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Comparison of Diagnostic Modalities in Early Dengue in a Tertiary Hospital in Mumbai

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ABSTRACT

Background: Dengue is regarded the most common arboviral infection in the world, which is transmitted by the bite of the Aedes mosquito. It usually causes a flu like illness, occasionally causing potentially fatal complications. Since dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Objective: The study was done to assess the performance of rapid NS1 Ag and NS1 ELISA in comparison to Dengue PCR in acute dengue cases. **Methods:** A comparative retrospective analysis of 200 dengue cases, who have been diagnosed by Rapid NS1, ELISA and PCR was done. The rapid tests were performed using Dengue NS1 Rapid test (Aspen) and ELISA by Recombilisa (CTK Biotech Inc, San Diego) and PCR test by TaqMan based real time RT-PCR. **Results:** The sensitivity and specificity of NS1 rapid was 81.33% and 58% respectively, while positive and negative predictive value were 85.31% and 50.87%. The sensitivity, specificity, positive predictive value and negative predictive value of NS1 ELISA were 82.66%, 66%, 87.94% and 55.93% respectively. The positivity of NS1 rapid was 71.5%, NS1 ELISA was 70.5%, and that of PCR was 75%. Overall positivity was higher in males than females. Higher positivity was seen in the age group 21-30 years. **Conclusion:** The overall performance of RT-PCR was better than NS1 antigen detection test. The sensitivity and specificity of various kits vary widely and this needs to be kept in mind while choosing and performing the dengue diagnostic test.

Keywords: Dengue, ELISA, NS1 Ag, PCR.

INTRODUCTION

Dengue is regarded the most common arboviral infection in the world, mainly in the tropical and subtropical countries, which is transmitted by the bite of the Aedes mosquito. There are 5 serotypes of Dengue virus 1-5, DENV-5 being discovered in 2013.[1] It belongs to the Flaviviridae family. It usually causes a flu like illness, occasionally causing potentially fatal complications like Dengue shock syndrome or Dengue haemorrhagic fever. As per World Health Organisation (WHO), dengue has shown a 30 fold increase incidence since last five decades, with 40% of the population at risk of infection, and causing around 20,000 deaths annually worldwide.[2] The incidence of dengue cases in India has grown over the years causing around 90,000 cases in 2018, leading to a death of 150 patients.[3] Severe dengue (previously known as dengue haemorrhagic fever) was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand.[4] Despite a risk of infection which exists in 128 countries, 70% of the actual burden is borne by Asia.[5]

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The primary mode of transmission of DENV between humans involves mosquito vectors (Aedes species). There is evidence however, of a possibility of maternal transmission (from a pregnant mother to her baby), occasionally resulting in preterm birth, low birth weight and fetal distress.[2] The induction of vascular permeability and thereby shock leading to severe dengue depends on multiple factors, such as.[6]

1. The presence or absence of enhancing and non-neutralizing antibodies,
2. Age (susceptibility to severe dengue drops considerably after 12 years of age)
3. Sex (females are more affected than males)
4. Race (whites more than blacks)
5. Nutritional status (malnutrition is protective)
6. Or sequence of infections (e.g., dengue virus 1 infection followed by dengue virus 2 infection seems to be more dangerous than dengue virus 4 infection followed by dengue virus 2 infection).[6]

Secondary infection of pre immune individuals with a different DENV serotype could exacerbate rather than mitigate disease, a phenomenon that is thought to be caused by non-neutralising antibodies and is termed antibody-dependent enhancement (ADE) of disease.[7] Several weeks after convalescence from primary infection with dengue, the transient

protection conferred by primary infection against reinfection with a heterotypic dengue virus usually wanes. Heterotypic reinfection may result in classic dengue or, less commonly, in severe dengue.[6]

Since dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Virus detection and serological conversion have been the main diagnostic modalities for many years, however cross-reactivity of antibody responses among the flaviviruses serves as a confounding issue in providing a differential diagnosis. A definite diagnosis includes isolation of the virus itself (virus isolation in culture or mosquitoes or the direct detection of viral genomic RNA), viral products (capture and detection of the secreted NS1 protein), or the host immune response to virus infection (through measurement of virus-specific IgM and IgG).[8]

There is no specific treatment for dengue, but early detection and access to proper medical care lowers fatality rates below 1%. Treatment is usually supportive management and that of the co-morbidities. The first dengue vaccine, Dengvaxia (CYD-TDV) developed by Sanofi Pasteur was licensed in December 2015 and has now been approved i around 20 countries.[2] The vaccine is targetted for persons living in endemic areas, ranging from 9-45 years of age, who have had at least one documented dengue virus infection previously.[2]

Aim and objective:

To assess the performance of Rapid NS1 Ag and NS1 ELISA in comparison to Dengue PCR in acute dengue cases.

