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Research Article

Incidence of dengue in a rural hospital, Chinakakani, Andhra Pradesh, South India and comparison of two commercially available enzyme linked immunosorbent assays with immunochromatographic rapid test

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ABSTRACT

Background: Dengue is one of the most serious mosquito borne arboviral infections affecting tropical and subtropical countries in the world. Since there is no immune prophylactic or specific anti-viral therapy available, timely and rapid diagnosis plays a vital role in patients management and implementation of control measures. This work has been taken up 1. To study the incidence of dengue cases in our rural hospital, Chinakakani, South India. 2. To compare the performances of Pan Bio capture ELISA [PanBio], J Mitra Microlisa [J Mitra] and SD BIO dengue duo rapid test [SD RT].
Methods: A total of 1180 serum samples from clinically suspected dengue cases were collected over a period of seven months. All the samples were subjected to NS1 antigen and IgM antibody Pan Bio ELISA. The same were tested for J Mitra Microlisa and SDRT and were compared with Pan Bio. Measure of discrimination i.e. sensitivity and specificity was calculated for each observed test value and an receiver operating characteristic [ROC] curve was constructed to compare the area under curve's [AUC] of different test kits thereby identifying the test with the best discriminative value.
Results: Out of 1180 samples tested, Pan Bio has shown an incidence rate of 284 [24.06%] (NS1 156+IgM 128), J Mitra 280 [23.72%] (NS1 156 + IgM 124) and SDRT 292 [24.74%] (NS1 156+IgM 136). As far as NS1 is concerned the same 156 samples were positive in all the three tests giving the sensitivity, specificity, positive and negative predictive values 100%. Remaining 1024 samples were negative for NS1. But this was not the case with IgM. AUC of IgM Pan Bio is 0.944 with sensitivity 90.32% and specificity 98.48%. AUC of IgM J Mitra is 0.932 with sensitivity 81.62% and specificity 98.75%. AUC of IgM SD RT is 0.992 with sensitivity 99.2% and specificity 99.1%. 'Z' test revealed that there is statistically significant difference between AUC's of SD RT when compared to Pan Bio (p value: 0.05) and J Mitra (p value 0.0001) The p values explain that SD RT is superior to Pan Bio and J Mitra in classifying between diseased and non-diseased.
Conclusion: High incidence rate was noticed in our region during monsoon and post-monsoon season which calls for timely preventive and control measures. SDRT is a valuable screening test in laboratories with minimal resources.

Keywords: Dengue, Capture ELISA, Microlisa, Rapid test, AUROCC analysis

INTRODUCTION

Dengue is one of the most serious mosquito borne arboviral infections.[6,16] Dengue infections are caused by four antigenically distinct dengue virus serotypes (DENV-1,2,3,4) and transmitted by the bite of an infected female mosquito- Aedes aegypti. Clinical spectrum of infection with dengue virus ranges from asymptomatic through

classical dengue fever to life threatening dengue hemorrhagic fever [DHF] / dengue shock syndrome [DSS].

According to WHO, 2/5th of worlds population are now at risk for dengue.[22] Gobally there are an estimated 50-100 million cases of dengue fever [DF] and several hundred thousand cases of DHF per year. There is an increase in global prevalence of dengue fever in recent decades particularly in the Americas and Western-Pacific with more serious manifestations in South-east Asia [7]. It is endemic in many parts of India and several epidemics too have occurred throughout the country. According to the National vector borne disease control programme, Directorate general of health services Ministry of Health and Family Welfare, India, an estimated 75,454 dengue cases and 167 deaths from India and 910 cases and 1 death from Andhra-Pradesh have been documented in 2013.[23]

In the absence of specific anti-viral and immune prophylactic therapy, vector control is the only method by which spread of dengue can be prevented. Effective control and preventive programmes depend upon improved surveillance data. Considering this as one of the objective, the present study was undertaken to bring out the incidence of dengue infection around NRI General Hospital.

The major diagnostic methods currently available are Viral culture, Viral RNA detection by reverse transcription-PCR [RT-PCR] and Serological tests such as capture ELISA (NSI, IgM, IgG). However all these assays have their own pit falls like restricted scope as a routine diagnostic procedure, low sensitivity, laborious procedure, time consumption and requires trained personnel.[9]

Now a days, detection of non-structural protein [NS1] antigen, Immunoglobulin M [IgM] and IgG antibodies on rapid tests offer an even faster route to a presumptive dengue diagnosis. NS1 is a highly conserved glycoprotein and is produced both in membrane associated and secretary forms by the virus.[3] The detection of this antigen represents a new approach to the diagnosis of dengue infection. Timely and rapid diagnosis plays a vital role in patients management. Keeping in view the performance of serological tests and their ease and rapidity to act as a point of care test [POCT] , the present study also aims to compare PanBio dengue capture ELISA (NS1, IgM), J Mitra Microlisa and SDRT.

