods i.e. rapid card test for NS1 and ELISA is common methods used in service laboratories for early detection of Dengue[7]. The epidemiology of dengue virus and its prevalent serotypes has been ever changing. The epidemic at Kanpur during 1968 was due to DV-4 and during 1969 epidemic, both DV-2 and DV-4 were isolated[8,9].

Hence this study was conducted to study serological diagnosis and serotyping of Dengue fever with ELISA and multiplex PCR respectively.

MATERIALS AND METHODS

The prospective study was carried out in Microbiology department of Rama Medical College Kanpur from the period November 2016 to June 2018. Ethical clearance was taken from institutional ethical committee. Four hundred (400) clinically suspected patients of dengue fever as per WHO criteria were included.[4] Patients with febrile fevers due to typhoid, HCV, bacterial disease were excluded from the study. There was no sampling bias to recruit patients for the study. Demographic details of the patient and clinical history with along with the relevant clinical investigations (haematological and biochemical) was recorded.

Five (5) ml of blood sample was collected aseptically by venipuncture in a plain vial (without EDTA) and EDTA vial.

For Serological test NS1 antigen assay and IgM & IgG antibody assay were performed by NS1 ELISA kit (PanBio) and IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) (PanBio) respectively. In case of, acute dengue fever cases (fever duration ≤ 5 days) detection of dengue NS1 antigen while if duration of fever was more than 5 days the samples were screened for the presence of dengue-specific IgM antibodies. Procedure was performed as per manufacturer instructions.

For Molecular test Real time PCR was done to detect DEN1, DEN2, DEN3, DEN4 gene by multiplex PCR. The viral RNA was extracted from 150µl of the serum/ plasma samples by using the QIAamp viral RNA mini kit (Qiagen, Germany). The presence of the Dengue specific RNA in the clinical samples was detected by using a Rotar Q Gene RT-PCR (Qiagen). Qiagen Dengue RT-PCR kit was used and procedure was followed according to manufacturer instructions of detection kit.

RESULTS

In the present study 400 samples were recorded, of these 284 were positive for either NS1 or IgM or IgG. Among the 284 patients whose case records were analyzed, the male (165) and female (119) ratio was 1:1.3. All the age group patients were included, and found 20-40 age group were most affected. The major clinical presentation was a febrile illness with or without skin rash and myalagia.**[Table 1]** The features of low blood pressure, low hemoglobin count, thrombocytopenia and elevated liver enzymes were also predominant in these patients, has been shown in **Table 1.** Maximum number of dengue cases found in

month of October. Monsoon and post monsoon season is favorable for dengue virus. April and may are least favorable month no dengue cases found in these month. **[Fig 1]** Among 400 collected samples 96(24%) showed sero-positivity with NS1 antigen by ELISA. IgM and IgG were diagnosed in 111(27.75%) and 150(37.5%) respectively. **[Fig 2]. Table 2** showed no. of cases of primary and secondary dengue infection**.** Of these 96 NS1 antigen positive cases 31 cases were diagnosed within 5 days of onset of fever and 4 serotypes were detected in only these patients only. RT-PCR revealed 6 as DENV- 2 positive and 4 as DENV-3 positive**.[Table 3]**

Table 1: Demographic details and clinical presentation of Dengue positive patients

1.	**Gender**	
	Male	165
	Female	119
2	**Age**	
	<20	100
	21-40	128
	41-60	40
	61-80	13
	>80	3
3	**Signs and symptoms**	
	Fever	284
	Headache	281
	Myalagia	277
	Rash	85
	Abdominal pain	57
	Retro orbital pain	28
	Backache	28
4	**Clinical presentation**	
	Hemoglobin (<9.0 gm%)	17
	Leucocytosis (> 12,000/ µl)	85
	Thrombocytopenia(1*103)	130
	Billirubin(>1.2mg%)	56
	SGPT/SGOT(>40IU/I)	156
	Serum Albumin (<3gm%)	85
	Hypotension (<100/60mm Hg%)	178

