ORIGINAL RESEARCH PAPER

Community Medicine

VALIDITY OF DENGUE RAPID IMMUNOCHROMATOGRAPHIC CARD (ICT) TESTS (NS1, IGM AND IGG) KITS COMPARED TO ELISA AS A COST EFFECTIVE TOOL FOR USE IN RESOURCE POOR PRIMARY HEALTH CARE SETTINGS.

KEY WORDS: Validity, Dengue, ICT, ELISA

Zinia T. Nujum* Associate Professor, Community Medicine, Government Medical College, Thiruvananthapuram *Corresponding Author

Jyothi R. Associate Professor, Department of Microbiology, Government Medical College, Thiruvananthapuram

Vijayakumar K. Secretary Health Action by People, Retd. Professor and Head, Community Medicine, Government Medical College, Thiruvananthapuram

Saradadevi K.L. Professor and Head, Department of Microbiology, Government Medical College, Thiruvananthapuram

ABSTRACT

Kerala is hyperendemic for Dengue. Rapid ICT tests is a onestep, time independendent assay to detect dengue virus NS1 antigen, IgM and IgG antibodies to dengue virus. The validation of ICT test kits will give a useful direction to its use. A diagnostic test evaluation was done in Medical College, Thiruvananthapuram, Kerala, India. 25 NS1 ELISA positive and negative samples each, 75 IgM ELISA positive and negative samples each and 100 IgG ELISA positive and negative samples were tested by ICT card test method. The NS1 component of the test kit performed the best with 100% sensitivity and specificity. The specificity of the IgM and IgG component were also 100%. The sensitivity of the test kits were low for IgM (89.33%; 80.34 -95.5) and IgG (86.00; 77.86-91.47). The high performance of Rapid ICT tests makes it a cost effective alternative to ELISA in primary settings with resource constraints.

Introduction

Dengue is a serious and emerging tropical disease among mosquito borne diseases. Its burden globally is 465000 DALY (Gubler DJ *et al.*, 1978) World-wide nearly 2.5-3 billion people (40% of the global population) live at risk of infection. The earliest estimate of dengue infection annually was 80- 100 million in 1988. This was subsequently revised to a more frequently used and WHO approved estimate of 50-100 million. Using cartographic methods, this figure has been estimated as 390 million (95% CI–254-528) recently (Samir Bhatt *et al.*, 2013). India is regularly reporting dengue outbreaks. India is becoming hyperendemic for dengue and newer areas are being struck by the disease (lall R *et al.*, 1996; Kadar A *et al.*,1997). Dengue cases and deaths were reported in Kerala in 1997 for the first time. The first epidemic occurred in 2003 with 3546 cases and 68 deaths. Thiruvananthapuram district was worst affected in this epidemic. In 2003, Kerala reported maximum deaths due to dengue in India. Over the years, the reported cases of dengue have been increasing (State Bulletin, 2010). Kerala is now a hyperendemic for Dengue. Multiple serotypes, high rates of co-infection and local genomic evolution of the virus are challenges for the state to encounter (Anoop M *et al.,* 2010)

Rapid ICT tests offer a onestep assay to detect dengue virus NS1 antigen and IgM and IgG antibodies to dengue virus in human serum, plasma or whole blood (Gubler DJ, 1996) Usually patients with dengue present for the first time in primary health care settings where there are no resources in terms of human resource and laboratory for diagnosis of Dengue using ELISA. There are 835 such institutions at the primary level (660 PHC and 175 24X7 PHCs) (Government of Kerala, 2011).The duration of onset of symptoms is usually vaguely defined and deciding on NS1 or IgM is difficult. Distinguishing between primary and secondary dengue may also be enabled by this technique. Hence the validation of ICT test kits in the field setting, other than that provided by the kit manufacturers will give a useful direction to its use in resource poor health care settings.

Materials and methods

Design – Diagnostic test evaluation
Setting – Medical College, Thiruvananthapuram, Kerala, India

Study subjects
Validation of these kits were done as part of a cohort study entitled "Incidence and outcomes of dengue in Pregnancy". In this study the ICT kits were used for screening for dengue pregnancy women with fever. Pregnant women were recruited during the first trimester. Initial blood samples were collected from a sample of these women for looking at seroprevalence. ELISA method was used for IgG testing. 100 IgG positive and 100 IgG negative samples were tested using the ICT card. For validating the Ns1 and IgM of the ICT card, samples were obtained from suspect and probable dengue cases from Medical College. Thiruvananthapuram. 25 NS1 positive and negative samples each, 75 IgM positive and negative samples each which were tested by ELISA method were randomly chosen from the register of patients during the study period. Whole blood samples were collected in vaccutainers containing heparin. Serum was separated. Specimens which were not tested the same day were stored at 2to 8 degree in refrigerator. These samples were also tested using the ICT cards.

