European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 11 Issue 5, 2024

" EVALUATION OF DIFFERENT LABORATORY MODALITIES FOR DIAGNOSIS OF CLINICALLY SUSPECTED CASES OF DENGUE FEVER IN TERTIARY CARE HOSPITAL, AHMEDABAD INDIA"

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Abstract

Background: Dengue fever is a mosquito-borne disease. Its cases are increasing in India year by year with an increasing mortality rate. Hence, prompt and accurate diagnosis is necessary to prevent morbidity and mortality. The early diagnosis of dengue is necessary for identifying an epidemic and also for implementing effective vector control measures.

Aims and Objectives: The present study was carried out to evaluate different laboratory methods like NS1 ELISA, IgM ELISA & dengue RT-PCR for early diagnosis of dengue infection.

Materials and Methods: This prospective study was conducted in a tertiary care hospital in Ahmedabad, Gujarat, from June 2022 to May 2023. In this study, we enrolled 605 clinically suspected cases of dengue. All the samples were processed by either NS1 ELISA or IgM ELISA. Detection of DEN viral RNA was performed on all samples positive by at least one of the two methods and samples from the rest of the patients with a history of ≤ 5 days of fever.

Results: Out of 605 clinically suspected dengue cases, 186 were confirmed cases either positive by RT-PCR, NS1 ELISA, or IgM ELISA. Among all the 186 cases, 126 were positive by RT-PCR, 110 were positive by NS1 antigen ELISA, and 72 were positive by IgM antibody capture ELISA.

Conclusion: Both NS1 and RT-PCR are useful for early dengue diagnosis, although in terms of cost, ease of performance, sensitivity, and rapidity, NS1 is superior to RT-PCR. NS1 in combination with IgM offers the most sensitive and cost-effective diagnostic modality for the detection of acute and late phase of dengue fever.

Keywords: Dengue, IgM ELISA, NS1 ELISA, RT-PCR

Introduction

Dengue is a mosquito-borne disease mainly in tropical and subtropical regions affecting humans [1]. It is spread by mosquito species, Aedes aegypti and Aedes albopictus. Dengue fever (DF) is occasionally complicated and causes severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). It affects public health in urban and suburban areas causing morbidity and mortality [2-4]. WHO has estimated that around 3 billion people worldwide reside in areas where there are risks of exposure to dengue virus, and every year about 50 million people are infected with dengue virus [5-7]. Dengue virus is an RNA virus, which is a hemorrhagic type. It possesses four serotypes 1, 2, 3, and 4. All serotypes cause infection. One serotype infection does not cross-protect against the

infection with other serotypes but may cause a severe form of infection [1]. Among all the four serotypes of DEN (DEN 1-4), Asian genotypes of DEN 2 and 3 are frequently associated with secondary DEN infections. A fifth serotype was also identified [8].

Early diagnosis of dengue plays an important role in detecting an epidemic or outbreak and in implementing effective vector control measures [9]. Controlling dengue infections is difficult because it requires effective control of vectors responsible for transmitting the virus and also accurate and rapid diagnosis. In many parts of the world, particularly in countries with limited resources, accurate and timely diagnosis of early detection of dengue virus is a big problem for the management of dengue-infected patients to date[10].

Dengue virus infections may be asymptomatic or may lead to undifferentiated fever, DF, or DHF/DSS. For dengue, the incubation period is four to six days. Infants and young children present with an undifferentiated febrile disease with a maculopapular rash. Older children and adults may develop either a mild febrile syndrome or classical dengue fever, characterized by fever, headache, myalgias, arthralgia, and rash. Three or four days after the onset of fever, when the fever falls, few patients develop bleeding manifestations (at least a positive tourniquet test), thrombocytopenia, and hemoconcentration. Hepatomegaly may also be found. Patients mostly improve after fluid and electrolyte therapy. In severe cases, shock is observed, characterized by signs of circulatory failure (weak and rapid pulse, hypotension or narrowing of the pulse pressure, cold and clammy skin, and restlessness). Shock is followed by death in 5-10% of cases if rehydration is not sufficient or delayed. Plasma leakage is the main characteristic of DHF/DSS [11,12].

