

## Original Research Article

# Diagnosis of dengue fever: roles of different laboratory test methods

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### ABSTRACT

**Background:** Dengue fever is currently the most important arthropod borne viral disease. Since occurrence of dengue infections has been an epidemic in many parts of India and complications like DHF and DSS are increasing, while at the same time the diagnosis is challenging, particularly the laboratory diagnosis is confusing, this study was conducted to evaluate the different laboratory test methods and to compare their respective efficacy, timing, advantages and disadvantages.

**Methods:** This study was done in the Department of Microbiology in collaboration with the Department of Medicine and Pediatrics in two tertiary care medical colleges and hospitals in eastern India. Blood samples from 319 patients with clinical features suggestive of Dengue fever were included in this study. Laboratory investigations were done which included immunological assays that were performed using commercially available kits - SD dengue duo NS1Ag + Ab combo rapid test, NS1 Ag capture ELISA, IgM capture ELISA, IgG capture ELISA test for dengue and other routine tests -full blood cell count, coagulation tests, routine biochemical and lipid profile were also done. Ethical considerations were taken care of and statistical evaluations were done.

**Results:** An increased detection of IgM antibody (46.15%) was seen in the early febrile period (1-5 days) as compared to the mid-febrile period (6-10 days), and late febrile period (6-10 days) when it is 6.89%. IgG antibody is much less in early febrile period (4.16%). Compared to mid-febrile period (24.13%), and late febrile period (62.5%). IgM antibodies were detected in 44.5% of the samples, IgG antibodies were detected in 43.5% of the samples, Rapid test was positive in 36.9% and NS1AG ELISA was detected in 43.5% of the samples in the study.

**Conclusions:** It can be inferred from our study that for detection of dengue in the early febrile period (1-5 days), estimation of dengue-specific serum IgM is the most sensitive antibody detection method.

**Keywords:** Comparison of lab tests in dengue, Diagnosis of dengue, NS1 antigen

### INTRODUCTION

Dengue fever is currently the most important arthropod borne viral disease because of its widespread distribution in more than 100 countries and its potential for extensive outbreaks of life-threatening diseases. A total 2500 million people or two-fifths of world's population are

now at risk for dengue and every year approximately 50-100 million cases occur worldwide.<sup>1</sup>

Dengue virus was first isolated in India in the year 1945; it is endemic in both urban, semi-urban areas. Once again Dengue virus has struck India and cases of Dengue fever/DHF have been reported from various parts of the country in the last 4 decades.<sup>2</sup> During dengue epidemics attack rates among susceptible are 40-90% and an

estimated 500,000 cases of DHF require hospitalization each year of whom a very large proportion are children.<sup>3</sup>

Dengue virus belong to genus *Flavivirus* and family *flaviviridae*, are mosquito borne viruses. Principal vector *Aedes aegypti* is a day biting mosquito of public importance that breeds in natural or artificial waters. Dengue illnesses are caused by any one of the four serologically related viruses, designated as DEN-1, DEN-2, DEN-3 and DEN-4.<sup>4</sup>

Infection by anyone of the serotypes mostly causes a mild, self-limiting febrile illness (classical dengue fever) however a few cases develop severe life-threatening dengue haemorrhagic fever and dengue shock syndrome.<sup>4</sup>

Classical dengue fever is seen 4 - 6 days after an infective mosquito bite, with sudden onset of fever (biphasic often), severe headache, chills, generalized pains in muscles and joints, often is associated with maculopapular rash. There is leukopenia, relative lymphocytosis, thrombocytopenia and haemorrhagic manifestations may occur.<sup>5</sup>

The diagnosis of Dengue fever and Dengue haemorrhagic fever are made on clinical and epidemiological grounds. In some areas, DHF overlaps the distribution of other viral haemorrhagic fevers, thereby causing confusion in the diagnosis.

Serological diagnosis by detection of IgM and IgG antibodies to dengue in the serum is essential for monitoring the treatment. Commercial kits are available, which can help in differentiating between primary and secondary dengue infections. A rapid dengue detection test kit is used for preliminary diagnosis. ELISA tests are very useful in dengue serology. They detect IgM and IgG in the serum and thus are able to distinguish between primary and secondary infections. NS1Ag detection, in early detection of dengue cases is also very helpful in serological diagnosis of dengue.

Since occurrence of dengue infections and complications like DHF and DSS are increasing, while at the same time the diagnosis is challenging, particularly the laboratory diagnosis is confusing, this study was conducted to evaluate the different laboratory test methods and to compare their respective efficacy, advantages and disadvantages, so that proper laboratory tests can be done effectively in suspected dengue cases in a tertiary care set up, and a rapid diagnosis can be reached and a follow up can be properly made.

## **METHODS**

This study was done in the Department of Microbiology in collaboration with the Department of Medicine and Pediatrics in two tertiary care medical colleges and hospitals in eastern India.