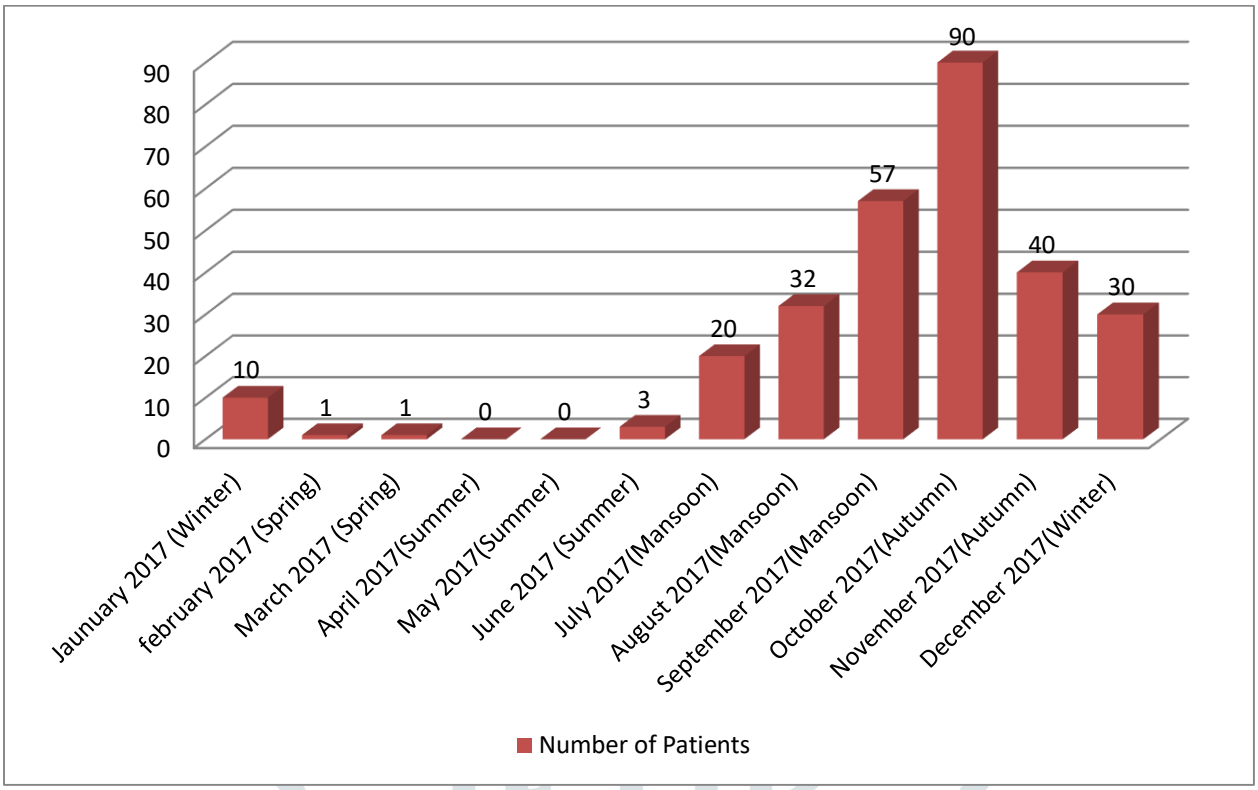

Fig 1 : Month wise and seasonal variation in dengue positive cases

Fig 2: Diagramatic representation of no. of Positive cases

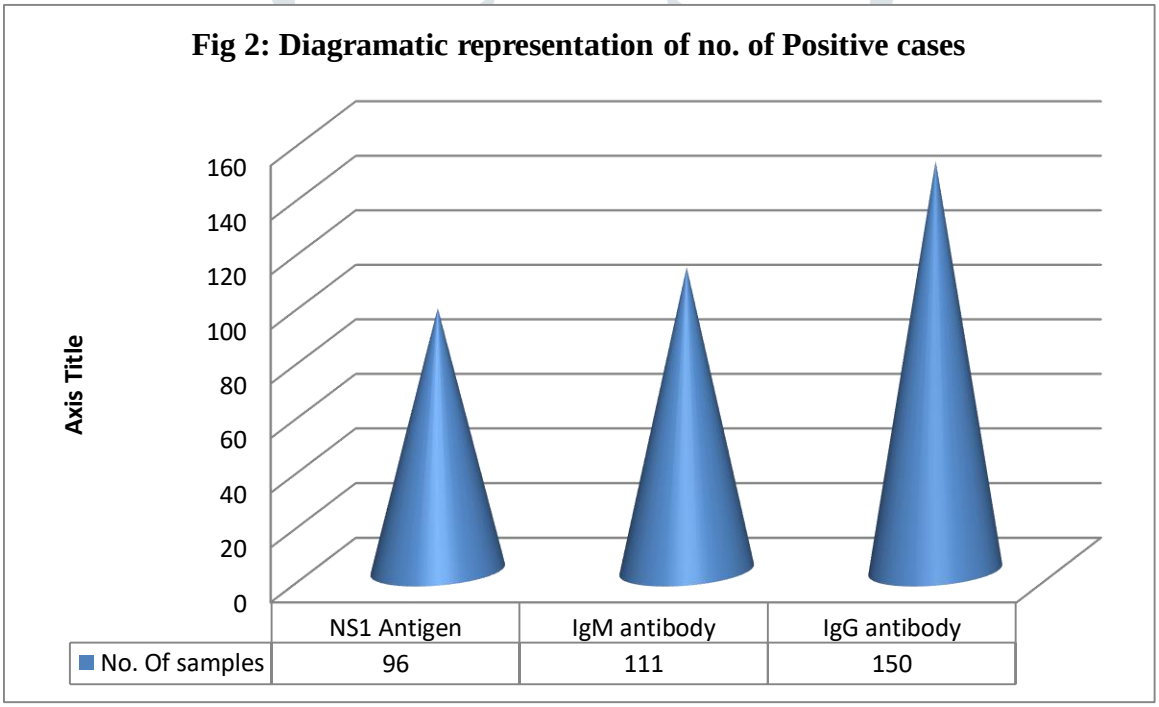

Table 2: Distribution of Primary and Secondary infection.

S.N.	Type of Infections	No. of Patients
1	Primary Infection (NS1, NS1+IgM, IgM)	154
2	Secondary Infection (NS1+IgG, IgM+IgG)	130

Table 3 : Tabular representation of Serotypes of Dengue

Dengue serotype	DENV-1	DENV- 2	DENV-3	DENV- 4	Negative for all
No of Patient	0	6	4	0	21

The median of Ct value of DEN 2 and DEN 3 were 20.4 and 22.7 respectively. The case records of the all 6 cases of DENV-2 were hospitalized while 2 out of 4 DEN 3 positive patients were IPD rest 2 were outpatients[Table 4]. The secondary infections, as per the serology, were predominant in these DENV-2 cases. None of the patients with DENV-3 have secondary infection. The primary infections were predominant in the cases with the DENV-3 infections.

Table 4: Distribution of OPD and IPD patients

S.N.	Serotype	OPD	IPD
1	DEN 1	0	0
2	DEN 2	0	6
3	DEN 3	2	2
4	DEN 4	0	0

DISCUSSION

In the south east asia, India is one of country reporting Dengue Fever regularly. The first case of Dengue fever was reported in 1946[10]. From then the DHF started simmering in various parts of India[11,12,13].

In our study 400 samples were recorded, of these 284 (71%) were positive for either NS1 or IgM or IgG. Our study showed 24% sero-positivity with NS1 by ELISA. 54.2% of cases harbor either only NS1 or NS1 with IgM or only IgM were classified as primary dengue infection and 45.7% were positive for either IgG with NS1 or IgG with IgM, recorded as secondary infection.

Among the 284 patients, 58% patients were male and majority of patients in this study belong to age group 20-40 years as seen in other studies[14,15].