There are many laboratory methods for the diagnosis of dengue infection such as viral isolation, detection of RNA, antigen, and antibody assays. In countries where dengue is prevalent, the diagnosis of dengue is mostly based on ELISA detecting either NS1 antigen or IgM antibody (MAC IgM) [13,14]. Molecular methods like reverse transcriptase polymerase chain reaction (RT PCR) for diagnosis of dengue virus are useful for early detection with its serotypes. RT PCR has been approved by WHO in the Dengue Bulletin 2009[15]. Both viral isolation and identifying viral RNA through RT-PCR are time-consuming and need a specialized laboratory with costly methods and well-trained personnel which may not be available in all hospital settings [7,9]. In most of the cases, serologic tests are used to detect IgM and IgG antibodies by ELISA. During the acute phase, the presence of IgM antibodies indicates primary infection and it appears after the viremia ends or after the fever subsides [16]. During primary infection, IgM appears after 5-6 days and IgG after 7-10 days. But, in secondary infections, IgG antibodies rise to high levels within the first week of infection and reduce over 3 to 6 months [2]. The detection of nonstructural protein 1 (NS1) antigen during the acute phase of disease in patients having primary and secondary infections has been studied in various laboratories across the world [9,17-19]. NS1 is a highly conserved glycoprotein for all the serotypes and is produced in both cell membrane-associated and secreted forms [7,17,19]. NS1 has no biological activity but It is essential for virus viability or replication and NS1 antigen does not yet have a precise function [17,20]. It stimulates a strong humoral response [21]. NS1 antigen is detectable in blood from the first day after the onset of fever up to day 9, and is also detectable in the presence of IgM antibodies and when viral RNA is negative by RT-PCR [18]. Ahmedabad is endemic for Dengue. This study was done to evaluate the utility of various diagnostic tests in patients with clinically suspected dengue fever.

Materials and Methods

Study design and site

It is a prospective study conducted at the Virology Laboratory, Department of Microbiology, GCS Medical College, Hospital and Research Centre, Ahmedabad, Gujarat, India from June 2022 to May 2023. This study was approved by the Institutional Ethics Committee. The inclusion and exclusion criteria were followed as mentioned below:

Inclusion criteria

• Age: All age groups.

• Clinically suspected cases of dengue fever visiting O.P.D. and I.P.D. of different clinical departments of GCSMCH & RH.

Exclusion criteria

All previously diagnosed cases of dengue infection and febrile cases with other proven etiology (malaria, typhoid, other febrile illnesses, etc.).

Study period, sample collection, and processing

A total of 605 cases were enrolled with clinical signs and symptoms (headache, fever, joint pain, etc.) suggestive of dengue fever during the outbreak from June 2022 to May 2023. Blood samples (3-4 ml) were collected under all aseptic precautions from patients who fulfilled the inclusion criteria. The samples (Serum/Plasma) were separated by centrifugation for serological and molecular diagnosis.

Patient signs and symptoms

The history of most common clinical signs and symptoms like fever, arthralgia, myalgia, retro-orbital pain, headache, rashes, epistaxis, melena, abdominal pain, and vomiting were taken from all the patients.

Dengue was confirmed by either NS1 antigen ELISA or IgM antibody ELISA.

NS1 antigen ELISA: Non-structural (NS1) antigen ELISA was performed using Oscar Medicare Dengue NS1 kit as per manufacturer protocol, (Oscar Medicare Pvt. Ltd. New Delhi, India).

Dengue IgM antibody capture (MAC) ELISA: Dengue antibody detection was done by NIV Dengue MAC ELISA kit as per manufacturer instructions, (National Institute of Virology - NIV, Pune, India).