## **Sample**

Blood samples from 319 patients with clinical features suggestive of Dengue fever were included in this study. The samples were collected aseptically, and serum was separated by centrifugation technique and stored at -70°C.

## **Inclusion criteria**

The clinical basis for diagnosing the patients as having Dengue fever was based on WHO criteria like presentation of febrile illness of 2-7 days duration with features like headache, myalgia, arthralgia, rash and/or haemorrhagic manifestations.<sup>6,7</sup>

## **Exclusion criteria**

Patients with clinical evidence of urinary tract infection, pneumonia, Abscess or any other apparent cause of fever were excluded.<sup>8</sup>

## **Source of sample**

The samples were received from inpatient and outpatient departments of the relevant tertiary care Medical Colleges and Hospitals.

## **Ethical considerations**

Written consent to participate in the study was obtained from the subjects or their guardians after the full explanation of the study was provided to them. This study was reviewed and approved by Institutional Ethical committee.

## **Statistical analysis**

The proportional data of this cross-sectional study was tested using Pearson's chi-square analysis test, two samples binomial proportion test and Statistical package for social sciences (SPSS).

Patients were identified as probable cases of dengue fever as per WHO criteria of probable DF i.e. acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leukopenia and supportive serology.

All clinical and investigation parameters were recorded from the time of admission to the time of discharge. Hypoproteinaemia was said to be present when serum albumin level was less than 3gm/dl. A haematocrit and platelet count was done at the time of admission. Platelet counts were repeated daily. Repeated haematocrit was done every alternate day except in serious patients with features of shock, for whom it was done every day. A tourniquet test was done on admission and in patients with shock, and it was repeated on recovery. Patients

were classified as DF, DHF, and DSS according to WHO guidelines.<sup>6</sup>

Blood was collected from patients and from that serum was separated as by the standard procedure and laboratory investigations were done which included immunological assays that were performed using commercially available kits - SD dengue duo NS1Ag + Ab combo rapid test, NS1 Ag capture ELISA, IgM capture ELISA, IgG capture ELISA test for dengue and other routine tests -full blood cell count, coagulation tests, routine biochemical and lipid profile were also done.

Peripheral thick and thin smears for malarial parasites were performed in all patients, whereas blood culture was done in selected cases

A bio data proforma was designed to get adequate information regarding personal profile, travel history, mosquito interaction, presence of any constitutional symptoms and the sewerage systems in surroundings.

## RESULTS

This study was done with 319 serum samples from patients with clinical symptoms suggestive of Dengue.

SD dengue duo NS1Ag + Ab combo rapid test, NS1ag capture ELISA, IgM capture ELISA, IgG capture ELISA

were done for all the suspected cases at the department of microbiology, of the relevant Medical College and Hospital. Other routine tests were done, and clinical data were collected for all patients.

An increased detection of IgM antibody (46.15%) was seen in the early febrile period (1-5 days) as compared to the mid-febrile period (6-10 days), and late febrile period (6-10 days) when it is 6.89%. IgG antibody is much less in early febrile period (4.16%). Compared to mid-febrile period (24.13%), and late febrile period (62.5%). Regarding both IgM and IgG antibodies, the values are seen at higher levels (68.96%) in mid-febrile period compared to that in both early (18.4%) and late febrile period (25%) (Table 2).

IgM antibodies were detected in 44.5% of the samples, IgG antibodies were detected in 43.5% of the samples, Rapid test was positive in 36.9% and NS1AG ELISA was detected in 43.5% of the samples in the study (Table 1).

**Table 1: Comparison between rapid test and ELISA procedure (NS1 Ag, IgM, IgG).**

Methods	Tested	Positive	Percentage (%)
Rapid test	319	118	39.9%
NS1Ag ELISA	319	139	43.5%
IgM ELISA	319	142	44.5%
IgG ELISA	319	129	40.4%

**Table 2: Antibody results in early, mid and late febrile period (n=110).**

Duration (day)	IgM ELISA	%	IgG ELISA	%	Both positive	%
1-5 (n=65)	30	46.15%	3	4.61%	12	18.4%
6-10 (n=29)	2	6.89%	7	24.13%	20	68.96%
>10 (n=16)	2	12.5%	10	62.5%	4	25%
Total % of antibody detection	IgM=30.9%		IgG=18.18%		Both =32.72%	

## DISCUSSION

Dengue has been recognized increasingly as an emerging infectious disease for the last four to five decades. The global burden of Dengue has shown dramatic growth in recent years.

The high prevalence of Dengue cases almost throughout this country in recent years marks it necessary to evaluate the seropositivity of Dengue cases.