MATERIALS AND METHODS

After obtaining Institutional Review board approval, a comparative retrospective analysis of 200 dengue cases, who have been diagnosed by Rapid, ELISA and PCR over a period of 4 months from July2018-October 2018, was initiated in the Microbiology Department of a tertiary level suburban teaching hospital in Mumbai,India. The rapid tests were performed at the department laboratory using Dengue NS1 Rapid test (Aspen) based on immunochromatography principle and ELISA by Recombilisa (CTK Biotech Inc, San Diego). The PCR test was performed at the reference molecular diagnostic laboratory by TaqMan based real time RT-PCR.

Study procedure:

Total 200 whole blood samples from patients were processed for detection of dengue virus antigen. Dengue NS1 Rapid test (Aspen) using immunochromatography principle and ELISA by Recombilisa (CTK Biotech Inc, San Diego) and PCR by TaqMan based real time RT-PCR.

Dengue NS1 Ag Rapid test

The Dengue NS1 rapid test is a qualitative, membrane based immunoassay for the detection of NS1 antigen in human serum/plasma. The rapid test membrane is pre-coated with a NS1 specific antibody on the test line region and utilizes a separate control to assure assay flow and performance. During testing, the test sample is added directly to the sample well followed by 2 drops of buffer. The buffer and sample mix and interact with NS1-specific monoclonal antibodies conjugated to gold nanoparticles. The solution migrates upward on the membrane (via capillary action) to react with the anti NS1 antibody on the membrane. If NS1 antigen is present, a pink/purple line will appear at the test line region. The pink/purple line at the control line region should always appear if the assay is performed correctly. The results are read after 15 minutes. Results obtained after 30 minutes are not valid.

Dengue NS1 Ag ELISA

The Recombilisa Dengue NS1 Ag ELISA is a solid-phase enzyme-linked immunosorbent assay based on the principle of the antibody sandwich technique for the detection of dengue NS1 antigen in human serum or plasma.

The Recombi LISA Dengue NS1 Ag ELISA is composed of two key components:

1. Solid microwells pre-coated with anti-pan dengue NS1 antibody,
2. Liquid conjugates composed of monoclonal antibodies recognize NS1 antigen from DENV1, 2, 3 and 4 conjugated with horseradish peroxidase (HRP-anti-dengue NS1 conjugates).

During the assay, the test specimen is first incubated in the coated microwell. The dengue NS1antigen, if present in the specimen, binds to the antibody coated on the microwell surface, and any unbound specimen is then removed by a wash step. During a second incubation with the HRP-anti-dengue NS1 conjugates, the dengue NS1 antigen adsorbed on the surface of microwell binds to antibody in the HRP conjugate, forming a conjugate complex. Unbound conjugates are then removed by washing. After addition of the TMB substrate, the presence of the conjugate complex is shown by development of a blue colour resulting from a reaction between the enzyme and substrate. This reaction is then quenched by addition of the stop solution, and the absorbance value for each microwell is determined using spectrophotometer at 450/620-690 nm.

Real time polymerase chain reaction (RT-PCR)

RNA extraction

Real time RT-PCR was done in 200 samples. RNA was extracted using the Techne qPCR kit for Dengue subtyping genomes, as per manufacturer's instructions.

Real time RT-PCR

The extracted RNA samples (n=200) were subjected to real time RT-PCR developed by Gurukumar et al targeting the 3′ UTR gene sequence.[9] The primers and probe used targeted a stretch of nucleotides conserved in the dengue virus serotypes. The probe used was TaqMan MGB probe labelled with FAM at 5′end and non-fluorescent quencher at 3′end. Cloned and amplified target sequence RNA as a positive control, and no template control were included in each run. The real time RT-PCR primers, probe, enzyme mix, reaction buffer and positive control were provided by National Institute of Virology, Pune, India. The reaction mixture containing 20µl of master mix and 5µl of sample (or control) RNA was subjected to the following reaction conditions in ABI 7500 real time PCR system: reverse transcription at 42° C for 10 mins, enzyme activation at 95 ° C for 2 mins, denaturation at 95 ° C for 10 mins and 50 cycles of 95° C for 15sec and 60° C for 1 min. The test was considered valid if positive control showed a CT value 28+/-3 are within normal limits.