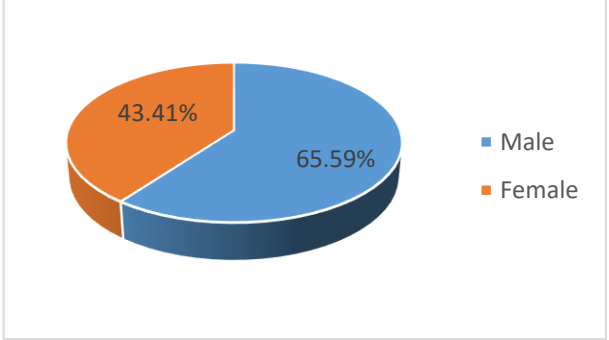
Detection of DEN viral RNA was performed on all samples positive by at least one of the two tests (NS1 antigen/ IgM antibody ELISA) and samples from the rest of the patients with a history of ≤ 5 days of fever.

RNA extraction and real-time reverse transcriptase PCR: The RNA was extracted by the standard procedure as per manufacturer protocol (Meril RNA extraction kit, Meril Diagnostics, Vapi, Gujarat, India). Real-time reverse transcriptase PCR for dengue virus detection was performed by TRUPCR Dengue/Chikungunya Detection Kit Version: 2.0 (3B BlackBio Biotech India Ltd, MP, India) in real-time PCR instrument (Qiagen Rotor-Gene Q).

Results:

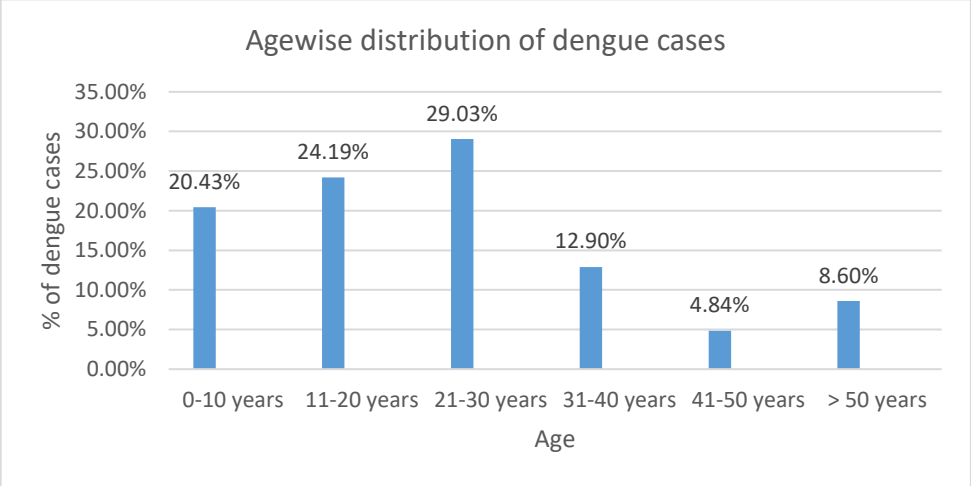
In this study, out of the total 605 clinically suspected dengue cases, 186 were confirmed cases (either positive by RT-PCR, NS1 ELISA, or IgM ELISA). Among 186 positive cases, 122 were male, and 64 were female, with M: F ratio of 1.9:1.

Figure 1: Sexwise distribution of Dengue patients (N = 186)