Aims and Objectives

1. To study the incidence of dengue cases in our rural hospital [NRI General Hospital] Chinakakani. (by detecting NS1 antigen and IgM antibody in the serum of clinically suspected dengue cases)
2. To compare the performances of PanBio capture ELISA [PanBio], J Mitra microlisa [J Mitra] and immunochromatographic rapid test.

METHODS

Study design: Cross–sectional study.

Setting: NRI General Hospital, Chinakakani, Guntur District. Patient's visiting our hospital include not only from Chinakakani, but also from in and around rural areas.

Participants

OPD and indoor patients clinically suspected of dengue or DHF or DSS were selected.

Study period: Seven months, 1st of June to 30th of Dec, 2013.

Materials

In this cross-sectional study, taking aseptic precautions 2-3 ml of blood samples from clinically suspected patients of dengue infection were collected from June to December 2013 of OPD and indoor patients of our hospital. A total of 1180 samples were subjected to centrifugation at 3000 rpm for 5 mins. to separate the serum and stored at -70^{0}C. The serum samples were tested for NS1 antigen and IgM antibodies using three commercially available kits -

1. SD Bio Dengue Duo rapid test (SD RT)
2. PanBio dengue capture ELISA (Pan Bio)
3. J MITRA Microlisa (J Mitra)

Interpretation of tests

1. SD RT

- It is an invitro immunochromatographic one step assay designed to detect both dengue NS1 antigen and IgM /IgG antibodies to dengue virus in human serum, plasma or whole blood.
- The presence of each one colour line (Control) within the result window indicates a negative result.
- If the control line [C] and NS1 antigen line is visible on the test device, the test is positive for NS1 antigen and indicates early acute dengue infection
- If the control line [C] and IgM line are visible on the test device, the test is positive for IgM antibodies and indicates primary dengue infection
- If the control line [C] and IgG line are visible on the test device, the test is positive for IgG antibodies and indicates secondary or past dengue infection
- If the control line [C], IgM line and IgG line (C) are visible, the test is positive for both IgM and IgG antibodies and indicates late primary or early secondary dengue infection.

2. Panbio capture ELISA

- The principle used both for NS1antigen and IgM antibody is capture ELISA.
- Cut off value and index value are calculated according to the manufacturers instructions and results are expressed in Panbio units.
- If the dengue NS1 antigen and IgM antibodies units are < 9 sample is interpreted as negative for both.
- If the dengue NS1 antigen and IgM antibodies units are 9-11, the sample is interpreted as equivocal for both.
- If it is > 11 for both, then it is interpreted as positive for both.

3. J MITRA Microlisa

Dengue NS1 antigen Microlisa: Solid phase ELISA based on the "Direct sandwich" principle.

Dengue IgM Microlisa: Is an enzyme immunoassay based on MAC capture ELISA.

The cut off values and index values are calculated and expressed in units.

(<9-negative, >11-positive, 9-11 equivoal for NS1 antigen and IgM antibodies)

All the three tests were performed as per the manufacturers instructions and the results obtained were calculated accordingly.

Methods

Methods of comparison of the three kits

To perform any comparative study, confirmatory tests are taken as the gold standard. This applies to viral studies too, where viral culture, RT PCR or one of the molecular methods should be taken as standby to evaluate the commercially available kits. But, due to unavailability of such facilities in our hospital, to overcome this deficiency, we have constructed receiver operating characteristic [ROC] curve for comparing the performances of the three kits taking PanBio ELISA as (the gold standard) standby.

Statistical analysis

Analysis was done with the help of both descriptive and inferential statistics. In descriptive statistics bar diagrams and line diagrams were used to present the demographic data. In inferential statistics ROC analysis was used to compare the efficiencies of ELISA's and RT. Sensitivities and specificities of each kit were also calculated in ROC analysis. ROC curve was drawn between true positive rate and false positive rate.

'Z' test was used to evaluate the difference in the area under the ROC curves, level of significance was fixed as 0.05 and the test is one sided test. The above mentioned statistical analysis was carried out by using Medcal C statistical software, Version 13.3.1 (15 days trial version).

RESULTS

A total of 1180 serum samples were analysed from June 2013 to December 2013. We observed a good number of seropositive cases (NS1 & IgM) in the age group between 1-60 years with maximum seropositivity between 15-50 years [Table-1, Fig-1].