Statistical analysis

Assessment of efficiency, sensitivity, specificity, positive and negative predictive values of the Rapid and ELISA were calculated by Fisher's exact test taking PCR as gold standard.

RESULTS

Table 1: Comparison between NS1 Rapid and NS1 ELISA

	Rapid NS1 (%)	ELISA NS1 (%)
Sensitivity	81.33	82.66
Specificity	58	66
Positive Predictive Value	85.31	87.94
Negative Predictive Value	50.87	55.93

Table 2: Positivity of NS1 rapid, ELISA, PCR tests.

Results	NS1 rapid	ELISA	PCR
Positive tests n (%)	143 (71.5%)	141 (70.5%)	150 (75%)
Negative tests n (%)	57 (28.5%)	59 (29.5%)	50 (25%)
Total N (%)	200 (100%)	200 (100%)	200 (100%)

[Table 2] Shows that the positivity of NS1 rapid was 71.5% (143/200), NS1 ELISA was 70.5% (141/200), and that of PCR was 75% (150/200).

[Table 3] shows that PCR has highest positivity 151/200 (75.5%), followed by NS1 Rapid (72%), followed by ELISA 142/200 (71%). 13/200 (6.5%) showed false positivity i.e positive by NS1 Rapid but negative by ELISA and PCR

Table 3: Showing test wise analysis of the results of all the samples.

No of tests (n = 200)	NS1 Rapid test	ELISA	PCR
103	Positive	Positive	Positive
19	Negative	Negative	Negative
6	Negative	Negative	Positive
8	Positive	Positive	Negative
13	Positive	Negative	Negative
22	Negative	Positive	Positive
9	Negative	Positive	Negative
20	Positive	Negative	Positive
Total = 200	144/200	142/200	151/200

The results were calculated using diagnostic tests that considered sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) as follows:

Sensitivity: a/a + c × 100%;

Specificity: d/d + b × 100%;

Negative predictive value: d/d + c × 100%;

Positive predictive value: a/a + b × 100%,

Where: a = number of true positives, b = number of false positives, c = number of false negatives, and d = number of true negatives. The rapid NS1 antigen and NS1 early ELISA were compared with RT-PCR.

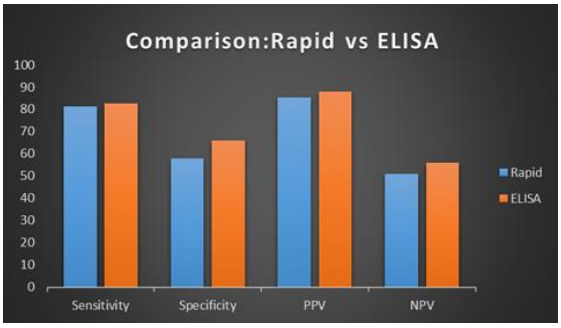

Figure 1: Comparison between NS1 Rapid and NS1 ELISA.

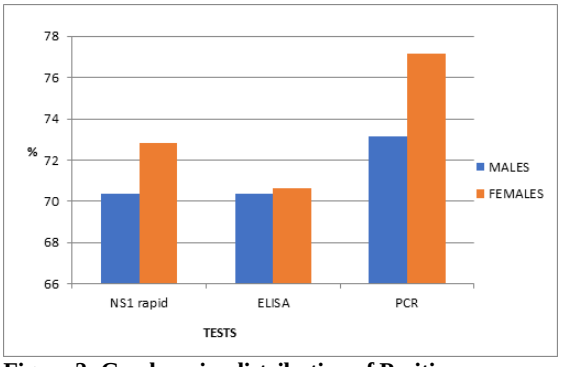

Figure 2: Gender wise distribution of Positive cases.

The sensitivity and specificity of NS1 rapid was 81.33% and 58% respectively, while positive and negative predictive prevalence were 85.31% and 50.87% in comparison to PCR. The sensitivity, specificity, positive predictive value and negative predictive value of NS1 ELISA were 82.66%, 66%, 87.94% and 55.93% respectively. The comparison between both is elicited in [Figure 1 and Table 1]. Male preponderance was found as shown in [Figure 2]. Maximum number of cases who were NS1

antigen positive were in the age group of 21-30 years showing 31 (21.68%) positive cases, as shown in [Figure 3]. The overall performance of RT-PCR was better than NS1 antigen detection test, as seen in [Table 2].

[Figure 2] Shows that the overall positivity was higher in males 108 (54%) as compared to females 92 (46%).