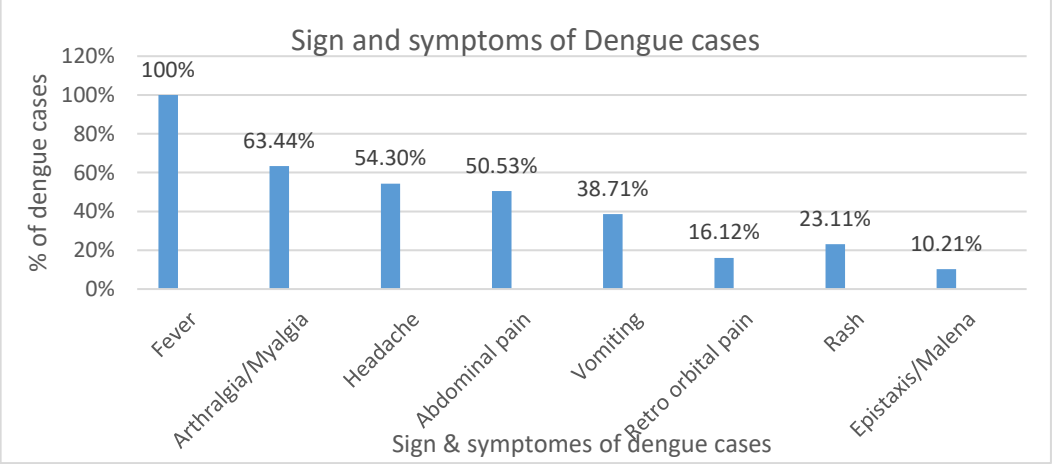
Out of 186 cases, 38 (20.43 %) were between age group 0-10 years, 45 (24.19 %) between age group 11-20 years, 54 (29.03 %) between age group 21-30 years,24 (12.90 %) between age group 31-40 years, 9 (4.84 %) between age group 41-50 years and 16 (8.60 %) above age 50 years.

Figure 2: Agewise distribution of Dengue patients (N = 186)



In this study most common symptom was fever present in all cases 186 (100%). In 108 (58.06 %) cases fever was ≤ 5 days and in 78 (41.94 %) cases fever was > 5 days, followed by arthralgia/myalgia present in 118 (63.44 %) cases, headache in 101(54.30 %) cases, vomiting in 72 (38.71 %) cases, abdominal pain in 94 (50.53 %) cases, skin rash in 43(23.11 %) cases, bleeding manifestation (epistaxis, Malena) in 19 (10.21 %) cases, retro-orbital pain in 30 (16 .12%)cases. Clinical signs and symptoms are shown in Figure 3.

Figure 3: Clinical signs and symptoms in dengue-positive cases (n=186).



Laboratory investigations show 134 (72.04 %) cases had thrombocytopaenia, 90 (48.39) had leucopenia and 82 (44.09) % cases had raised liver enzymes (AST/ALT)

In this study among all the 186 cases, 126 cases were positive by RT-PCR, 110 cases were positive by NS1 antigen ELISA, and 72 cases were positive by IgM antibody capture ELISA. Out of 605 fever cases, 289 had a history of ≤ 5 days fever & and 316 cases had a history of > 5 days fever. Out of 110 NS1 positive cases, 92 had a fever ≤ 5 days, and 18 had a > 5 days fever. Out of 72 IgM-positive cases, 12 had a fever of ≤ 5 days, and 60 had a > 5 days fever.

Table 1: Correlation between number of days of fever and serological tests

Serological Test	Fever ≤ 5 days	Fever > 5 days	Total cases
NS1 ELISA +	92	18	110
IgM ELISA +	12	60	72
Negative	185	238	423
Total	289	316	605

In this study, out of 185 serological negative cases of fever ≤ 5 days, RT PCR was positive in 4 cases.

Table 2: Dengue-positive cases by different testing methods

Test	Fever ≤ 5 days	Fever > 5 days	Total Positive
NS1 ELISA +	92	18	110
IgM ELISA +	12	60	72
RT PCR + NS1 Negative, IgM Negative	4	-	4
Total	108	78	186

Out of a total of 186 dengue-positive cases 182 were detected by serological methods NS1 or IgM ELISA & and 4 cases by RT PCR method.
In this study out of 92 NS1 positive cases of fever ≤ 5 days, 75 (81.52 %) were positive for dengue RT PCR, and out of 18 NS1 positive cases of fever > 5 days, 11 (61.11%) were positive for dengue RT PCR. Out of 72 IgM-positive cases, 36 (50 %) were dengue RT PCR positive. Out of 185 patients with a history of fever 5 days or less and serology negative 4 (2.16 %) were dengue RT PCR positive.