Table 1: Age wise distribution of NS1 and IgM positive cases.

Age	NS1 positive cases	IgM positive cases
1 -- 20	36	21
21--40	81	74
41--60	31	33
61--80	7	9
> 80	1	1

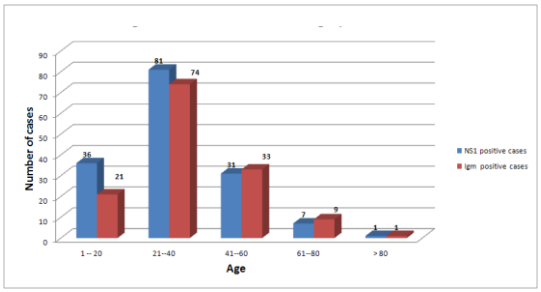

Figure 1: Age wise distribution of NS-1 and IgM positive cases.

Table 2: Gender wise distribution of positive cases.

	Number	%
Males	617	52.29
Females	563	47.71

Table 3: Gender wise distribution of positive cases in NS1 and IgM.

	NS-1		IgM	
Gender	Males	Females	Males	Females
Number of positive cases	103	53	85	51
% of positive cases	16.09%	9.41%	13.78%	9.06%

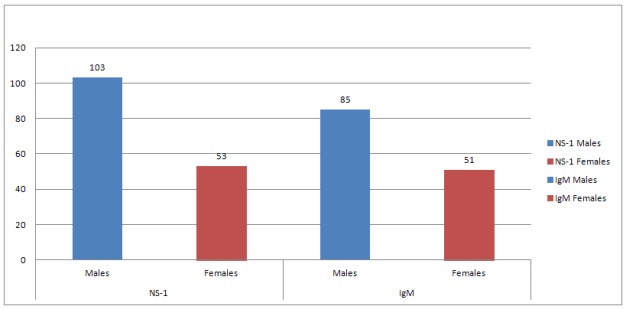

Figure 2: Genderwise distribution of positive cases in NS-1 and IgM.

There was male patient predominance 617 (52.29%) over female patient 563 (47.71%) accounting for 103 (16.69%) NS1 and 85 (13.78%) IgM positivity among males and 53 (9.41%) NS1 and 51(9.06%) IgM positivity among females [Table-2,3; Fig-2].

Table 4: Seasonal distribution of number of positive cases.

Season	Number	%
Pre monsoon	3	1
Monsoon	228	78
Post monsoon	61	21
Total	292	100

Table 5: Month wise distribution of number of positive cases.

Month	Number of cases
June	3
July	43
Aug	94
Sep	91
Oct	43
Nov	16
Dec	2
Total	292

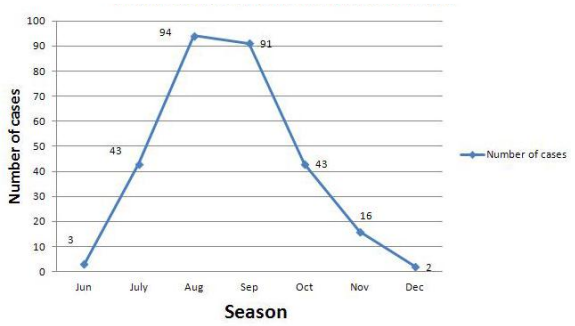

Figure 3: Seasonal Distribution of positive cases.

Coming to seasonal distribution, maximum number of seropositive cases 228 (78%) were observed during monsoon period (July, August, September) with decrease 61(21%) in the post monsoon period (October, November) [Table-4,5; Fig-3].

During this study period of seven months, 1180 serum samples were tested by subjecting them to Pan Bio, J Mitra and SD RT for NS1 and IgM parameters.

Out of 1180 samples, 156 (13.2%) were positive for NS1 and 128 (10.84%) were positive for IgM giving a total of 284 [24.06%] seropositive cases for Pan Bio. J Mitra has shown 156 (13.2%) NS1 and 124 (10.5%) IgM positives with total positivity of 280 (23.72%) and SD RT has come up with 156 (13.2%) NS1 and 136 (11.52%) IgM positives, giving a total positivity of 292 (24.74%) [Incidence rate].

As far as NS1 is concerned, out of 1180 samples, 156 samples were positive in all the three tests giving the sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV] 100% for all the three kits [Online sensitivity and specificity calculator]. Remaining 1024 samples were negative for NS1. But this was not the case with IgM.

