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Original Research Article

Evaluation of rapid immunochromatographic card test in comparison with IgM ELISA in diagnosis of dengue fever at a tertiary care hospital, South India

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ABSTRACT

Background: Dengue has emerged as a major public health concern throughout India because of the mortality and morbidity associated with it. It is the most common mosquito-borne viral disease of humans. Hence early and rapid laboratory diagnosis of dengue is crucial. This study aims to determine demographic, clinical and laboratory investigations of all the suspected cases of dengue fever and comparison of two commercial tests routinely useful in diagnosis of dengue fever. This study was conducted to determine seropositivity of dengue samples in patients suspected of dengue illness and to compare immunochromatographic card test (ICT) test and IgM ELISA test.
Methods: A total of 702 serum samples from patients with suspected dengue infection were included and the study was undertaken at department of microbiology at a tertiary care hospital, Hyderabad from July to December 2021. All samples were subjected to rapid ICT and confirmed by dengue IgM-capture ELISA.
Results: Out of 702 cases suspected of dengue, 85 (12%) samples were positive by IgM ELISA method. The most affected age group was 21-40 years with 55 cases (64.3%) were positive, followed by the age group 0f 0-20 years with 25% of the cases. Males were affected more than females with a percentage of 54% and 46% respectively. The highest number of suspected dengue patients admitted was in the month of September, i.e., 140 with 16 positive (14.81%) followed by August 122 samples (12.16%) and October 110 samples with 14 (11.03%) positive. The sensitivity and specificity of ICT was 95.5% and 100% when compared with IgM-ELISA.
Conclusions: Dengue cases were more during August to November in the monsoon and post monsoon season which is useful to plan special preventive strategies. This study draws attention toward the male, young and adult age group. To conclude, in countries lacking infrastructure for the diagnostic labs especially in the rural and remote areas, the rapid dengue ICT tests can play a major role in diagnosis and in patient management of acute dengue infection. The rapid ICTs are very simple, easy to perform, and can be used as point of care tests. We suggest that the rapid ICT for dengue detection may be used in patients presenting with febrile illness.

Keywords: Dengue fever, ICT, IgM-ELISA, Seroprevalence

INTRODUCTION

Viral hemorrhagic fevers are becoming increasingly common in the tropics and subtropics. 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 disease. Two-fifths of world's population or 2500 million people are now at risk for dengue and every year approximately 50 million new cases occur worldwide.[1]

Dengue virus was first isolated in India in the year 1945 and is endemic in both urban and semi-urban areas. Dengue fever has struck again in India and cases of dengue fever (DF)/dengue hemorrhagic fever (DHF) have

been reported from various parts of the country during the last 4 decades.[2] Dengue virus, belonging to the genus *Flavivirus* and family *Flaviviridae*, are mosquito borne viruses and the 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 4 serologically related viruses designated as DENV-1, DENV-2, DENV-3 and DENV-4.[3]

Primary infection with one of the four serotypes confers lasting immunity to that serotype. Secondary infection with a different serotype is associated with an increased risk of DHF.[4] Classical dengue fever is seen 4-6 days after an infective mosquito bite, with sudden onset of fever (often biphasic), severe headache, chills, generalized pains in muscles and joints, often associated with maculopapular rash. There is leucopenia, relative lymphocytosis, thrombocytopenia and hemorrhagic manifestations may occur. [5]

Non-structural protein 1 (NS1)-glycoprotein antigen which is abundantly produced by dengue virus in the early stage of infection can be detected in the serum or plasma of the patients.[6] After the onset of illness, the NS 1 antigen of virus can be detected in serum or plasma in 4-5 days. After 05 days i.e., at the end of the initial phase of infection, IgM/ IgG detection is choice for diagnosis.[7]

Aims and objectives

Detection of dengue infection by rapid immune-chromatography and by IgM ELISA and comparison of rapid ICT detection test and IgM ELISA test.

METHODS

This is a cross-sectional study was done at Premier hospital a tertiary care hospital from January 2021 to December 2021. Blood samples from 702 patients with clinical features suggestive of dengue, were included in this study. Samples were collected aseptically and serum was separated by centrifugation technique and stored at -70˚C. All samples put for dengue serology testing and subjected to Dengucheck Combo rapid method by Tulip diagnostics and IgM ELISA kit (Panbio).

Inclusion criteria

The clinical basis for diagnosing the patients as having dengue fever was based on standard criteria like presentation of febrile illness of 2-7 days duration, with features like headache, myalgia, arthralgia, rash, hemorrhagic manifestations and leucopenia.[8,9]

Exclusion criteria

Patients with clinical features of urinary tract infection, pneumonia, abscess or any other apparent cause of fever were excluded from study.[10]

Source of sample

The samples were received from outpatient department and from in-patients with features suggestive of dengue at Premier hospital, Hyderabad.

