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Original Research Article

Detection of Dengue NS1 by a Comparative Analysis of Panbio Elisa and Rapid Diagnostic Test

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Abstract: Early diagnosis of Dengue, decrease the incidence of Dengue hemorrhagic fever, Dengue shock syndrome. With the quick diagnostic methods, initiating early treatment to dengue patients, can decrease the dengue mortality rate from 20-30% in severe cases to less than 1%. Here in this study we have tried to analyze the prevalence of dengue infection by identifying NS1 detection using Rapid test and ELISA. A Prospective study on Dengue NS1 detection was done for two months (October, November 2015) in the Department of Microbiology at Government Medical College, Anantapuramu. At Microbiology Laboratory, serum of all samples was assessed for NS1 detection using two tests (PANBIO ELISA test, J. Mitra Rapid Dengue test) simultaneously. All the results were analyzed, entered into spread excel sheet and were compared between two tests. Out of 885 serum samples collected from suspected cases of Dengue in and around Anantapuramu, 134 (15.1%) were positive for Panbio ELISA and by Rapid diagnostic test, 113 (12.7%) samples were positive. On assessment of sensitivity and specificity of two tests for detection of Dengue NS1, it was observed that there is a slight higher sensitivity and specificity using Panbio ELISA than Rapid Diagnostic Test kit. NS1 ELISA test takes several steps and more time. RDT's require very less time about 15-30 mins, single step procedure. Even though ELISA has superior performance than RDT. In countries with fewer infrastructures and in remote areas, RDT's are more useful for early diagnosis and management of dengue with less expertise within a short time.
Keywords: Dengue, ELISA, NS1, Rapid Diagnostic Test kit

INTRODUCTION:

Dengue is caused by four serotypes of dengue virus namely DEN-1, DEN-2, DEN-3 and DEN-4 and transmitted by Aedes mosquitoes (Aedes egypti is a main vector). Dengue virus is a single positive strand RNA virus, about 11000 bases genome, belonging to the family flaviviridae; genus flavi virus. Its genome codes for three structural proteins (capsid protein C, membrane protein M, envelope protein E), seven Non structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) and also short non coding regions on both the 5' and 3' ends [1, 2]. Dengue affects nearly 50-270 million people every year worldwide [3]. Dengue is an endemic disease and appears to be overtaking malaria in terms of morbidity and economic impact of the disease [4]. It has become an international public health concern, even travelers who travel to the dengue affected areas are expected the possibility of infection [5]. Most of the dengue cases are asymptomatic, which can also presents as dengue hemorrhagic fever and dengue shock syndrome [6]. Asymptomatic dengue cases are mostly seen in children and also adults with first infection [5]. Symptoms of dengue are fever, headache, fatigue, body pains, rash, petechiae and bleeding through nose, gums and gastrointestinal tract [7]. A number of atypical manifestations were also reported; include encephalitis, encephalopathy, myocarditis, hepatitis and cholecystitis [8].

In three countries (Braxil, Mexico, Philippines), one vaccine is currently approved. Still under trails by many private and public researchers [9]. Developing a vaccine against four different serotypes is quite a big challenge. Based on the clinical history it is difficult to diagnosis dengue, there is a much need of aid from diagnostic side for confirmation of dengue. Dengue can be diagnosed in various ways. Rapid, accurate diagnostic method is needed to evaluate dengue for initiating supportive therapies and early treatment. Early diagnosis also decreases the incidence of Dengue hemorrhagic fever, Dengue shock syndrome. With the quick diagnostic methods, initiating early treatment to dengue patients can decrease the dengue mortality rate from 20-30% in severe cases to less than 1% [10, 11].

During first five days of illness, dengue can be diagnosed by Antigen detection, Nucleic acid detection. Antibodies IgM, and IgG can be detected reliably after 3-4 days of post symptom onset [12-14]. RT-PCR (Reverse transcription - Polymerase Chain Reaction), IFA (Indirect Immunoflorescence Assay) are expensive and available only in higher centers. ELISA and (Enzyme Linked Immunosorbent Assay), RDT's (Rapid Diagnostic Methods) is the most commonly preferred method for both antigen and antibody detection (IgM and IgG).

