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Original Research Article

Dengue Diagnostic Test for the Resource Constraint Developing World: Validity of Rapid Immunochromatographic Card Test against ELISA

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Abstract: Dengue infection is a serious global public health problem, and the dengue viruses are widely distributed throughout the tropical and subtropical areas of the world. In the Indian subcontinent, the epidemiology of dengue fever has been very complex. High prevalence rate in our region particularly in pre-monsoon and monsoon season is alarming and necessitates early and accurate diagnosis of dengue virus infection to prevent its complications and further morbidity. Now-a-days detection by rapid tests offers an even faster route to a presumptive dengue diagnosis. Hence, this study aims to assess the agreement between the rapid immune-chromatographic card test (RICT) for dengue and antibody capture ELISA, the latter being used as gold standard. A prospective study was conducted from July to November 2014 and July to November 2015 in a secondary care hospital. Probable dengue cases were diagnosed as per the WHO criteria and RICT and ELISA were conducted on the same serum samples for the detection of NS1 antigen and IgM & IgG antibodies. The results were analyzed. The sensitivities of RICT for NS1antigen and IgM & IgG antibodies were 100%, 89.6% and 81.3%, respectively and all showed 100% specificity. In the present study, the accuracy parameters were comparable with ELISA. Also, RICT met the WHO ASSURED criteria, hence this study recommends introduction of RICT in all levels of health care system for an early and accurate diagnosis and confirmation of the clinical suspicion of dengue**.**

Keywords: Dengue, rapid immunochromatographic card test, ELISA, serological markers.

INTRODUCTION

Dengue infection is a serious global public health problem, with 2.5 billion people at risk and an annual range of 50 to 390 million infections, which include dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [1-4]. Dengue viruses, transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, are widely distributed throughout the tropical and subtropical areas of the world [5] and have four distinct dengue virus serotypes (dengue virus 1, 2, 3, and 4) [6]. If the patient is infected a second time with a different serotype, a more severe disease, dengue hemorrhagic fever (DHF) or dengue shock syndrome(DSS), is more likely to occur.

The epidemiology of dengue fever in the Indian subcontinent has been very complex and has substantially changed over past six decades in respect to prevalent strains, affected geographical locations and disease severity. The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proven epidemic of dengue fever in India occurred in Calcutta and Eastern Coast of India in 1963-1964 [7-9]. Though dengue affects urban and suburban population, outbreaks have been reported in rural areas of northern, western and southern India, probably due to the changing life style of the rural population as a result of urbanization process [10]. National Vector Borne Disease Control Programme, reported an annual average of 60,792 dengue cases and less than 1% dengue related deaths between 2010 to 2016.

High prevalence rate in our region particularly in pre-monsoon and monsoon season is alarming to the health professionals and necessitates early and accurate diagnosis of dengue virus infection to prevent its complications and further morbidity. Since there is no immunoprophylaxis or specific antiviral therapy available, timely and rapid diagnosis plays a vital role

in patient management and implementation of vector control activities in the community so as to mitigate further transmission [11].

It is well established that enzyme linked immunosorbent assay (ELISA) is a valuable screening test for the detection of antibodies to dengue, in diagnostic laboratories because of its high sensitivity and specificity rates. However, it is time consuming and a relatively costly procedure and facilities may not be available in peripheral primary and secondary health care centres to diagnose dengue.Now-a-days detection by rapid tests offers an even faster route to a presumptive dengue diagnosis. Hence, this study aims to assess the agreement between the rapid immunochromatographic card test (RICT) for dengue and antibody capture ELISA.

MATERIAL AND METHODS

The validity of card test against ELISA (gold standard) was tested over a total 780 blood samples obtained for suspected dengue cases during monsoon and early post monsoon, over a period of two years, from July to November 2014 and July to November 2015. Patients of all age groups, clinically suspected of dengue, presenting within 1-9 days of fever were included in the study, which was carried out in a secondary care hospital. The serum was separated and subjected to RICT as well as ELISA for detection of NS1Ag and IgM & IgG antibodies depending on duration of fever. The accuracy indices of test performance were calculated. Results of both methods were compared. Other relevant clinical information including past medical history, history of travel and vaccination record were recorded.