Dengue fever is named as break bone fever so it is therefore not surprising that majority of the patients presented fever with myalgia or arthralgia along with fever. Fever is the most common presenting symptom with or without rashes followed by headache, abdominal pain, retro-orbitalpain, symptom seen in other studies also[16,17]. The features of thrombocytopenia, elevated liver enzymes, hypotension and low hemoglobin count were also predominant in these patients. The association of the Dengue positivity with thrombocytopenia was in concordance with the findings of another study which was done by R.D.Kulkarni et al., and Neeraja M et al[18,19] Dengue vector density varies according to seasons therefore in India.[20] Therefore, in India, dengue fever cases rises in monsoon and post monsoon season, least cases reported in spring and summers. Primary infection of DEN results in immunity to that serotype but does not confer

immunity against other serotypes. Thus, infection can occur with heterologous serotypes (secondary infection). Secondary DENV infection has been shown to be a significant risk factor for the development of severe disease, including DHF and dengue shock syndrome (DSS).

The first serotype of dengue infection in India was DEN-1. DEN-2 emerged as the predominant serotype from the early 1970s to 2000. One of the largest dengue epidemic in North India occurred in Delhi and adjoining areas in the year 1996 which was mainly due to DEN 2[21,22].

Our study, showed two circulating serotypes DEN 2 and DEN 3. DEN-2 was the most common serotype in our study which is in concordance to the study done by Vinodkumar et al., Padbidri et al. and Damodar et al. which concluded that DEN-2 was the predominant circulating serotype. followed by DEN-3[23,24,25].

