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SEROPREVALENCE AND COMPARISON OF RAPID IMMUNOCHROMATOGRAPHIC TEST AND ELISA FOR THE DETECTION OF NS1 ANTIGEN FOR THE EARLY DIAGNOSIS OF DENGUE FEVER

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ABSTRACT

***Background**: Dengue is one of the rapidly emerging global threats. Many outbreaks are being noticed nowadays all around the world. In situations of epidemics and routine cases, early diagnosis is the key to successful management of dengue cases. Many diagnostic kits are available commercially for the same purpose. But their validity is unknown. The standard is ELISA, though it is time consuming. In the present study, the test results of commercially available rapid immune chromatographic card test(Dengue Day 1 Kit, J.Mitra) is compared with ELISA(J. Mitra) as the standard for the detection of NS1 antigen. **Aims:**1. Screening the blood samples for dengue virus infection in suspected patients. 2. To compare rapid immune chromatographic test assay with ELISA for detection of NS1 antigen for early diagnosis of dengue infection. **Setting and Design:** It was a cross-sectional study conducted from December 2012 to August 2014 at Shri B M Patil Medical College, Hospital & Research Centre, BLDE University, Bijapur. **Materials & methods:** Probable dengue cases were diagnosed as per the WHO criteria and rapid immune chromatographic card test and ELISA were conducted on the same serum samples for the detection of NS1 antigen and results were analyzed. **Statistical analysis**: Sensitivity, Specificity was calculated. Statistical analysis was done by software-SPSS17 Version. **Results:** A total of 90 probable dengue cases were selected. 25 (27.8%) cases were positive by rapid test as well as ELISA. The sensitivity of rapid test was 100%& specificity was 100%. **Conclusion:** The study showed that the rapid card test has sensitivity of 100%&specificity 100%. Thus the card test can be used for screening. Highly suspicious cases should be subjected to investigations with standard test ELISA.*

KEY WORDS

Dengue, ELISA, NS1 antigen, Rapidimmunochromatographic test

INTRODUCTION

Dengue fever is an important arthropod borne viral disease of public health significance. In recent decades the global prevalence has grown dramatically with estimated 2.5 billion people at risk of acquiring dengue viral infection and more than 50 million new infections projected annually.[1] In 2013, dengue ranks as the most important mosquito-borne viral disease in the world. The emergence and spread of all four dengue viruses ("serotypes") from Asia to the Americas, Africa and the Eastern Mediterranean regions represent a global pandemic threat. Although the full global burden of the disease is

still uncertain, the patterns are alarming for both human health and the economy.[2]According to World Health Organization estimates, the incidence of dengue disease has increased by a factor of 30 over the past 50years.[3] An increased disease burden has been linked to the resurgence of mosquito vector Aedesaegypti, overcrowding, urbanization and increasing travel. Despite its significant health and economic impacts, as of yet there is no specific treatment or therapy for dengue infection and the outcome depends on medical care provided by the doctor to the patient.[4]

Dengue virus is flavi virus which is enveloped, single stranded positive sense RNA virus. The genomic RNA is 11 kb long and contains 10 genes encoding 3 structural proteins capsid (C), membrane(M), an envelope(E) and seven non structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).Dengue symptoms range from mild fever , the most common form, to potentially fatal dengue haemorrhagic syndrome(DHF) and dengue shock syndrome(DSS) or encephalitis and hepatitis.[4]

Currently the three basic methods used by most laboratories for the diagnosis of dengue virus infection are virus isolation, detection of viral genomic sequence by a nucleic acid amplification technology assay (reverse transcription polymerase chain reaction) and detection of dengue virus specific IgM antibodies by the IgM capture enzyme linked immunosorbentassay (MAC-ELISA) and/or the rapid dengue immunochromatographic test (ICT).[5]

A rapid test kit would be useful to provide early diagnosis of acute dengue infection.[6] The rapid test does not involve any specific laboratory equipments except micro centrifuge for serum separation. So the rapid test may prove to be useful aid in screening, in clinical diagnosis of dengue infection, more so in the resource poor peripheral health setting. It can prove to be a useful tool to hasten the initiation of the first line of the management and thereby can be great help to the health care providers in the rural area.[7]

Hence this study was carried out to evaluate the performance of a rapid immunochromato graphic test device for the detection of NS1 response in comparison with enzyme linked immunosorbent assay (ELISA) for NS1 detection for the early diagnosis of dengue infection.