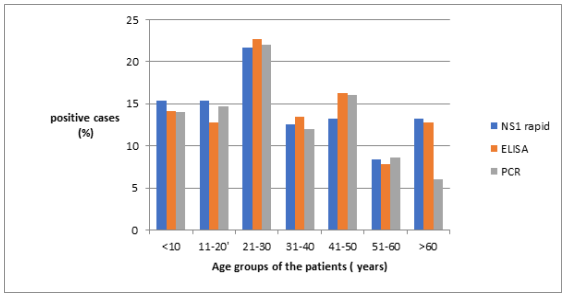

Figure 3: Age wise distribution of positive cases.

[Figure 3] shows that the higher positivity was in the age group 21-30 years.

DISCUSSION

Acute dengue is highly endemic in India. The usual symptom of dengue infection can be easily misdiagnosed with other infections prevalent during the monsoon season like malaria, typhoid fever, chikungunya etc. It is acknowledged that the management of dengue fever is conservative; nevertheless, strict monitoring of clinical condition and hematological parameters is required to prevent complications, which makes early diagnosis pertinent. Furthermore, early diagnosis plays a crucial role in forecasting a timely warning of an epidemic and in undertaking effective vector control measures.[10]

NS1 protein is highly conserved for all dengue serotypes, circulating in high levels during the first few days of illness. There is no cross-reaction of dengue NS1 protein with those of other related flaviviruses.[11] NS1 antigen detection ELISA can be implemented in routine diagnostic laboratories and can be easily automated. The test has great potential value for use in epidemic situations, as it could facilitate the early screening of patients and limit disease expansion.[10]

Virus isolation is carried out only by reference laboratories and is a time-consuming and expensive technique. The use of dengue RT-PCR in most laboratories is currently difficult, due largely to the cumbersome procedure, difficult interpretation and the time taken.

We have evaluated the utility of real time RT-PCR, which is more sensitive, can be automated, interpretation is easier and the hands on time is less. The real time RT-PCR can also be used to quantitate the viral load in blood samples, making it a useful tool to investigate the role of viremia in pathogenesis of dengue.[10]

The specificity and sensitivity of NS1 Rapid test in our study was 58% and 81.33%, whereas it was 100% and 73.53% in the study of Nishant et al (2014).[10] The specificity and sensitivity of ELISA in our study was 66% and 82.66%, whereas Nishant et al reported as 100% and 79.41% respectively.[10]

When the rapid NS1 antigen was compared with NS1 antigen capture ELISA, it showed that the sensitivity of NS1 ELISA was slightly higher than NS1 rapid, however specificity of ELISA was significantly higher in comparison to Rapid test. A study by Najiuollah et al found the sensitivities of ELISA and Rapid as 61.2% and 49.4% respectively.[12] A study by Subhamoy Pal et al found a significant higher sensitivity of NS1 ELISA in comparison to NS1 Rapid, however there wasn't any difference between the specificities.[13] However a study by Xiaoyun Shan et al found a greater sensitivity of NS1 Rapid in comparison to NS1 ELISA.[14]

Maximum number of cases who were NS1 antigen positive were in the age group of 21-30 years showing 31 (21.68%) positive rates, as against R. Mahesh Reddy et al found maximum NS1 antigen positive cases in the age group of 10-50 years of age.[15] Minimum age at which dengue NS1 antigen detected was in a 12months old child as against Harshavardhini Palanivel et al, who found dengue NS1 antigen positive in 6 month old child.[16]

The overall positivity was higher in males (108/200 (54%) as compared to females (92/200 (46%), similar male preponderance was found by Reddy et al and Pal S et al.[15,13]

In our study we found PPV of rapid NS1 antigen was 85.31%, which corroborates with other studies, which have shown the PPV of rapid NS1antigen test to be more than 80%.[15] This indicates that the probability of patient having acute dengue infection, if the NS1 rapid tests are positive is almost the same as the ELISA based tests. The rapid NS1 antigen test have major advantage in that, they are easy to perform, they need less expertise and can be done within minutes.

CONCLUSION

Early and accurate laboratory diagnosis of DENV infection is critical to effective patient management and can be done using several tests. The choice of tests however depends upon the purpose of the test (clinical, epidemiological survey), type of laboratory facilities and technical expertise, costs and time of collection.

In our study, we found that the overall performance of RT-PCR to be better than NS1 antigen detection test. The sensitivity and specificity of various kits in the market today vary widely and this needs to be

kept in mind while choosing and performing the dengue diagnostic test.

In the future, newer modalities like microsphere based immunoassay (MIC), biosensor technology and microarray techniques need to be explored for precise and early diagnosis.

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