Inclusion Criteria

All patients presenting to the OPD or admitted in the hospital with fever and clinically high suspicion of dengue fever (1-9 days of the onset of fever, headache, retro-orbital pain, rash or myalgia/arthralgia) or DHF(patients with symptoms of DF and one or more of the following symptoms: thrombocytopenia, platelets < 1,00,000/cmm or evidence of capillary leak with hematocrit rising >20%) were included in the study .

Exclusion Criteria

Patients with established cause of acute febrile illness like malaria, chikungunya and typhoid were excluded from the study. We included 30 controls, 15 from other PUO cases and 15 healthy individuals. The tests were performed adhering to the kits' specifications.

Ethical considerations

Blood collection was not done specifically for research/study purpose. All the analyzed samples were being used for diagnosis purpose required by the care centre for any patient presenting- dengue like symptoms. All patients (or their parents in case less than 18 years of age) were informed prior regarding the test and had given no oral objection.

Rapid Immunochromatic Test: SD BIO LINE dengue Duo-Standard Diagnostics, Korea) (dengue NS1+ Ab Combo)

This rapid kit has two separate cassettes, one for detection of NS1 antigen and another for IgM and IgG antibodies. 10ul of serum was placed in each of the wells and readings interpreted after 20 min. The appearance of control band along with the test band for NS1 antigen in the first cassette and 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 and invalid if control line failed to appear. Sera were stored at –20°C for further testing by ELISA.

Panbio Australia Dengue Early ELISA for NS1 & Dengue IgM and IgG Capture ELISA

It is a qualitative test. Once the NS1 antigen/IgM/IgG antibody in patient's serum was bound, conjugated monoclonal antibody was detected by adding the tetramethylbenzidine (TMB) substrate. The reading was taken at 450 nm wavelength with 620 nm reference filter. The cut-off value, index value and Panbio units were calculated for each sample. Panbio units of ≥11 were taken as positive and ≤9 as negative. Panbio units of 9-11 were considered equivocal and were repeated twice.

RESULTS

Out of a total of 780 suspected cases of dengue, 101 cases (12.9%) tested positive with age ranging from 2 years old female child to 78 year old male. An increased incidence was observed in 2^{nd} – 4^{th} decade (66.33%) followed by the 1^{st} and 5^{th} decade (25.74%). Only 7.92 % of the febrile elderly tested positive for dengue. Overall, there was a male preponderance (Table 1).

Table 1: Age and sex distribution of dengue positive cases

Age	Male	Female	Total	Percentage (%)
0-10	7	6	13	12.87
11-20-	12	8	20	19.8
21-30	15	10	25	24.75
31-40	12	10	22	21.78
41-50	5	8	13	12.87
51-60	2	2	4	3.96
61-70	2	1	3	2.97
71-80	1	0	1	0.99
Total	56	45	101	

The cases peaked in the months from July to September and decreased through October to November. All patients presented with fever with myalgia, morbiliform/erythematous rash and headache were most common associated symptoms. Gastrointestinal manifestations, nausea vomiting, abdominal pain and hepatomegaly were present in less than half patients. Twenty seven patients (7 children and 20 adults) had associated mild to moderate thrombocytopenia and they showed spontaneous recovery. One 5yr old and two elderly (68 yrs & 72 yrs old) presented with dengue haemorrhagic fever (DHF) manifesting with epistaxis and severe thrombocytopenia. The two elderly patients had secondary dengue infection. All three were referred to tertiary care level hospital for further management (Table 2).

Table 2: Major signs and symptoms in Dengue

Clinical manifestations *(n=101)*	Percentage *n=101*	Percentage(*No*) 0-10years	Percentage(*No*) 11-50years	Percentage(*No*) 51-80years
Fever	100	100 (13)	100 (80)	100(8)
Myalgia	98	84.6 (11)	100 (80)	100(8)
Headache	80.2	38.4 (5)	85 (68)	100(8)
Nausea/Vomiting	34.7	38.4 (5)	31.2 (25)	62.5(5)
Abdominal pain	11.8	15.4 (2)	8.7(7)	37.5(3)
Hepatomegaly	41.6	46.2 (6)	33.6(34)	37.5(3)
Bleeding manifestations	2.9	7.6 (1)	-	25(2)
Rash	92	100(13)	90(72)	87.5(7)

RICT results were categorised depending upon the negativity/positivity of NS1 antigen either alone or with IgM and/or IgG and results compared with dengue ELISA kit which was kept as the standard due to its higher sensitivity and specificity (Table 3).