Table 3: Comparison of NS1 ELISA & IgM ELISA against RT PCR

Category	Cases processed by RT PCR	Cases positive by RT PCR
NS1 Positive & fever ≤ 5 days	92	75 (81.52 %)
NS1 Positive & fever > 5 days	18	11(61.11 %)
IgM Positive	72	36 (50 %)
Serologically Negative & fever ≤ 5 days	185	4 (2.16 %)

Discussion

Dengue is an emerging vector-borne disease associated with high morbidity and mortality. Rapid urbanization, globalization, poor solid waste and water management, and increasing population have given rise to new habitats for mosquito breeding thereby increasing the number of cases. Increased awareness among treating physicians and communities has brought down the mortality due to dengue, however increasing number of cases year after year is an area of concern. The diagnosis of dengue is still difficult in developing countries due to a lack of resources, infrastructure, and skilled manpower. A study on the Dengue burden in India shows that the prevalence of dengue fever occurred in increasing order each year [22]. Dengue is a major public health problem in India and should be early diagnosed and treated to prevent any related morbidity and mortality.
In this study, 122 dengue cases were male, and 64 females were affected, out of 186 dengue-positive cases. Male preponderance was seen which may be due to more outdoor activities of males as compared to females. Like this study, a study from north India also demonstrated that males were more commonly affected than females [23]. More number of dengue patients were in the age group 21-30 years followed by 11-20 years & then 0-10 years. Less number of cases in the elderly age group may be due to fewer outdoor activities [24,25].

Dengue Fever is nicknamed 'break-bone fever' [26] so it is common that the majority of patients present with myalgia or arthralgia along with fever. The clinical feature of dengue shows that fever was the most common presenting symptom 186 (100%) in our study. Our results are similar to the study by Damodar et al[27]. In the present study, the other symptoms next to fever were, arthralgia/myalgia 118 (63.44%), headache 101(54.30 %), and abdominal pain 94 (50.53 %). Similar results were observed by Goel et al, and Anuradha et al [28,29]. Fever is the most common presenting symptom followed by musculoskeletal symptoms & headache [30,31].
Retroorbital pain is considered a cardinal sign in the clinical diagnosis of dengue. An Indian study by Laul et al. observed that 41% of patients with dengue have retro-orbital pain. But in this study only 30 (16.12 %) of cases have this symptom. Rashes were reported in 43 (23.11 %) cases in this study, which is similar to other studies that have observed it as a less common symptom occurring in about 21% of cases only [32,33]. It seems that the trend of clinical presentation is changing year by year in dengue patients with increasing severity.
The association of dengue positivity with thrombocytopenia was the same as the findings of other recent Indian studies [34,35].

The effective and accurate diagnosis of dengue is important for clinical care and management. Serological methods, like NS1 ELISA and IgM ELISA, are routinely being used globally. In this study, we also used NS1 and IgM ELISA as the main diagnostic tool. In our study 92 dengue cases have been diagnosed with NS1 ELISA, out of a total of 108 dengue cases with fever ≤ 5 days and 60 dengue cases have been diagnosed by IgM ELISA in patients with fever with >5 days, out of a total 78 dengue cases with fever >5 days. Out of 185 serology negative patients with fever ≤ 5 days only 4 cases of dengue are diagnosed by RT PCR. Out of 92 NS1-positive dengue cases, only 75 (81.52 %) are RT PCR positive. So NS1 is more useful than RT PCR for early diagnosis of dengue cases. Even out of 72 IgM ELISA positive dengue cases only 36 (50 %) were RT PCR positive.
The sensitivity and specificity for NS1 antigen ELISA were found to be as 87% and 91.88 % respectively with fever ≤ 5 days in our study. An Indian study also supports our higher positivity rate of NS1 as they found that the positive detection rate of NS1 antigen ELISA was 80.9% [36].
The NS1 and IgM ELISA methods show higher sensitivity and specificity either alone or in combination with both tests. In our study NS1 ELISA and IgM ELISA combined sensitivity and specificity are 96.42% and 97.84 % respectively. Many studies observed their higher sensitivity and specificity range from 53-96% [36-38]
The maximum positivity of RT PCR was observed in NS1-positive samples, which showed a strong correlation between them. It is due to high viremia in the initial days. In our study, 78.83 % sensitivity and 86.19% specificity were observed by RT PCR.

