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Original Research Article

Utility of rapid diagnostic kit tests for diagnosis of suspected dengue fever

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ABSTRACT

Background: Dengue fever often presents as an undifferentiated febrile illness requiring a laboratory test for identification. Serological tests particularly on rapid kits for the detection of NS1Antigen, IgG and IgM antibodies are the most commonly performed test across the country.
Methods: The serum samples of suspected dengue cases were tested by Rapid test kits for assessing all the three parameters as well as by ELISA for NS1 antigen test. The platelet count of the patients was obtained from automated coulter counter. The results thus obtained were analyzed in Excel format.

Results: The serum samples from 304 suspected Dengue fever cases were received in the lab, of which 190 samples were positive either by rapid or ELISA and 176 when rapid card test was considered alone Highest seropositivity of dengue cases were observed in the age group of ≥60 years (79.2%) followed by 45-59 years (70.7%). On rapid test, 78 cases were NS1 antigen positive of which 60 cases were positive only for NS1 antigen. When NS1 rapid and ELISA tests when compared, 16 kit negative tests were positive on ELISA while 34 kit positive tests were ELISA negative. Sensitivity, specificity, PPV and NPV when only NS1 was considered on rapid test kits when compared with ELISA were 78.9%, 87.8%, 63.8% and 93.8%. 33.5% of serologically positive cases of Dengue had low platelet count on admission while only among negative cases 17.2% had a low platelet.

Conclusions: Rapid kits often show variable results thus needing a validation of them from end user. As a positive dengue test result is an essential prerequisite for diagnosis thus it is essential that for serological tests ELISA technique should be used for reporting. Thus, it also mandates more efforts at decentralization of NVBDCP to include both government and non government institutions.

Keywords: Dengue fever, ELISA, Rapid diagnostic test kits, Serological tests

INTRODUCTION

Dengue, a viral infection often presents as an acute febrile illness and is endemic to the Indian sub-continent.[1] It has a wide spectrum of manifestation in humans varies from inapparent infection to mild fever to potentially fatal dengue shock syndrome. Dengue virus is a single-stranded, positive sense enveloped RNA virus belonging to the family Flaviviridae. Japanese encephalitis (JEV), chikungunya (CHIKV) and West Nile virus (WNV) are often indistinguishable clinically thus needing a laboratory confirmation of dengue suspected cases.[2] Serological diagnostic methods to ascertain presence of

NS1, IgM and IgG are the best available tests and are often carried out in laboratories across the country by Rapid immunochromatographic tests. The present study attempts to compare the results of the easy to perform ICT tests with that of a more demanding ELISA method.

METHODS

Serum samples were collected from clinically suspected cases of dengue patients from July to November 2018.These the months of wide seroprevalence of Dengue virus with a notable number of cases in our area. Patients with established cause of acute febrile illness like malaria, chikungunya and typhoid were excluded from the study.

NS1 antigen along with IgM and IgG antibodies were detected using the rapid test kit by J. Mitra. The card contains 2 wells one for NS1 Antigen and other for IgG and IgM antibodies. 10µl of serum was placed in each of the wells and readings interpreted after 20min. The appearance of control band along with the test band for NS1 antigen in the first cassette or IgM and/or IgG antibodies in the second was interpreted as positive. The results were considered negative if one line "C" appeared in result window for both these casettes and invalid if control line failed to appear.

ELISA was done for detection of NS1 Antigen at Capital Hospital, Bhubaneswar, a designated NVBDCP centre for Dengue serological testing and reporting.

Platelet count was done from blood collected in EDTA vials by CBC counter and whenever necessary were rechecked manually by examination of smears. Count <100,000/cmm was interpreted as abnormal (WHO cut off for platelet count for DHF).

Data thus obtained were analyzed and interpreted in Microsoft Excel format.

RESULTS

The serum samples from 304 suspected dengue fever cases were received in the lab. Of them, 138 were males while 116 were females. Most common (56.9%) of samples were in age group of 15-44. But highest seropositivity of dengue (by any of the two methods used) cases were observed in the age group of ≥60 years (79.2%) followed by 45-59 years (70.7%) (Table 1).

Of these 190 samples were positive either by rapid or ELISA. When rapid card test was considered alone, 176 samples were found to be positive (Table 2). 78 cases were NS1 antigen positive. 60 cases were positive only for NS1 antigen while 18 cases were positive for NS1 antigen as well as antibodies.

When result of rapid tests were compared with NS1 ELISA 10 cases which were negative by rapid tests on both antigen and antibody detection were positive on ELISA while a vast number of kit positive tests (126) turned out to be ELISA negative. Sensitivity, specificity, PPV and NPV of rapid test kits when compared with ELISA were 85.7%, 65.9%, 32.3% and 96.1% (Table 3).

Table 1: Demography of the patients.

Age in year	Male		Female	
	Total	Positive	Total	Positive
0-1	05	02	02	02
2-14	28	14	14	05
15-44	104	73	69	34
45-59	38	25	20	16
≥60	13	13	11	06
Total	188	127	116	63

Table 2: Result of rapid tests.

Rapid test	No. of positives
NS1 only	60
IgM only	14
IgG only	0
NS1 and IgM	14
IgM and IgG	05
NS1, IgM and IgG	04
Total	176

Table 3: Comparison of rapid tests with ELISA.