DEN-2 has been implicated as the causative agent in most of the outbreaks of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) the incidence of which have increased in the last few decades[26,27,28].

Hence, rapid detection and differentiation between primary and secondary DENV infections and determination of the infecting serotypes in past and current infections are vital for patient management.

In the year 2003 outbreak occurred in Delhi in which all the four serotypes were found[29,30].

However, DEN-3 was reported to predominate in certain parts of North India. It has been reported in different studies from various places all over India like the study in Lucknow (North India), Kerala and Delhi[31,32,33].

A study by Khan et al., (2014)31 from the North Eastern most state of Arunachal Pradesh also stated DEN-3 to be the most prevalent serotype in that year[34]. The median of Ct value of DEN 2 and DEN 3 were 20.4 and 22.7 respectively that indicate high viral load in recent acute dengue virus infected samples. In our study all the 06 cases with recent acute dengue virus infection and DEN-2 were hospitalized and 2 out of 4 DEN-3 positive patients were IPD rest 2 were outpatients. The secondary infections, as per the serology, were predominant in these DENV-2 cases. None of the patients with DENV-3 have secondary infection. The primary infections were predominant in the cases with the DENV-3 infections. We demonstrated that DENV-2 infection may be more severe compared with DENV-3 infection. DEN 1 and DEN-4 was not isolated from any of the cases in our population. While in previous studies all four serotypes were detected in Kanpur [8, 9], so further study may require with large population.

CONCLUSION

We found that only DEN 2 and DEN 3 were circulated in Kanpur. For serological diagnosis, detection of the NS1 antigen in acute-phase serum samples has shown promising results. Further studies are needed to evaluate its usefulness and to compare it with real-time one-step RT-PCR with respect to its sensitivity and specificity.

REFERENCES

1. WHO. WHO Scientific Working Group Report on Dengue (2007)
2. S.B. Halstead Dengue Lancet, 370, 2007, pp. 1644-1652

3. G.N. Malavige, S. Fernando, D.J. Fernando, S.L. Seneviratne. Dengue viral infections, Postgrad Med J,2004; 80: 588-601
4. C.P. Simmons, J.J. Farrar, v.V. Nguyen, B. Wills Dengue N Engl J Med,2012; 366:1423-1432
5. M.G. Guzman, S.B. Halstead, H. Artsob, P. Buchy, J. Farrar, D.J. Gubler, et al. Dengue: a continuing global threat Nat Rev Microbiol,2010; 8:7-16
6. S.O. De Paula, B.A. Fonseca Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis. Braz J Infect Dis,2004; 8:390-398
7. R. Lima Mda, R.M. Nogueira, H.G. Schatzmayr, F.B. dos Santos Comparison of three commercially available dengue NS1 antigen capture assays for acute diagnosis of dengue in Brazil PLoS Negl Trop Dis, 2010;4 (7): e738
8. Virological study of an epidemic of febrile illness with haemorrhagic manifestations at Kanpur, India, during 1968. Chaturvedi UC, Mathur A, Kapoor AK, Mehrotra NK, Mehrotra RM Bull World Health Organ. 1970; 43(2):289-93.
9. Clinico-virological study of the recurrence of dengue epidemic with haemorrhagic manifestations at Kanpur during 1969.Chaturvedi UC, Mathur A, Kapoor AK, Tandon HO, Mehrotra RM Indian J Med Res. 1972 Mar; 60(3):329-33.
10. Dengue group of fevers in India. Karamchandani PVLancet. 1946 Jan 19; 1(6386):92.
11. Dengue haemorrhagic fever in children in Delhi. Kabra SK, Verma IC, Arora NK, Jain Y, Kalra V,Bull World Health Organ. 1992; 70(1):105-8.
12. Bhattacharjee N, Mukherjee KK, Chakravarti SK, Mukherjee MK, De PN, Sengupta M, et al. Dengue haemorrhagic fever (DHF) outbreak in Calcutta - 1990. J Commun Dis. 1993;25:10–4.
13. Cherian T, Ponnuraj E, Kuruvilla T, Kirubakaran C, John TJ, Raghupathy P. An epidemic of dengue haemorrhagic fever & dengue shock syndrome in & around Vellore. Indian J Med Res. 1994;100:51–6.
14. Kashinkunti MD, Shiddappa, Dhananjaya M. A study of clinical profile of dengue fever in a tertiary care teaching hospital. Sch J Appl Med Sci 2013;1:280-2.
15. Chandralekha, Gupta P, Trikha A. The North Indian dengue outbreak 2006: A retrospective analysis of Intensive Care Unit admissions in a tertiary care hospital. Trans R Soc Trop Med Hyg 2008;102:143-7.
16. Khan SA, Dutta P, Topno R, Soni M, Mahanta J. Dengue outbreak in a hilly state of Arunachal Pradesh in Northeast India. ScientificWorldJournal 2014;2014:584093.
17. Kumar A, Rao CR, Pandit V, Shetty S, Bammigatti C, Samarasinghe CM. Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udupi district, Karnataka. Indian J Community Med 2010;35:386-90
18. Kulkarni RD, Patil SS, Ajantha GS, Upadhya AK, Kalabhavi AS, Shubhada RM, et al. Association of platelet count and serological markers of Dengue infection- importance of NS1 antigen. Indian J Med Microbiol. 2011 Oct-Dec;29(4):359-62.