Strength of the study
The study was performed for a longer duration of one year and the number of patients is also more in number.

Limitations of the study
We can not able to perform RT PCR tests on all the clinically suspected dengue cases.

Conclusion
Dengue is the most common mosquito-borne viral infection occurring in both endemic and outbreak forms in Ahmedabad. There is no specific treatment available for dengue, therefore early diagnosis is important for individual case management and also for planning and implementing control strategies. Out of three different methods used for the diagnosis of dengue, NS1 antigen detection had the highest sensitivity in the early stages while IgM detection was more sensitive in the later half of the illness. Both NS1 and RT-PCR are useful for early dengue diagnosis. However, NS1 antigen detection was

found to have a better sensitivity than viral RNA detection by PCR for early detection of DEN infection. Also, NS1 is superior to RT-PCR in terms of cost, ease of performance, and rapidity. NS1 antigen when combined with IgM capture ELISA increased the diagnostic efficacy.

Ethical approval

The study was approved by the Institutional Ethics Committee.

Funding: No funding sources.

Conflict of interest: None.

References

[1] Chua KB, Mustafa B, Abdul Wahab AH, Chem YK, Khairul AH, Kumarasamy V, et al. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. Malays J Pathol. 2011;33(1):13–20.

[2] WHO | Dengue hemorrhagic fever: diagnosis, treatment, prevention, and control. 2nd edition. Geneva: World Health Organization. [Internet]. WHO [cited 2012 May 6]; Available from:http://www.who.int/csr/resources/publications/dengue/ Denguepublication/en/.

[3] Gibbons RV, Vaughn DW. Dengue: an escalating problem. BMJ. 2002;324(7353):1563–66.

[4] Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv. Virus Res. 1999;53:35–70.

[5] Tang KF, Ooi EE. Diagnosis of dengue: an update. Expert Rev Anti Infect Ther. 2012;10(8):895-907.

[6] WHO. Dengue Guidelines for diagnosis, treatment, prevention and control. 2009.

[7] Kassim FM, Izati MN, TgRogayah TAR, Apandi YM, Saat Z. Use of dengue NS1 antigen for early diagnosis of dengue virus infection. The Southeast Asian Journal of Tropical Medicine and Public Health. 2011;42(3):562.

[8] Lee J, Kim HY, Chong CK, Song HO. Development and clinical evaluation of a highly accurate dengue NS1 rapid test: from the preparation of a soluble NS1 antigen to the construction of an RDT. Diagn Microbiol Infect Dis. 2015;82(2):128– 34. doi: 10.1016/j.diagmicrobio.2015.03.009. Epub 2015 Mar 18.

[9] Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Evaluation of a commercial Dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. Indian J Med Microbiol. 2011;29(1):51–55.

[10] Kassim FM, Izati MN, TgRogayah, et al. Use of Dengue NS1 antigen for early diagnosis of dengue virus infection. Southeast Asian J Trop Med Pub Health 2011; 42(3): 562- 569. Link

[11] Rigau-Pérez J, Clark GG, Gubler DJ, et al. Dengue and dengue hemorrhagic fever. Lancet 1998; 352: 971-77. Link

[12] Guzmán MG, Kouri G. Dengue diagnosis, advances and challenges. International Journal of Infectious Diseases 2004; 8: 69-80. Link

[13] Neeraja M, V Lakshmi, VD Teja, P Umabala, MV Subbalakshmi. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol. 2006;24:280-82.

[14] Dash PK, Parida MM, Saxena P, Abhyankar A, Singh CP, Tewari KN, et al. The reemergence of dengue virus type-3 (subtype-III) in India: Implications for increased incidence of DHF and DSS. Virology J. 2006;3:55-65.