Procedure for ICT tests
Using capillary pipette 10 microlitres of serum was added to the sample well for Ns1 tseting. Using disposable dropper, 3 drops of serum (100 microlitres) of the same serum was added to the sample well for IgM and IgG. Four drops of assay diluent was added to the assay diluent well. The test results were interpreted after 20 minutes. The presence of only one colour line within the NS1 result was considered a negative test. The presence of two lines ("T' band and "C" line) within the result window was considered a positive test. The presence of two lines ("C" and "M" line) in the result window was considered as IgM positive and presence of two lines ("C" and "G" line) was considered IgG positive. The SD Bioline dengue duo rapid ICT test kits were used and the ELISA kits used as reference were from PanBio.

Statistical analysis
Validity measures like Sensitivity, Specificity, Positive and Negative Predictive values and Likelihood ratios and their 95% Confidence intervals were calculated.

Ethical consideration
The study has been approved by the Human Ethics Committee of Government Medical College, Thiruvananthapuram (IEC No: 02/42/MCT dated 14/02/2014). Informed written consent was obtained from the study participants. Confidentiality and privacy has been maintained at every stage of the study.

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Results

The mean age of study participants (figure 1) for validation of IgG component of kit was 24.59 (SD-4.421). For the IgM component the mean age (figure 2) was 32.51 (SD- 19.2). Females formed 57 % (86/150) and Males constituted 42.7 % (64/150) of the study subjects. The mean age of participants (figure 3) for the NS1 component was 36.21 (SD- 17.28). The male samples were greater than females (56% and 44%).

The NS1 component of the test kit performed the best with 100% sensitivity and specificity. The specificity of the IgM and IgG component were also 100%. The sensitivity of the test kits were low for IgM and IgG. The corresponding negative predictive values were also low (see table 2).

Kappa was calculated as a measure of agreement. It was 1.00, 0.893 and 0.860 for NS1, IgM and IgG component of the test kit respectively. There is significant (<0.001) agreement between the test kit and ELISA for all the three components of the kit namely NS1, IgM and IgG.

Discussion

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 lifesaving. Only 5 % of cases are severe. The less severe cases can be managed at the primary level provided there are no warning signs as per the protocol, if the diagnosis can be confirmed. The referral done to a higher centre for confirmation of diagnosis only, can be avoided if the primary settings are equipped with these cards. Secondary dengue is more likely to develop complications. We can distinguish them with these card tests at the primary level itself.

The major diagnostic methods currently available are viral culture, viral RNA detection by Reverse Transcriptase Polymerase Chain Reaction (RTPCR) 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 immunofluorescence though a gold standard, cannot be used as a routine diagnostic procedure due to its low sensitivity, laborious procedure & time consumption. 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 (Pragya Sharma *et al*., 2017)by this method. Rapid serological tests have become available since the past many years but their accuracy is thought to be uncertain because of lack of proper validation (Gubler DJ, 1996).This study is an tried to validate the rapid tests with ELISA.

The ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users) criteria, outlined by the World Health Organization (WHO), provide a good framework for evaluating point of care devices for resource-limited environments. Tools that satisfy the ASSURED criteria primarily aim to provide same-day diagnosis and facilitate immediate decision-making (Mabey D et al., 2004)

The sensitivity of NS1 component obtained in our study is high compared to many other studies (Kumar S *et al*., 2016; Fry SR *et al*., 2011, Pal S *et al*., 2014, Ivan E *et al*.,2015, Osorio L *et al*, 2010) This may be because of the reference standard used. The NS1 levels and thus its positivity is related to the viraemia. Reference standard which use virus isolation detect even low levels of viraemia which may not be picked up by the NS1 antigen detection methods. Also there is interference with IgG antiboides. Perhaps our samples tested for NS1 were more likely to have primary infection and therefore less interference with IgG. (Ivan E *et al*.,2015, Gan VC et al.,2014, Sanchez- Vargas LA *et al*.,2013, Blacksell SD *et al*., 2011,Andries AC *et al*.,2012, Blacksell SD *et al*., 2012, Hang VT *et al*., 2009, Hunsperger EA, et al.,2009) The performance of the IgM component is also higher compared to other studies. (Ivan E *et al*., 2015, Hunsperger EA *et al.* 2009)