An accurate clinical assessment of all included cases was also performed during the study, since it was necessary for the proposed case definition of suspected dengue patients.

MATERIALS & METHODS

Inclusion criteria for this study were fever less than 7days, two or more of the following manifestations like headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestation, leucopenia.

Exclusion criteria were focal source of infection (otitis media, pneumonia, meningitis), chronic illness including anemia and unstable vital signs.

METHOD OF COLLECTION

Study design:

Cross-sectional study from December 2012 to August 2014. The study was conducted at Shri B M Patil Medical College, Hospital & Research Centre, BLDE University, Bijapur.

Ethical clearance was taken for this study from the ethical committee of BLDE University.

A total of 90 clinically suspected dengue viral fever satisfying inclusion and exclusion criteria were included in the study. History was taken from the patients.

Blood samples were collected after taking informed and written consent from study participants or from parents/guardian in case of children. 2ml of Blood was collected following venepuncture with all aseptic precautions in plain red vacutainers in adults as well as children. This was sent to laboratory immediately and the serum was separated. Serum samples were removed from the clot as soon as possible to avoid haemolysis. Rapid kit test (Dengue Day 1 test kit, J mitra) was performed and the serum was frozen at -20˚C for performing ELISA (NS1, J.mitra, 48 tests kit).

Rapid immunochromatographic card test and ELISA were conducted on the same serum samples for the detection of NS1 antigen as per manufacturer's instructions.

Dengue Day 1 Kit test:

Dengue Day 1 Test kit (J. Mitra) is a rapid solid phase immunochromatographic test for the qualitative detection of Dengue NS1 Antigen. 1 kit was used at a time for single sample.

Dengue day1 test foil pouches & test specimen was kept at room temperature before tests were performed. Once the assay started, the complete procedure was done without interruption.

Dengue NS1 antigen device:

Dengue NS1 Antigen device contains two lines; 'C' (Control line) & "T" (Dengue NS1 Antigen test line). Test line is coated with anti-dengue NS1 Ag.

2 drops of sample was added using dengue antigen test sample dropper to the sample well of antigen device. Reaction was allowed to occur for 20minutes. Results were read at 20 minutes. Dengue NS1 antigen if present in the sample will bind to the anti-dengue NS1 gold colloidal conjugate making antigen antibodies complex. This complex migrates along the membrane to the test region and forms the visible pink line at "T" as antibody-antigen-antibody gold colloid forms. Interpretation of test was as per manufacturer's instructions.

NS1 Ag MICROLISA:

1 ELISA KIT (48 test pack each) was used at a time. The frozen serum samples were allowed to thaw in a vertical position in the rack.

NS1 serotype specific IgG ELISA was performed as follows,

Components & test specimen was kept at room temperature before tests were performed. Once

the assay started, the complete procedure was done without interruption.

Stripholder was fitted with 48 number of anti dengue NS1 antibody coated strips. The assay control wells were arranged in vertical configuration.

First 50µl of diluent was added in all wells, then 50 µl of negative control, 50µl of positive control, then 50µl of calibrator was added. Then samples of 50 µl in all the other wells. Meanwhile fresh working conjugate was prepared and 100µl of working conjugate was added in all the wells. Thorough mixing of control and samples with the conjugate was done. Cover seal was applied and incubated at 37˚C for 90min. While being incubated working wash solution was prepared with the reagents. After 90 min of incubation, taking out the plate, washing of the wells 6 times with working wash solution was done. Then 150µl of working substrate solution was added in each well & incubated at room temperature for 30min in dark, and then stop solution 100µl was added. Absorbance was read at 450nm within 30minutes in ELISA reader. Interpretation of test was as per manufacturer's instructions. Results were analyzed.