[15] World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: World Health Organization;2009.

[16] Halstead SB. Dengue. The Lancet. 2007;370(9599):1644–52.

[17] Kumarasamy V, Chua SK, Hassan Z, Wahab AHA, Chem YK, Mohamad M, et al. Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. Singapore Med J. 2007;48(7):669–73.

[18] Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol. 2002;40(2):376–81.

[19] Young PR, Hilditch PA, Bletchly C, Halloran W. An Antigen Capture EnzymeLinked Immunosorbent Assay Reveals High Levels of the Dengue Virus Protein NS1 in the Sera of Infected Patients. J Clin Microbiol. 2000;38(3):1053–57.

[20] Dussart P, Labeau B, Lagathu G, Louis P, Nunes MRT, Rodrigues SG, et al. Evaluation of an Enzyme Immunoassay for Detection of Dengue Virus NS1 Antigen in Human Serum. Clin. Vaccine Immunol. 2006;13(11):1185–89.

[21] Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, et al. Evaluation of diagnostic tests: dengue. Nature Reviews Microbiology. 2010;8:S30–37.

[22] Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM, Dengue burden in India: recent trends and importance of climatic parameters. Emerg Microbes Infect. 2017;6:e70.

[23] Prakash O, Singh DD, Mishra G, Prakash S, Singh A, Gupta S, et al. Observation on dengue cases from a virus diagnostic laboratory of a tertiary care hospital in north India. Indian J Med Res. 2015;142:7-11.

[24] Kashinkkunti MD, Shiddappa, Dhananjaya M. A study of clinical profile of dengue fever in a tertiary care hospital. Sch J Appl Med Sci 2013;1:280-2.

[25] Chandralekha, Gupta P, Trikha A. The North India 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.

[26] Halsay ES ,Marks MA, Gotuzzo E, Fiestas V, Suarez L, Vargas J, *et al*. Correlation of serotype-specific dengue virus infection with clinical manifestations.PLoS Negl Trop Dis 2012;6:e1638.

[27] Damodar T, Dias M, Mani R, Shilpa KA, Anand AM, Ravi V, et al. Clinical and laboratory profile of dengue viral infections in and around Mangalore. Indian J Med Microbiol. 2017;35:256-61.

[28] Goel A, Patel DN, Lakhani KK, Agarwal SB, Agarwal A, Singla S, et al. Dengue fever- A dangerous Foe. J Ind Acad Clin. Med. 2004;5:247- 58.

[29] 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;29:503-06.

[30] 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.

[31] 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.

[32] Laul A, Laul P, Merugumala V, Pathak R, Miglani U, Saxena P. Clinical profiles of dengue infection during an outbreak in Northern India. J Trop Med. 2016; 5917934:1-7.

[33] Kumar A, Rao CR, Pandit V, Shetty S, Chanaveerappa B, 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.

[34] Neeraja M, Lakshmi V, Dash PK, Parida MM, Rao PV. The clinical, serological, and molecular diagnosis of emerging dengue infection at a tertiary care institute in Southern, India. J Clin Diagn Res 2013;7:457-61.

[35] 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;29:359-62.

[36]. Anand AM, Sistla S, Dhodapkar, Hamide A, Biswal N, Srinivasan. Evaluation of NS1 antigen detection of dengue in a tertiary hospital in Southern India. J Clin Diagn Res. 2016;10:01-4.

[37] Ahmed NH, Broor S. Comparison of NS1 antigen detection ELISA, real-time RT-PCR and virus isolation for rapid diagnosis of dengue infection in the acute phase. J Vector Borne Dis. 2014;51:194-99.

[38] Khan E, Mehraj V, Nasir A, Khan N A, Billoo Bushra, Moatter T, et al. Evaluation of two ELISA assay kits against RT-PCR for diagnosis of dengue virus infection in a hospital setting in Karachi, Pakistan. J Pak Med Assoc. 2009;51:390-94.