Dengue check combo rapid cassette method

Dengue check combo test is a rapid qualitative Immunochromatographic test system for detection of dengue NS1, IgM and IgG antibodies to dengue virus in huma serum or plasma. It consists of two devices held in tray, one device for detection of dengue NS1 and second device for differential detection of IgG and IgM antibodies. The detection system utilizes the principal of agglutination of antibodies/ antiserum with respective antigen in immune-chromatography format along with the use of nano gold particles as agglutination revealing agent. In each NS 1 and IgG/IgM device, a built-in control band in the control are a marked 'C' appears when the test has been performed correctly, regardless of the presence or absence of the dengue NS 1 antigen and or 'anti-dengue virus' antibodies in the specimen. It serves to validate the test performance of each device.

Procedure for dengue NS 1 antigen testing[11]

Holding the sample applicator (provided in the pouch) vertically, carefully dispense exactly 3 drops (75 µl) of the serum/plasma specimen into the specimen port 'S'. Immediately start the stopwatch and read the results at the end of 15 minutes.

For IgG/IgM dengue antibody testing[11]

By using precision micropipette carefully add 5 µl serum or plasma specimen into the specimen port 'S'. Add two drops of sample running buffer into the same specimen port 'S' and immediately start the stopwatch. Read the result at the end of 15 minutes.

Interpretation of results

Two pink bands appear in NS1 test device, at control line C and Est region T-early infection. Primary infection-two pink bands appear in the test region M and control area C. Secondary infection -two pink bands appear in IgM, IgG and control regions or pink band appears in IgG and control regions. Negative-A pink band appears in the control region only. Invalid-no pink band appears in control region.

Dengue antibody ELISA requirements

Anti-human IgM coated microwells (Assay plate), dengue 1-4 antigens (Recombinant), wash buffer concentrate-20X concentrate of phosphate buffered saline (PBS), pH 7.2-7.6 with tween 20 and 0.1% proclin as preservative, serum diluent-Tris buffered saline with preservatives and additives, antigen diluent-PBS with

preservative and 0.005% gentamycin, horse radish peroxidase (HRP) conjugated monoclonal antibody tracer, tetramethyl benzidine (TMB)- 3,3',5,5'-the substrate, tetramethyl benzidine, hydrogen peroxide in a citric-acid citrate buffer (pH 3.5-3.8), positive control serum, negative control serum, and cut-off calibrator-Human serum with 0.1% sodium azide and 0.005% gentamycin sulphate and stop solution-1Mole Phosphoric acid.

Dengue IgM capture ELISA procedure

Serum predilution

The microwells are inserted into the strip holder. 5 microwells are required for positive control (PC), negative control (NC) and cut-off calibrator (CO) in triplicate. The PC, NC and CO and patient samples are diluted using suitable test tubes or microtiter plate. The 1000 µl or 1 ml of serum diluent is added to 10 µl of serum and mixed well.

ELISA procedure: (instructions as per Panbio kit insert)

Antigen is diluted 1/250 using the antigen diluent. i.e., 10 µl of antigen + 2.5 ml of antigen diluent. A volume of 0.5 ml of diluted antigen is required per strip. Required volume of diluted antigen is mixed with equal volume of MAb tracer (Horse radish peroxidase conjugated Monoclonal antibody tracer) in a test tube and kept at room temperature (20- 25˚C) until required. The 100µl of diluted patient sample and controls (one positive control, one negative control and three cut-off calibrators) are pipetted into their respective microwells of the assay plate. 4. The plate is covered and incubated for 1 hour at 37˚C. After incubation, the plate is washed 6 times with diluted wash buffer. The antigen- MAb tracer solution is mixed well and 100µl is transferred to microtiter wells. The plate is covered and incubated for 1 hour at 37˚ C. The plates are washed 6 times with diluted wash buffer after incubation. The 100 µl of TMB (Tetramethylbenzidine) is pipetted into each well and a blue colour develops. The plate is incubated for 10 min at room temperature. At the end of 10 min, 100 µl of stop solution is pipetted into all wells. The blue colour will change into yellow. The absorbance of each well is read at a wavelength of 450 nm with a reference filter of 600-650 nm, using a dual wavelength spectrophotometer.

Calculation

The cut-off value was determined by calculating the average absorbance of the triplicate of the cut-off calibrator. The index value was calculated by dividing the sample absorbance by the cut-off value. Panbio units can be calculated by multiplying the index value by 10. Index value=sample absorbance/ cut-off value. Panbio units=index value×10. Test validity: calibrator mean ≥1.5×negative absorbance. Positive control=1.1-6.0 cut-off, negative control<0.350. Interpretation of results: Index Panbio units results 1.1 >11 positive. Sensitivity of this test is 94.7%, specificity is 100%.