The combination of NS1, IgM and IgG rapid testing is often referred as dengue package. It is very useful in hospitals and community settings for screening patients with suspect / probable dengue. The diagnosis and management of acute undifferentiated febrile illness (AUFI) can be greatly improved by its use. Dengue can be diagnosed in the initial febrile phase itself irrespective of whether the infection is Primary or Secondary dengue, acute or convalescent (Ivan E *et al*., 2015, Subash *et al*., 2011)

Dengue NS1 antigen detection in combination with anti-glycoprotein E IgM and IgG serology can significantly increase the sensitivity of acute dengue diagnosis (from 62.0% using Ns1 alone to 93% when used in combination with IgM and IgG). (Fry SR et al., 2011) Another study reports that this combination increase in sensitivity to 97.5 % (95 % CI: 92.9-99.2) and 98.9 % (95 % CI: 96.0-99.7), respectively. These higher sensitivities were achieved without any decrease in specificities. (Ivan *et al*., 2015)

Cost and time efficiency

The cost of doing NS1, IgM and IgG by ELISA method is in the range of Rs. 600 to 800 for each. These can also be done by the card test at around the same cost. The use of the test kits were all three tests can be done together reduces the cost to 1/3rd. The result of the card tests is available in 20 mts, whereas the ELISA tests take four hours each for the result. In terms of cost and time, these rapid tests offer a better diagnostic advantage compared to ELISA.

Strengths and Limitation

The tests were compared with ELISA. The sensitivity of ELISA compared to virus isolation is 85.6–95.9%. (Pal S *et al*., 2014). This could be a reason for high performance. Since different samples were used for validating NS1, IgM and IgG we did not look at combined sensitivities and specificities. We did not check for cross reaction with other flaviviruses. The strength of the study is that there are no conflicts of interest.

Conclusion

The performance of Rapid ICT tests is almost at par with ELISA methods for diagnosing dengue infection. This may be used as an alternative to ELISA in primary and secondary health care settings with resource constraints for using ELISA. This test kit is most valuable for detecting early dengue infection and mortality prevention since NS1 component of the test has the highest accuracy indicators.

Table 1: Performance of the dengue kit compared to ELISA

		ELISA		
Kit		Ns1 postive	NS1 Negative	
	NS1 positive	25	0	25
	Ns1 negative	0	25	25
Kit		IgM postive	IgM Negative	
	IgM positive	67	0	67
	IgM negative	8	75	83
Kit		IgG postive	IgG Negative	
	IgG positive	86	0	86
	IgG negative	14	100	114

Table 2: Validity measures of the test Kit compared to ELISA

	NS1	IgM	IgG
Sensitivity	100	89.33 (80.34-94.5)	86.00(77.86-91.47)
Specificity	100	100	100
Positive Predictive Value	100	100	100
Negative Predictive Value	100	90.36	87.72
Negative Likelihood ratio	0	0.107 (0.065 - 0.205	0.140(0.086-0.228)

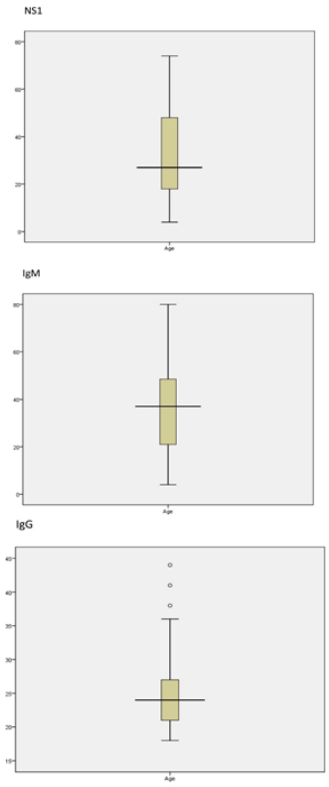

Figure 1: Age distribution of study participants for validation of NS1, IgM and IgG components of Kit

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Conflict of Interest

None

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