8 samples were negative for IgM in Pan Bio, but were positive in both SD RT and J Mitra. Similarly 16 samples which were negative for IgM in J Mitra were positive in SD RT and Pan Bio. Moreover, the 24 samples [8+16] which were negative for IgM were not the same in any of the three tests. So PPV and NPV for IgM were excluded from discussion because confirmatory test was not taken as gold standard for comparison of the three kits. Instead Pan Bio was taken and ROC curve was constructed for analyzing the results.

Receiver operating characteristic [ROC] curve analysis:

ROC analysis was applied to evaluate the performance of ELISA's with RT. Area under the ROC curve was compared between the three kits, thereby sensitivity and specificity of the kits were obtained.

Range of area under the curve [AUC] in ROC analysis:

- 0.90-1 = Excellent in classifying between disease and non-diseased
- 0.80-0.90 = Good in classifying between disease and non-diseased
- 0.70-0.80 = Fair in classifying between disease and non-diseased
- 0.60-0.70 = Poor in classifying between disease and non-diseased
- 0.50-0.60 = Fail in classifying between disease and non-diseased

Comparison of IgM Pan Bio ELISA with SD RT: [Fig-4]

AU ROC curve of Pan Bio ELISA is 0.944. Sensitivity and Specificity: 90.32% and 98.48%.

AU ROC curve of SD RT is 0.992. Sensitivity and Specificity is 99.2% and 99.1%

The difference in AUC of SD RT and Pan Bio ELISA is evaluated with the help of 'Z' test

(Z value: 1.964). The difference in areas is statistically significant with p value 0.05.

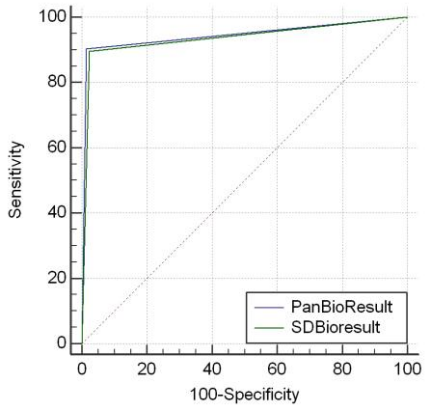

Figure 4: Comparison of IgM PanBio with SD RT.

Comparison of IgM J Mitra Microlisa with SD RT: [Fig-5]

AUC curve of J Mitra Microlisa is 0.932. Sensitivity and Specificity is 81.62% and 98.75%

AU ROC curve of SD RT is 0.992. Sensitivity and Specificity is 99.2% and 99.1%

'Z' value is 3.81 and p value is 0.0001. The difference is highly significant

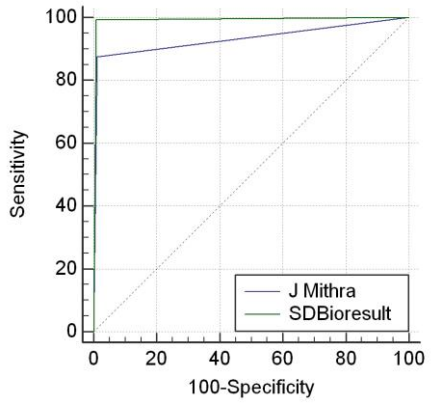

Figure 5: Comparison of IgM J Mitra Microlisa with SD RT.

Comparision of IgM PanBio with J Mitra: [Fig-6]

AUC curve of Pan Bio is 0.944. Sensitivity and Specificity is 90.32% and 98.48%

AUC ROC curve of J Mitra is 0.932. Sensitivity and Specificity is 81.62% and 98.75%

'Z' value is 4.625 and p value is < 0.0001. High statistical significance was noted here also.

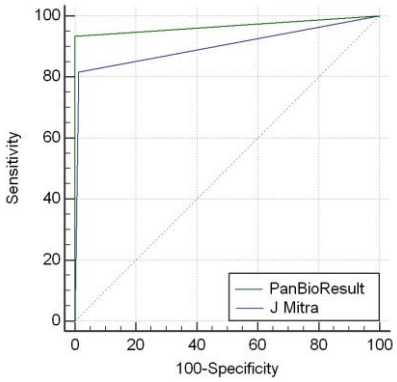

Figure 6: Comparison of IgM PanBio with J Mitra.