NS1 (DENV Non-structural protein 1) found in both membrane and soluble forms which is highly conserved [15].During both primary and secondary Dengue infection, NS1 releases into human serum and increase in its concentration up to 50µg/ml [16-18]. Detection of NS1 helps to diagnose dengue early and also to predict the risk associated with Dengue such as Dengue Hemorrhagic Fever (DHF). NS1 antigen can be detected by ELISA and Rapid Diagnostic Tests. Here in this study we have tried to analyze the prevalence of dengue infection by identifying NS1 detection using Rapid test and ELISA.

MATERIALS AND METHODS:

A Prospective study on Dengue NS1 detection was done for two months (October, November 2015) in the Department of Microbiology at Government Medical College, Ananthapuram. Before starting the study, institutional ethical committee approval and informed consent from patients was taken.

Patients presented with clinical history of Dengue were selected to do this study. A total of 885 patients were advised to go with laboratory testing for confirmation of Dengue. Blood samples of patients who have come to General Medicine OPD within 5days of fever onset were collected and sent to laboratory for NS1 detection. At Microbiology Laboratory, serum of all samples was assessed for NS1 detection using two tests simultaneously.

Test 1: PANBIO ELISA test kit for detection NS1 - Serum samples about 100µl each, was assessed according to manufacturer's instructions using ELISA Reader and ELISA Washer. The test assessment takes around 2-3 hours

Test 2: J. MITRA RAPID DENGUE TEST Kit for detection of NS1 - Serum sample (50µl) was loaded into Rapid Dengue Test kit as per manufacturer's instructions. The test result can be read in 15-30 minutes. Both the test kits have to store at refrigerator 2-8^{0}C. All the results was analyzed and entered into spread excel sheet.

RESULTS:

A total of 885 serum samples were tested using two tests (Panbio ELISA and RDT) for Dengue identification. The Mean Age of Dengue patients were 29±3.05. Both female and male were almost equally affected with dengue. The Average days of Fever or dengue illness or post symptom onset was 2-3 days (Table No: 1). Most of patients belongs to middle class and upper lower class according to Modified Kuppuswamy's classification.

Table 1: Demographic data of selected population

Characteristic	No. of Patients	Percentage
Mean Age in years	29±3.05	
Sex		
Male	51	51%
Female	62	54.8%
Days of Fever/Dengue illness		
1-2 days	11	18.5%
2-3 days	55	48.6%
3-4 days	30	17.6%
4-5 days	17	15%

All serum samples were tested for NS1 using PANBIO ELISA and RDT. Out of 885 serum samples, 134 (15.1%) were positive for Panbio ELISA and by Rapid diagnostic test, 113 (12.7%) samples were positive (Table No: 2).

Table 2: Dengue NS1 analysis by Panbio ELISA and Rapid Diagnostic Test.

	PANBIO ELISA (n=885)		RDT (n=885)	
	No. of positives	**Percentage**	**No. of positives**	**Percentage**
Dengue NS1 Antigen	134	15.1%	113	12.7%

On analyzing, statistical significance between Panbio ELISA test and RDT test, shown Extremely Statistical significant. Using Graph pad software, p value was <0.0001 by Fischer's exact test (Table No:3). On assessment of sensitivity and specificity of two tests for detection of Dengue NS1, it was observed that there is a slight higher sensitivity and specificity using Panbio ELISA than Rapid Diagnostic Test kit.

Table 3: Showing significance of Panbio ELISA and Rapid Diagnostic Test kit

	RDT		Total
Panbio ELISA	**Positive**	**Negative**	
Positive	106	28	134
Negative	7	744	751
Total	113	772	885

The sensitivity and specificity of Panbio ELISA for NS1 detection was 92.9% and 97.8% and of RDT kit for NS1 detection was 88% and 97.8%.