19. Mamidi Neeraja et al., The Clinical, Serological and the Molecular Diagnosis of the Emerging Dengue Infection Journal of Clinical and Diagnostic Research. 2013 March;Vol-7(3): 457-461
20. N.A. Honorio, M.G. Castro, F.S. Barros, A. Magalhaes Mde, P.C. Sabroza The spatial distribution of Aedes aegypti and Aedes albopictus in a transition zone, Rio de Janeiro, Brazil Cad Saude Publica, 2009;25(6): 1203-1214
21. Broor S, Dar L, Sengupta S, Chakaraborty M, Wali JP, Biswas A, Kabra SK, Jain Y, Seth P: Recent dengue epidemic in Delhi, India. In Factors in the emergence of arbovirus diseases Edited by: Saluzzo JE, Dodet B. Paris: Elsevier; 1997:P123-7.
22. Dar L, Broor S, Sengupta S, Xess I, Seth P: The first major outbreak of dengue hemorrhagic fever in Delhi, India. Emerg Infect Dis 1999;5:589-90.
23. Gunasekaran P, Kaveri K, Mohana S, Arunagiri K, Babu BV, Priya PP, et al. Dengue disease status in Chennai (2006-2008): A retrospective analysis. Indian J Med Res 2011;133:322-5.
24. Vinodkumar CS, Kalapannavar NK, Basavarajappa KG, Sanjay D, Gowli C, Nadig NG, et al. Episode of coexisting infections with multiple dengue virus serotypes in Central Karnataka, India. J Infect Public Health 2013;6:302-6.
25. Damodar, et al.: Dengue in and around Mangalore, India Indian Journal of Medical Microbiology.2017;35(2):256-261.
26. Guzmán MG, Kourí G. Dengue: An update. Lancet Infect Dis 2002;2:33-42
27. Dash PK, Parida MM, Saxena P, Abhyankar A, Singh CP, Tewari KN, et al. Reemergence of dengue virus type-3 (subtype-III) in India: Implications for increased incidence of DHF & DSS. Virol J 2006;3:55.
28. Kabilan L, Balasubramanian S, Keshava SM, Thenmozhi V, Sekar G, Tewari SC, et al. Dengue disease spectrum among infants in the 2001 dengue epidemic in Chennai, Tamil Nadu, India. J Clin Microbiol 2003;41:3919-21.
29. Gupta E, Dar L, Narang P, Srivastava VK, Broor S: Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. Indian J Med Res 2005;121:36-8.
30. Dar L, Gupta E, Narang P, Broor S: Cocirculation of dengueserotypes 1,2,3 and 4 during the 2003 outbreak in Delhi, India. Emerg Infect Dis 2006;12:352-3.
31. Prakash O, Singh DD, Mishra G, Prakash S, Singh A, Gupta S, Singh J, Khan DN, Jain P, Vishal A, Pandey MK, Jain A. Observation on dengue cases from a virus diagnostic laboratory of a tertiary care hospital in North India. Indian J Med Res 142 (Supplement), December 2015, pp 7-11.
32. Kumar NP, Jayakumar PR, George K, Kamaraj T, Krishnamoorthy K, Sabesan S, Jambulingam P. Genetic characterization of dengue viruses prevalent in Kerala State, India. Journal of Medical Microbiology 2013;62:545–552.
33. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. Virol J 2006; 3: 92
34. Khan SA, Dutta P, Topno R, Soni M, Mahanta J. Dengue outbreak in a hilly state of Arunachal Pradesh in Northeast India. Scientific World Journal 2014; 2014: 584093.