Ethical considerations

Ethical clearance was taken from hospital ethics committee for the study. This study was conducted on the existing samples and no repeat blood sample was taken from the patient. All data were handled confidentially and anonymously.

Statistical analysis

Concordance, specificity and sensitivity with 95% confidence intervals, and positive predictive value (PPV) and negative predictive value (NPV) of the rapid ICT test were calculated by using IgM ELISA results as the reference test with the help of XLSTAT statistical software.

RESULTS

Out of 702 cases, 85 (12%) were found to be positive by IgM ELISA (Figure 1). 87% of positive samples were from outpatient department while 13% from inpatient cases (Figure 2). The most affected age group was between 21-40 years, with 55 (64.3%) positive samples followed by the age group of 0-20 years with 25% of all positive cases (Table 1). Males were affected more than females with a percentage of 54% and 46% respectively (Figure 3). 61% of positive samples were dengue fever with thrombocytopenia (DFT) i.e., platelet count less than 100,000/ml and 39% of dengue fever (DF) with platelet count more than 100,000 / ml (Table 2).

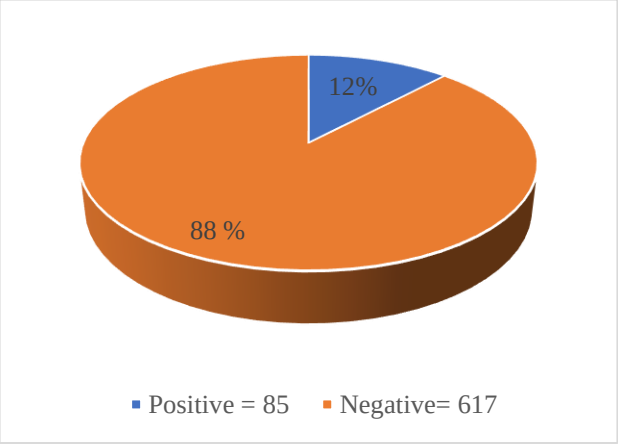

Figure 1: Seropositivity of dengue cases (total cases=702).

The highest number of suspected dengue patients admitted in the month of September, i.e., 140 with 16 positive (14.81%) followed by August 122 samples (12.16%) and October 110 samples with 14 (11.03%) positive (Figure 4). 702 samples were subjected for both the tests i.e., rapid ICT and IgM ELISA. For the 85

samples which were positive for IgM ELISA, 82 were positive by rapid ICT. Rapid ICT test was compared with the IgM ELISA. The result showed a sensitivity of 95.5%, specificity of 100 %, PPV of 100 % and NPV of 98.8% for the rapid ICT test as (Table 3).

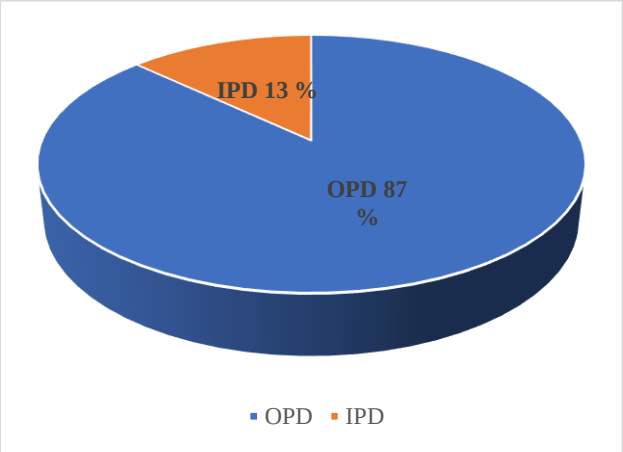

Figure 2: Ward wise distribution of cases.

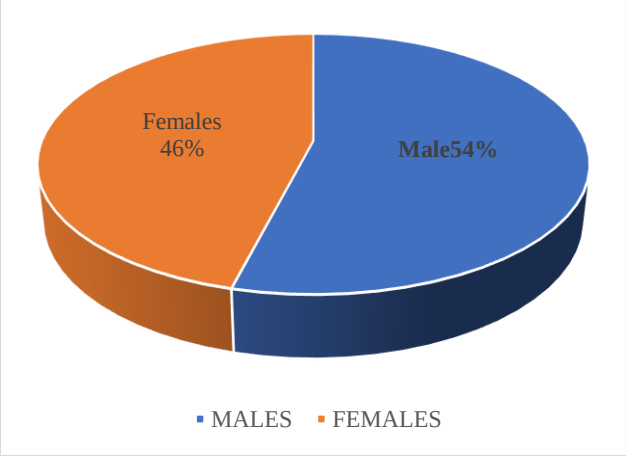

Figure 3: Gender wise distribution of cases.