DISCUSSION:

In this study the Mean Age of Dengue patients was 29±3.05. Both female and male were almost equally affected with dengue, as there is slight female preponderance. The Average days of Fever or dengue illness or post symptom onset were 2-3 days. Subhamoy pal *et al.;* [19] also observed that the mean age of patients suffering with dengue was 30 years and was distributed more evenly between male (51%) and female (54%). Ankita Nisarta *et al.;* [20] also reported that females were more commonly affected than males, with male to female ratio of 1:1.35. In contrast to our study, Male preponderance was observed by Gupta *et al.;* [21], chakravarthi *et al.;* [22]. Most of the studies shown maximum sensitivity of NS1 detection in 2-4 days [19]. During primary infections, NS1 levels peak around 4-5 post symptom onset, but it wanes earlier during secondary infections [23].

Many diagnostic methods are assessing for early diagnosis of dengue. Few studies recommend NS1 antigen detection and few studies suggests antibody detection is better for dengue diagnosis. Now-a-days detection of NS1 is an emerging diagnostic method, as NS1 starts appearing right at the beginning of feverish period and also appears before IgM appears in serum samples [24]. Contrast to this, other studies suggests a combination of NS1 and IgM detection during the first few days of illness to increase the sensitivity for dengue diagnosis [25, 26]. Out of 885 serum samples, 134 (15.1%) were positive for Panbio ELISA and by Rapid diagnostic test, 113 (12.7%) samples were positive. A study by Mahesh Reddy *et al.;* [27] observed the overall prevalence of Dengue as confirmed by ELISA was 46.1%. Ankita Nisarta *et al.;* [20] observed that 23.3% of dengue positive cases, among 90 serum samples and also documented that 58% positive samples were from 16 to 35 years of age group. Similar dengue prevalence was reported by Garg [28] and paramasivan [29].

As per this study, on assessment of sensitivity and specificity of two tests for detection of Dengue NS1, it was observed that there is a slight higher sensitivity and specificity using Panbio ELISA than Rapid Diagnostic Test kit. The sensitivity and specificity of Panbio ELISA for NS1 detection was 92.9% and 97.8% and of RDT kit for NS1 detection was 88% and 97.8%. Both tests on comparison are extremely statistically significant. Sensitivity and specificity of different tests varies between companies. Subhamoy pal *et al.;* [19] documented that the overall sensitivity of RDT's of various companies ranged from 71.9% - 79.1% and the sensitivity of ELISA's varied between 85.6%-95.9%. Mahesh Reddy R *et al.;* [27] also done a comparative analysis of Dengue NS1 ELISA and NS1 RDT and reported as sensitivity of 90.11%, specificity of 98.45%, positive predictive value of 98.15% and Negative predictive value of 91.57%. Ankita Nisarta et al [20] studied on Dengue NS1 ELISA with sensitivity of 95.8%, 54.3% specificity, 81.6%positive predictive value, 88.9% negative predictive value.

ELISA shows good sensitivity, yield is best, so it is recommended diagnostic method to use in fully equipped laboratories with ELISA washer and reader and need of well trained personnel. In ELISA large number of samples can be run simultaneously. RDT's need less expertise and can complete within minutes. Detection of NS1 can also be done in vector population, while this test helps in improvement of both clinical management and vector surveillance. Even though ELISA has superior performance than RDT. In countries with fewer infrastructures and in remote areas, RDT's are more useful for early diagnosis and management of dengue with less expertise within a short time.

CONCLUSION:

On comparison of Panbio ELISA and Rapid Dengue Test kits, ELISA has slightly higher sensitivity and specificity than single step Immunochromatographic test kit. NS1 ELISA test takes several steps and more time. RDT's require very less time about 15-30 minutes, single step procedure. RDT's are more useful in resource limited settings and can also carry easily to remote areas for diagnosing dengue.

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