RESULTS

Statistical analysis: Sensitivity, Specificity was calculated. Statistical analysis was done by software-SPSS17 Version.

A total of 90 serum samples were collected from dengue suspected patients.

In our study, incidence of dengue infection was 28%. Majority of the cases i.e 21 (23.3%) of the dengue suspected cases belonged to age group of 11-20 yrs & 21-30 yrs followed by 16 (17.7%) cases belonged to age group of 1-10yrs. Among males 15 (25.42%) cases belonged to 21-30yrs of age followed by 13 (22.03%) cases were of age group of 1-10yrs. Among females 9 (29.03%) cases belonged to age group of above 11-20yrs followed by 7 (22.5%) belonged to age group of >50yrs [**Table 1**].

Males (65.6%) were affected more than females (34.4%). Among the males involved in the study, 76% were positive and among females, 24% were positive by rapid test.[Table 2].

Out of 90cases, 25 (27.8%) were NS1 antigen positive and 65(72.2%) were NS1 antigen negative by both rapid test and ELISA [Table 3].The sensitivity and specificity of the test was 100%.

DISCUSSION

Dengue virus infection was first reported in India from Chennai in 1780. Today dengue virus and all its clinical forms are documented in almost all parts of India.

In the present study, a total of 90 serum samples from patients with clinical features of dengue were analyzed for Dengue rapid test & ELISA for detection of NS1 antigen.

Present study showed that majority of the cases i.e 21 (23.3%) of the dengue suspected cases belonged to age group of 11-20yrs & 21-30yrs.

This was comparable to other studies of Gore MM[8], Raju BJ[9], NeerjaM[10] and Dash PK[11] et al. The disease was predominantly seen in case of males (59%) than females (41%) i.e 1.4: 1 which was corresponding to the other studies done by Raju B[9], Dash PK et al[11], Vijaya kumar TS et al[12], Neeraja M et al[10].Male preponderance and the age group of 15-30 years indicate more transmission of dengue infections at work sites.[14]In a study conducted by Kamal S et al[13] females were more commonly affected.

In our study the incidence of Dengue was 28%. Low incidence (8.69%) was recorded by Banerjee et al[15] from Lucknow in 2005 & high incidence (62.2%-70%) was recorded by Huber K et al[16] from South Vietnam in 1996-97. This increase in incidence might be explained by the possible impact of ecological characteristics of the areas on the natural cycles of the arthropod-borne viruses under consideration.[17]

In the present study out of 90 cases involved, 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by rapid test, & 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by ELISA which is similar to StephenS[18] but study by Dussart P et al[4]has shown high positivity with rapid test as compared to ELISA.

Table 1: Age wise distribution of cases w.r.t sex

Age	Total cases	Male	Female
0-10yrs	16 (17.7%)	13 (22.03%)	3 (9.6%)
11-20yrs	21 (23.3%)	12 (20.33%)	9 (29.03%)
21-30yrs	21(23.3%)	15 (25.42%)	6 (19.3%)
31-40yrs	09 (10%)	06 (10.1%)	3 (9.6%)
41-50yrs	10 (11.1%)	07 (11.8%)	3 (9.6%)
>50yrs	13 (14.4%)	06 (10.1%)	7 (22.5%)
Total	90	59	31

w.r.t – with respect to

Table 2: Sex wise distribution of Dengue positive & negative cases

Sex	Dengue positive	Dengue negative	Total
Male	19 (76%)	40 (62%)	59
Female	6 (24%)	25(38%)	31
Total	25	65	90

Table 3: Distribution of Dengue cases in Rapid test & ELISA w.r.t NS1 Antigen

NS1 antigen	Rapid test	ELISA
Positive	25 (27.8%)	25 (27.8%)
Negative	65 (72.2%)	65 (72.2%)
Total	90	90

w.r.t – with respect to

CONCLUSION

The rapid test can be used for screening for the early diagnosis of dengue infection. But highly suspicious cases should be subjected to investigations with standard test ELISA.

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