Table 3: Comparison of seropositivity between RICT and Dengue ELISA

Grouping of patients based on Panbio ELISA	RICT SD(NS1)	RICT SD(IgM)	RICT SD(IgG	PANBIO (NS1)	PANBIO (IgM)	PANBIO (IgG)
NS1 only positive n= 68	68	0	0	68	0	0
NS1 and IgM positive n= 10	10	8	0	10	10	0
NS1, IgM and IgG positive n= 3	3	3	3	3	3	3
IgM and IgG positive n= 9	0	8	6	0	9	9
IgM positive n= 7	0	7	0	0	7	0
IgG positive n= 3	0	0	2	0	0	3
NS1 and IgG positive n= 1	1	0	1	1	0	1

Out of these 101 cases, 82(81.2%) were positive by both RICT and Panbio ELISA for NS1. IgM antibody was detected in 29(28.71%) cases by Panbio ELISA and IgG antibody in 16(15.84%) cases whereas RICT detected 26(25.74%) IgM and 13(12.87%) IgG antibody positive cases. Three IgM and four IgG

antibody positive cases were missed by RICT. All 30 controls were negative for NS1Ag, IgM and IgG by the two diagnostic methods. In our study, the sensitivity and specificity of RICT when compared with Panbio ELISA for detecting NS1 Ag was 100% while, Panbio ELISA was more sensitive, specific with higher diagnostic accuracy(100%) for detecting IgM and IgG antibodies as compared to RICT (Table 4).

Table 4: Accuracy indices of serological markers by RICT

	NS1 Ag	IgM	IgG
Sensitivity(%)	100	89.6	81.3
Specificity(%)	100	100	100
PPV(%)	100	100	100
NPV(%)	100	99.6	99.6

The statistical significance between RICT and ELISA was calculated by Fisher Exact Test. The *P* values however, for RICT and Panbio ELISA were <0.01, which is statistically insignificant. Thus, the performance of both kits was comparable. Kappa coefficient was calculated to evaluate the concordance between the RICT and panbio ELISA, which came out to be 1 for NS1, 0.95 for IgM and 0.96 for IgG.

DISCUSSION

Dengue virus infection has emerged as a notable public health problem in India in recent decades and has become endemic with outbreaks occurring frequently and explosively almost annually [12].

It is considered to be the most important arthropod-borne viral disease due to the human morbidity and mortality it causes [13]. Dengue virus infection produces a broad spectrum of symptoms, many of which are non-specific. Thus, a diagnosis based only on clinical symptoms is unreliable. Early laboratory confirmation of clinical diagnosis is valuable as some patients may progress over a short period from mild to severe disease and sometimes to death. Early intervention may be life saving [14].

The major diagnostic methods currently available are viral culture, viral RNA detection by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and serological tests such as Non Structural Protein (NS1), IgM Capture & IgG Capture ELISA(enzyme linked immunosorbent assay). However early dengue diagnosis still remains a major problem as all these assays have their own pitfalls. The first two assays have restricted scope as a routine diagnostic procedure. Viral isolation by immunoflourescence though a gold standard, cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure & time consumption [15].

It is important to diagnose dengue early with the help of specific and inexpensive diagnostic serological markers that would permit early intervention to treat patients and prevent or control epidemics as no vaccine is yet available for protection and the vector control measures are inadequate. Ideally, all new point of care diagnostics, including diagnostics for infectious diseases should meet the WHO ASSURED criteria. They should be:

A- Affordable by those at risk of infection
S- Sensitive with very few false negatives
S- Specific with very few false positives
U-User friendly tests that are simple to perform and require minimal training
R-Rapid, to enable treatment at first visit. Robust, for example not requiring refrigerated storage
E-Equipment free
D-Delivered to those who need it

Hence, our study was aimed to assess RICT kit's sensitivity and specificity in comparison to conventional ELISA so that it can be used in peripheral primary and secondary health care centres to diagnose dengue as conventional ELISA is time consuming, needs testing in batches by trained technicians, not suitable for small laboratories and can be performed only in central testing laboratories.