DISCUSSION

In our study though a good amount of seropositive cases were noted in the age group 1-60 years, maximum number is between 21 - 40 years. This is in accordance with some studies which have shown maximum number of cases between 15 - 30 years [24,25] and in few studies it was mainly a paediatric public health problem.[4] This could be attributed to the health care seeking behavior of the patients, changes in locations where disease transmission takes place and endemic nature of the infection.[19]

Male patient predominance is seen over female. The reasons may be – (1) Females stay at home and or less exposed to infection. [11,12] (2) Either immune response in females is competent than in males, resulting in greater production of cytokines, or the capillary bed of females is prone to increased permeability. Males predominate with milder disease but females account for more severe illness.[8]

Our study has shown maximum number of seropositive cases during monsoon (July, August, September) and postmonsoon (October, November) period of 2013. This is in par with other reported patterns of dengue transmission.[10,17,19] Major contributory factors to this may be -

(1) Breeding habit of Aedes aegypti which is highest during pre and post monsoon period. [20] Heavy rains result in stagnant water that serves as breeding ground for vectors of this virus and lead to increased activity. [4] (2) Geographical region with prime occupation of the people being agriculture. These months are paddy sowing months which needs large stores of water. But, sporadic cases extend upto december which indicates endemicity of infection upto December

In the present study, we have compared the performances of the three kits by studying the parameters NS1 antigen and IgM antibody. The reasons for selecting these two parameters were, (1) Our aim was to highlight the incidence [recent and new cases] of dengue cases (2) Our study included single sample study because of difficulty in follow up of cases. (3) The combination,increases the detection rate in identifying positive cases thereby increasing the diagnostic efficacy for early diagnosis of dengue infection.[2, 11, 15,20]

NS1 protein is essential for viral replication and the protein is detectable as early as first day till the ninth day of onset of fever. It doesn't appear to be hindered by the presence of anti-dengue IgM antibodies[1] and the amount of NS1 secreted in the sera of individuals infected with dengue directly correlates with viraemia.[14] During acute active infection, NS1 has shown promise as a novel diagnostic marker whatever the serotype responsible for the disease.[18] More over DENV-2 secretory NS1 levels have shown to be higher in patients with DHF making diagnosis by NS1 a valuable predictive tool for severe disease.[14]

Primary dengue infection is characterised by the presence of significant or rising levels of IgM 3-5 days [21] after the onset of infection which can persist for more than 3-5 months. In early and some secondary infections, detectable levels of IgM antibodies may be low. By this, we confirmly say that all the positive cases in our study belong to acute (NS1 positive) and primary (IgM positive) infection because all cases were either positive for NS1 or have shown very high titres of IgM antibodies. We have not included IgG because it is not sensitive early in the illness, requires paired specimens, lacks specificity (Cross- reaction with other flaviviruses) and also cost effective.

We made comparative evaluation of Pan Bio, J. Mitra and SDRT. SDRT showed 100% sensitivity and specificity for NS1 antigen and 99.2% sensitivity and 99.1% specificity for IgM. 'Z' test reveals that there is statistically significant difference between AUC's of Pan Bio and SD RT. SD RT seems to be better in classifying between diseased and non-diseased. The case was the same with J. Mitra and SD RT also.

The difference in AUC's of Pan Bio and J. Mitra were also evaluated, and it was noted that the 'Z' and 'P' values explain that Pan Bio is superior to J. Mitra in classifying between diseased and non-diseased.

In our study, the results of NS1 SDRT correlated with the Pan Bio and J.Mitra showing sensitivity and specificity of 100% [15]. These findings were in par with other published studies.[2] But, it was the result of IgM which differed in all the three tests. But, in spite of this, the results of SD RT IgM were superior to ELISA's with 8 negatives in Pan Bio and 16 negatives in J. Mitra and none in SD RT test.[3,15] This discrepancy between the tests may be attributed to the type of antibodies used, different principles of the assays, conjugates, stored samples and use of individual (single) sample.[10, 13]

The excellent sensitivities and specificities of the three kits for NS1 suggest that NS1 based may not be subject to the problems of cross – reactivity. Consequently, NS1 may prove to be a useful differential diagnostic tool in areas where flaviviruses are endemic.[13]

CONCLUSION

Among the kits evaluated, SDRT has proved to be the most efficient in confirming dengue infections. Moreso, it is convenient and easy to perform, results obtained in 15 minutes, doesn't require special equipment and trained personel. In hospitals with minimum facilities which lack RT PCR / viral culture, SD RT serves as an alternative and important tool. The addition of IgM window increases the detection rate thereby increasing the diagnostic efficacy for early diagnosis.

The high incidence rate in our region during monsoon and post monsoon season gives an alarm not only to the doctors regarding early and accurate diagnosis of dengue and its complications but also calls for timely preventive and control measures like fever alert surveillance, sentinel surveillance sites with laboratory support, strengthening of referral services, involvement of private sector in surveillance, epidemic preparedness and rapid response.

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