	Rapid Tests		NS1 Rapid	
NS1 ELISA	+	-	+	-
+	50	10	44	16
-	126	118	34	210

NS1 rapid and ELISA tests when compared, 16 kit negative tests were positive on ELISA while 34 kit positive tests were ELISA negative. Sensitivity, specificity, PPV and NPV when only NS1 was considered on rapid test kits when compared with ELISA were 78.9%, 87.8%, 63.8% and 93.8%. Tabulated chi square statistic is 89.0776 and the *p*-value is significant at $p<0.05$ (Table 3).

Table 4: Comparison of platelet count with serology of dengue.

Platelet count (in lakhs)	Positive serology	Negative serology
<1	64	20
>1	126	94

The chi-square statistic is 89.0776. The *p*-value is significant at $p<0.05$. 33.5% of serologically positive cases of dengue had low platelet count on admission while only among negative cases 17.2% had a low platelet. Of the patients who were positive for NS1 Ag only 42% had an associated thrombocytopenia. But of

those having IgM positivity only 22.9% had associated thrombocytopenia (Table 4).

DISCUSSION

Early and accurate diagnosis of dengue fever is important to direct clinical attention to the appearance of major warning signs of severe or even life-threatening complications as well as to prevent unnecessary antibiotic usage. Virus isolation and Dengue PCR are methods of choice for detection but they are limited by the cost and ease of availability of the technology to the end user. Thus, the serological diagnostic methods to ascertain presence of NS1 antigen and IgM, IgG antibodies are the tests available in peripheral laboratories across the country for diagnosis of dengue fever.

NS1 Ag, highly conserved glycoprotein produced in both membrane-associated and secretion forms, is abundant in the patient's serum during early stage of infection.[3] Several studies reveal the detection rate of NS1 Ag is higher in acute primary dengue than in acute secondary DI.[4-6] Libraty et al, observed that a very high concentration of NS1 antigen within 72 hours of illness identified patients at risk of developing DHF.[7]

A case is labelled as "probable dengue case" if he satisfies the clinical criteria during dengue outbreak or positive on non-ELISA based immuno-chromatography tests (ICT) for NS1 antigen (Ag)/IgM ("National Guidelines for Clinical Management of Dengue Fever, Dec 2014.") The diagnosis is "confirmed" when NS1 Ag/Ig M is positive by ELISA or fourfold rise of IgG titre is demonstrated or viral nucleic acids or virus itself is isolated.[2]

Our locality has been reporting high prevalence of dengue fever during the months of July to October, the period during which the study was carried out. In this study the incidence of positive tests from clinically suspected dengue cases was 62.5% when positivity by any of the two methods was considered. This is higher than those of other studies.[8,9] 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.[10]

Rapid ICT need very little technical expertise to perform with a turnaround time of minutes in contrast to approximately 4hrs for ELISA. A single sample whether serum/plasma/whole blood can be run without waiting for the samples to be gathered and processed as in ELISA. As an added advantage in the rapid test kits there is provision for performing both NS1 Antigen, IgM and IgG tests at once.[11,12] Limitations of RICT include improper storage conditions giving rise false results. At times the very faint bands are seen, but these indicate a positive test. It is a common mistake to read these as negative. Our laboratory being a NABL accredited laboratory all parameters like storage conditions and uniformity of interpretation was all well taken care of.

Sensitivity, specificity, PPV and NPV of rapid test kits in our study when compared with ELISA were 85.7%, 65.9%, 32.3% and 96.1% respectively. A meta-analysis[13] have reported accuracy of RICTs being highly heterogenous with sensitivity ranging between 45-100% and specificity range of 57-100% when compared with ELISA. Other studies have reported, 100% concordance between SD RICT and Panbio ELISA for detecting NS1 Ag in primary and secondary dengue infections.[14]

Study by Selvaraj et al, also showed a sensitivity and specificity of 97.54% and 98.33%, respectively for SD RICT when compared with Panbio ELISA for detection of NS1Ag.[15] Many other studies also show the PPV of rapid ICTs to be more than 85%.[13,16,17] It is said that NS1 Ag gives positive results in first 4 days of illness, there is no need of repeat testing as NS1 is highly specific marker for the diagnosis of Dengue infection.[18] Among the IgG and IgM antibodies, as both clinical and subclinical infection can produce IgG, which may persist for several years affecting the interpretation of testing results.[19] Further obtaining paired sera for confirmation is often not possible. Thus, it is very important that a test of optimum sensitivity and specificity at least for NS1 be used for the diagnosis.

The manufacturer of the RICT kit used in our laboratory claims to have a sensitivity of 96%, specificity 98%. This varies from the values obtained by the study. As the sensitivity and specificity of various kits available in the market in a developing country like ours 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. As per the rule of the state every sample of dengue in any laboratory by needs to be confirmed by ELISA in a government approved centre before handing out the results forbidding many tertiary care centers to conduct routine ELISA for dengue testing.

In the present study 42% of cases which were positive for NS1 Ag only were having thrombocytopenia. Only 22.9% cases with IgM of positivity had associated thrombocytopenia. On the other hand, association of thrombocytopenia with IgM was found to be higher in other studies.[20] This is probably due to the associated scare of dengue in the rainy months which has leads to early attendance in clinics, thus an early diagnosis. Studies claim that in addition to an early diagnosis, NS1 antigen may be an indicator of disease severity.[21]

In this work the association of low platelet and the NS1 positivity of the patient with development of further complications have not been considered, which may be a drawback in our case.

CONCLUSION

This study reiterates the fact that the accuracy of rapid diagnostic kits vary. Dengue presents as an undifferentiated acute febrile illness. Keeping in mind the necessity of an early and accurate test to detect dengue, all cases should be screened by ELISA instead of RICT kits. If these rapid kits are being considered it should be validated in the laboratory before use.

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