Our study showed 100% concordance between SD RICT and Panbio ELISA for detecting NS1 Ag in primary and secondary dengue infections. Study by Selvaraj *et al* [16] showed a sensitivity and specificity of 97.54% and 98.33%, respectively for SD RICT when compared with Panbio ELISA for detection of NS1Ag. Several other studies showed 100% specificity for detecting NS1 Ag by RICT when compared with ELISA. However, the sensitivity of RICT for NS1 Ag varied from 62.5% to 81.5% [17-19].

In most studies, almost similar specificity between RICT for NS1 antigen and other conventional ELISA kits was observed. However, the sensitivity of the rapid kits was low to moderate. Usually, NS1 antigen has low sensitivity in case of secondary infection. NS1 levels may be quickly masked by circulating antibody and/or cleared from circulation. Hence, the most effective diagnostic application of NS1 detection is when it is combined with antibody detection. Together, they provide a broader window of detection [20, 21].

In our study, the sensitivity of RICT for detection of IgM Ab was 89.6% with 100% specificity

when compared to Panbio ELISA. Three cases were missed by RICT. There were no false positive cases. Similar study done by Seok Mui *et al* showed a relatively lower sensitivity of 53.5% for IgM detection by RICT. However, specificity and PPV were 100% each [22]. Sahu et al [23] got a higher sensitivity and specificity of 93.90% and 99.53% in detecting IgM in early convalescent sera while Kaylan D, *et.al* have recorded a higher positivity with RICT than ELISA [24]. Though Dengue IgM Ab is a marker of recent infection it has cross-reactivity with other circulating Flaviviruses. Seok Mui *et al* proposed that SD Dengue Duo NS1/IgM combined can increase the sensitivity to 88.65% with specificity of 98.75%.So RICT can be useful, sensitive, and specific for the diagnosis of acute dengue infection [22].

Moorthy *et al* showed the sensitivity and specificity of RICT for detection of IgM and IgG as 81.8% and 75% & 87.5% and 66.6%, respectively [25]. Several other studies showed differences in sensitivity and specificity of ELISA and rapid tests and their difference might be due to the different principles of these assays [26].

In the present study, the accuracy parameters were comparable with ELISA though few other studies showed more variable results. Nevertheless, RICT have advantages and disadvantages over conventional ELISA kits.

RICT is simple, point of care test for detection of NS1 Ag and both dengue-specific IgM and IgG antibodies can be detected in a test card format with results available within 20min.It does not require complicated technical expertise. It can be performed on blood. No pre-treatment of sera to remove competing IgG or rheumatoid factor is required. It can be performed on single or small number of samples and is cost effective.

The ELISA, on the contrary is an expensive test which can be used to test large number of samples in batches. The test can be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established. Also, icteric or lipaemic sera, or sera exhibiting haemolysis cannot be used. Moreover, the assay is temperature sensitive (20 - 25ºC). From the separation of serum to preparation and dilution of reagents along with multiple incubation steps and interpretation of results, the ELISA takes a minimum of four hours. Hence, though ELISA may exhibit higher sensitivity and specificity as compared to RICT, it is suitable for central laboratories and tertiary care centres which have trained technicians. RICT, which is an easy test, can be used in primary and secondary care centres so that early diagnosis can be made and morbidity & mortality could be significantly brought down.

Limitations of RICT include improper storage conditions, which may give false results. Also, reading and interpreting of a rapid test result is not always unequivocal. At times the bands are very faint, but these do indicate a positive test. It is a common mistake to read these as negative. Similarly, if an indentation is produced on the strips due to a manufacturing or handling error, it is possible that a coloured line appears but on careful observation, these are mostly observed to be either thin or located at the wrong place on the strip. In such circumstances, a repeat test is recommended.

CONCLUSION

Dengue is a notifiable disease and requires intervention in its early stage, to prevent serious complications. Detection of NS1 antigen is important and for acute dengue diagnosis, a combined analysis of both NS1 and IgM/IgG has allowed for a more sensitive early diagnosis of acute dengue infection. Since performing ELISA for NS1 antigen, IgM and IgG, for each patient has practical challenges and RICT meets the WHO ASSURED criteria, this study recommends introduction of RICT in all levels of health care system for an early and accurate diagnosis and confirmation of the clinical suspicion of dengue. This would also aid the public health authorities in timely initiation of control measures.

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