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Original Article

Rapid immunochromatographic test to detect NS1antigen, Ig M and Ig G: An alternative to enzyme linked immunosorbent assay in diagnosis of dengue at primary care level.

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Abstract

Background: During the recent outbreak of Dengue, there was an alarming increase in the morbidity and mortality in the southern districts of Tamilnadu. Early diagnosis and differentiation of primary and secondary dengue is essential for treat- ment and control measures. **Objective:** This study was done to compare rapid immunochromatography (ICT) and ELISA in the early diagnosis of dengue by the simultaneous detection of IgM, IgG and NS1 antigen. ***Methods:*** Sera from 120 suspected dengue fever patients were tested for IgM, IgG and NS1 antigen by ICT and ELISA methods. ***Results:*** Among the tested 120 samples, 37 (31%) for any one marker, 18 (15%) for NS1antigen, 18(15%) for IgM and 25 (21%) for IgG were positive by ICT. By ELISA 58(48%) for any one marker, 22(18%) for NS1, 42(35%) for IgM, and 48 (40%) for IgG were positive. ***Conclusion:*** In view of high degree of agreement (96.7%) between ICT and ELISA for NS1 detection, the rapid ICT may be useful at field level particularly where ELISA is not available for the early diagnosis and effective intervention.

Introduction

Despite effective public health measures, emerging and re-emerging infectious dis- eases continue to crop up in southern districts of Tamilnadu posing problems in early diagnosis. Fur- ther, there was an upsurge in the severe form and mortality due to dengue during the recent epidemic (2011-2012). The reasons for the epidemic could be rapid urbanization, travel, trade and water scarcity leading to a tendency to store water for longer periods of time. Poor sewage system and vector re- sistance which facilitate the survival and transmission of the vector, Aedes aegpti may be other factors. [1] The clinical spectrum of dengue is indistinguishable from other febrile illnesses. The current diagnostic method MAC ELISA fails to detect some cases since IgM starts appearing on day 3 or 5 of illness. [2] ELISA is not avail- able in most of the rural hospitals. Further, it is very difficult to perform ELISA during emergency which requires expertise and experience. Simultaneous detection of NS1 antigen, IgM and Ig G, antibodies is helpful to distinguish primary and secondary infection. Hence a rapid, sensitive, cost effective method in the diagnosis of dengue is the need of the day. In this background, this study was done to compare the value of rapid immunochromatography (ICT) and ELISA for these three markers and the importance of NS1antigen in the early diagnosis.

Material and Methods

A total of 120 blood samples were collected from patients with fever attending a private multi special- ty hospital from October 2011 to February 2012. Informed consent and Institutional Ethical Commit- tee approval was obtained. Sera were separated and tested for evidence of dengue by ICT to detect NS1 antigen , Ig M and Ig G Antibodies. (SD Bioline, Dengue Duo manufactured by SD Standard Diagnostics, Inc., korea).

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Sera was stored at –20 ^{0}C and tested for Ig M and Ig G antibodies to dengue virus by ELISA (standard Diagnostics, Inc., Korea) and NS1 (PanBio, France). Tests to rule out other causes of fever were done wherever necessary. The results were analyzed for agreement , Sensitivity , Specificity, positive predictive value and negative predictive value (Table2).

Results

The serological markers detected by ICT and ELISA were as follows: Of the 120 serum samples tested by ICT 8(7%) were positive for NS1 antigen only, 2(2%) were positive for IgM only and 10(8%) were positive for IgG only. More than one marker was detected in 17 (14%) of the samples. All the three markers were detected in 7(6%) sera (Table1). Of the 120 serum samples tested by ELISA, 2(2%) were positive for NS1 antigen only, 4(3%) were positive for IgM only and 12(10%) positive for IgG only. More than one marker was detected in 40(33%) samples. All the three markers were detected in 14 (12%) sera (Table1).

Markers	ICT (%)	ELISA (%)
NS1 Only	08 (7)	02(2)
IgM Only	02(2)	04(3)
IgG Only	10(8)	12(10)
NS1 and IgM	02(2)	04(3)
IgM and IgG	07(6)	20(17)
NS1, IgM and IgG	07(6)	14(12)
NS1+IgG	01(1)	02(2)
Total	37(31)	58(48)

Table: 1- Detection of Dengue specific markers by ICT and ELISA

The results of ICT and ELISA were compared. Of the 120 samples tested, 31% (37) were positive by ICT and 48% (58) were positive by ELISA for any one of the markers. Among them 15% (18) were positive for NS1 antigen by ICT and 18% (22) by ELISA. 15% (18) were positive for Ig M by ICT and 35% (42) by ELISA. 21% (25) were positive for IgG by ICT and 40% (48) by ELISA. Samples positive by ICT were also positive by ELISA (Table 2).