Rapid diagnosis of Dengue is important for proper patient care. The appearance of IgM antibody early during the disease course mandates its detection as an important tool for rapid diagnosis. NS1Ag detection is also an important diagnostic tool in early diagnosis of Dengue fever. This

study was done with 319 serum samples from patients with clinical symptoms suggestive of Dengue.

In the present study an increased detection of IgM antibody (46.15%) was seen in the early febrile period (1-5 days) as compared to the mid-febrile period (6-10 days), and late febrile period (6-10 days) when it is 6.89%. IgG antibody is much less in early febrile period (4.16%). Compared to mid-febrile period (24.13%), and late febrile period (62.5%). Regarding both IgM and IgG antibodies, the values are seen at higher levels (68.96%) in mid-febrile period compared to that in both early (18.4%) and late febrile period (25%) (Table 2).

It can be inferred from present study that for detection of dengue in the early febrile period (1-5 days) estimation of

dengue-specific serum IgM is the most sensitive antibody detection method. However, for detection of dengue in later febrile period, which is from 6<sup>th</sup> day onwards, both IgG and IgM should be estimated.

IgM antibodies were detected in 44.5% of the samples, IgG antibodies were detected in 43.5% of the samples, Rapid test was positive in 36.9% and NS1AG ELISA was detected in 43.5% of the samples in the study (Table 1).

In the study by Neeraja et al in 2006, done at Andhra Pradesh, IgG was detected in 40.28% cases, IgM in 3.8% of cases and both IgG and IgM in 55.9% of cases.<sup>9</sup>

The detection of dengue cases was more by ELISA method (whether antibody or antigen) than rapid test method in this study. In the study by Kumar et al at Kasturba medical college in 2003, the results found out were similar to this study.<sup>7</sup>

The “gold standard” for diagnosis of dengue in a febrile patient is obviously the specific virus detection, virus isolation and virus identification after by cell culture. However, this is gradually being replaced by real time reverse transcriptase polymerase chain reaction (RT-PCR) method for more rapid diagnosis.

The isolation of viruses and their culture from clinical samples can be conveniently carried out with mosquito cells such as: AP-61, Tra-284, C6/36, AP-64, CLA-1 cell lines or mammalian cells such as LLCMK2, Vero, BHK-21 cell lines.<sup>10</sup>

Because of its higher sensitivity, the mosquito inoculation is still the method of choice for attempting dengue virus isolation from severe and fatal cases or from patients with severe haemorrhagic disease.<sup>11,12</sup> *Aedes albopictus* and *toxorhynchites* spends have been shown to be useful for dengue virus recovery.<sup>12-14</sup> At present, virus isolation with the C6/36 cell line with the acute phase serum or plasma from patients is the method of choice for routine virus isolation.

Both cytopathic effects (CPE) (resounding, refractability and cell sloughing) and plaque formation are observed in these cells. Growth in cell culture consists of a rapid adoption phase followed by an eclipse phase of approximately 10-12 hrs. after which infectious virus first appears and enters a log phase of replication lasting 18-24 hours.<sup>15</sup>

However, the above tests are almost impractical in most places because they feasible only in rare centers of national importance, and hence in most or almost all places serological diagnosis is resorted to. Serological diagnosis in dengue virus infection is a most challenging matter due to its cross-reactivity to homologous and heterologous Flavivirus antigens. However great advances in analyzing the complicated viral antigens and antibody responses have recently been made by the

development of various methods that target different structural and non- structural proteins for sero-diagnosis and sero-epidemiological studies of dengue virus infection.<sup>16</sup>

Of these, the NS1 non-structured antigen detection is the most favored one because of the ease and rapidity of the test. The Flavivirus NS1 is a 46-50 kilo Dalton glycoprotein which is expressed in both membrane-associated (mNS1) and secreted (sNS1) forms and possesses both group- specific and type- specific determinants. The procedure for both detection and estimation of NS1 antigen by ELISA has been developed for detection of Flavivirus NS1 in patients sera.<sup>16</sup> This test is particularly important for early diagnosis of DHF in early febrile patients yet without symptoms of DHF as it has been found that a high NS1 serum titer statistically nicely correlates with later DHF.<sup>17</sup>

The other tests are Rapid immunochromatography test and Enzyme- Linked Immuno Sorbent Assay (ELISA) for both IgG and IgM.<sup>18,19</sup>

The studies here have hopefully thrown some light on selection of appropriate diagnostic tests for detection of dengue. Further studies, however, are required to confirm and improve upon.

## CONCLUSION

It can be inferred from our study that for detection of dengue in the early febrile period (1-5 days) estimation of dengue-specific serum IgM is the most sensitive antibody detection method. However, for detection of dengue in later febrile period, which is from 6<sup>th</sup> day onwards, both IgG and IgM should be estimated. NS 1 antigen by ELISA is a good test for dengue detection and also for early prediction of DHF. Compared to Rapid test, it is also a better diagnostic test.

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