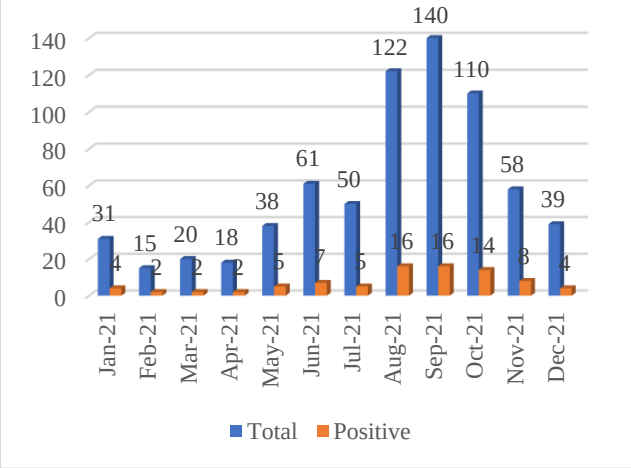

Figure 4: Month wise distribution of dengue cases in 2021.

Table 1: Age wise distribution of dengue cases.

Age group (Years)	N	Percentage (%)
0-20	21	25
21-40	55	64.3
41-60	06	7.1
61-80	03	3.6
81-100	00	0

Table 2: Platelet count in dengue positive patients.

Clinical manifestation (NRHM classification)	Platelet count (ml)	N	Percentage (%)
DFT	<100,000	52	61
DF	>100,000	33	39

Table 3: Sensitivity and specificity of ICT.

ICT	Sensitivity	Specificity	PPV	NPV
	95.5%	100%	100%	99.5%

DISCUSSION

The high prevalence of dengue cases in South India in the recent years, makes it necessary to evaluate the seroprevalence of dengue cases. The seropositivity of dengue cases in the present study among clinically suspected fever cases was 12% similar to a study by Kalpana et al with 8.46% seropositivity.[12] In our study highest prevalence was seen in the age groups between 21-40 years (64.3%) followed by the age group of 0-20 years (25%) of all positive cases. This was similar to the study conducted by Bharaj et al in which the common age group involved was 20-40 years (35.4%), followed by 0-20 years group (20.8%).[13] Another study by Gupta et al also showed that the maximum number of cases in a 3-year study period was seen in the 21-40-year age group.[14] In our study, an increased incidence of dengue was found among male patients 46 (54%), as compared to females 39 (46%). In the study done by Raja et al they observed an incidence of 51.55% in males and 48% in females.[15]

In present study, thrombocytopenia with platelet count of less than 1 lakh was seen in 52 cases (61%) and more than 1 lakh in 33 (39%) cases. In the study done by Min-Sheng et al at Taiwan, thrombocytopenia was seen in 78.9% of patients.[16] In the present study, a clear-cut increase in incidence of dengue cases was seen between August to December when South India receives heavy rainfall during monsoon. In a study conducted by John et al the incidence of dengue in Tamilnadu increased from June to December, confirming that the active transmission period is during monsoon and post–monsoon period.[17]

Out of the 702 samples taken in our study, 85(12%) were reactive for dengue infection by ELISA. Out of these 85 positive samples, 82 samples were reactive by rapid ICT

test. In 82 samples NS1 Ag was positive for 73, the rest of the positive samples were either positive for IgM alone or both IgM and IgG by ICT method. When rapid test was compared with IgM ELISA, 85 were true positives out of 702 samples and 617 were true negative while 3 were false negative and 0 were false positive. The specificity was 100%, PPV was 100%. This was in concordance with another similar study.[18]

Few drawbacks of ELISA are not only technical expertise but also requirement of fully equipped lab instruments like ELISA washer and reader. To make it a cost-effective larger quantity of samples are required during a single processing time. Rapid ICT on the other hand doesn't need technical expertise, individual test can be done this makes the procedure a cost effective one. The biggest advantage with ICT is shortest turn-around time with results available in minutes. And another advantage with ICT antigen detection is that it can be done with a single sample and doesn't need gathering of many samples as is the case with ELISA. Also, the combination test kits give the provision for performing NS1, IgM and IgG test in a single go.

Limitations

Our study showed that the rapid ICT kit that we used for testing NS1Ag and IgM performed well when compared with ELISA based tests. The sensitivity and specificity of various other kits in the market in a developing country like India vary widely and this needs to be kept in mind while choosing and performing the dengue diagnostic tests. An initial validation of the rapid kits with the ELISA will definitely help.

CONCLUSION

In countries lacking adequate infrastructure for the diagnostic labs, the rapid dengue ICT tests can play a major role in diagnosis and in patient management of acute dengue infection. The rapid ICTs are very simple, easy to perform, and can be used as point of care tests. We suggest that the rapid ICT Combo test for NS1Ag, IgM, IgG detection may be used in patients presenting with suspected dengue infection.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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