Marker	ICT (%)	ELISA (%)	Agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
NS1	15	18	96.7	81.8	100	100	96
IgM	15	35	80	42.9	100	100	76.5
IgG	21	40	80.8	52	100	100	75.8

Table: 2- Comparative evaluation of dengue positive by ICT and ELISA.

Discussion

Laboratory testing is imperative in the diagnosis of dengue infection owing to the co - circulation of other agents causing febrile infections in endemic areas. Dengue infections are now being increasingly report- ed during the post monsoon period. If effective pre- ventive measures are not taken, this may occur con- tinuously throughout the year leading to serious pub- lic health problems. Further, southern districts of Tamil Nadu have witnessed severe form of dengue during the 2011-2012 epidemic which may be due to combined infection with more than one serotype in patients [3] or introduction of new mutant serotype. Absence of effective tetravalent vaccine and specific viral anti therapy further worsen the situation. Unless screening is done for all three serological markers simultaneously, dengue diagnosis may be missed in some cases, because the detection of each marker is influenced by the stage of the disease. Hence a rapid ICT for all the three markers as a diagnostic tool is compared with ELISA for the early diagnosis of dengue.

Kulkarni RD *et al* reported 40.6% positivity for any one of the dengue marker by ICT. [4] In the present study, 31% were positive by ICT and 48% by ELISA. Markers positive by ICT were also positive by ELISA (Table1).This was supported by Shrivastava A et al indicating there is no false positive ICT. [5] Simultane- ous detection of all the three markers is useful to dis- tinguish primary and secondary dengue infection. [6] In the present study all the three markers were de- tected in 6% (7) by ICT and 12% (14) by ELISA. For NS1antigen, 15% (18) were positive by ICT and 18% (22) by ELISA (Table1). Shrivatsava A *et al* reported 16.5% for NS1 antigen by ICT and 26% by ELISA. [5] Many studies reported that NS1 antigen was detected in 82-83% of dengue infection from day 1 upto day 9-18 after the onset of fever. [7,8] .

In this study, the agreement of NS1 between ICT and ELISA was 96.7%. In endemic areas, NS1 detection by a simple, point of care test like rapid ICT in the first five days of fever will be a useful tool. This would aid in the prevention of complications.

For IgM, 15% were positive by ICT and 35% by ELISA. (Table2) Kulkarni RD *et al* reported 64% positive by ICT.[4] Since IgM persists for 8 months, in endemic areas single serological detection of IgM is indicative of dengue infection. A single serum sample positive for IgM antibodies from a clinically suspected dengue patient gives a probable diagnosis of dengue. It should not be interpreted as confirmed acute dengue infection without a paired second serum sample showing seroconversion. However, IgM is more specific than IgG to detect acute infection. [9, 10] The ICT may be evaluated for this purpose at field level by testing paired samples.

For IgG, 21% were positive by ICT and 40% by ELISA(Table2). Kulkarni RD *et al* reported 9% by ICT.[4] IgG is much useful to distinguish primary and secondary dengue infection. IgG also persists for 10 months to life long in many patients with secondary dengue infection. [2] Cross reactivity of Ig G is also well documented.[10] Agreement for IgM / IgG between ICT and ELISA were only 80% and 80.8% respectively in this study.

ICT may also be useful to investigate suspected epidemics of dengue as it is a rapid, easy to perform method to detect the markers of dengue at field level as a substitute to ELISA, so that control measures may be instituted early.

Conclusion

Since there was high degree of agreement (96.7%) for NS1antigen, between ICT and ELISA in this study ICT which is a rapid, easy to perform, specific and cost effective may be used for the simultaneous detection of all the three markers (NS1, IgM & IgG) at field level. But negative ICT should be confirmed by ELISA when there is a strong clinical suspicion because sensitivity was 81.8% for NS1antigen, 42.9% for IgM and 52% for IgG. So acute and convalescent sera should be tested for significant rise in antibody titre.

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Conflict of interest: The authors claim to have no conflict of interests in the context of this work.