SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 5.
Issue: 2.
Page N. 93-98
Year: 2021

Original Article www.wjahr.com

A COMPARATIVE STUDY OF RAPID IMMUNO CHROMATOGRAPHY TEST WITH ELISA FOR DETECTION OF DENGUE NS1 ANTIGEN, IGM AND IGG ANTIBODY

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Received date: 09 January 2021 **Revised date:** 29 January 2021 **Accepted date:** 19 February 2021

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ABSTRACT

Aim of The Study: To analyse the efficacy of Immunochromatography with ELISA for detection of Dengue parameters (NS1 antigen, IgM and IgG antibodies) in Dengue suspected cases. **Materials and Methods:** A total of 100 blood samples obtained from adults with fever >4 days presenting with thrombocytopaenia (<1,00,000) were taken for the study from the Department of Medicine, Government Medical College Hospital, Tirunelveli after obtaining informed written consent. The samples were tested by Rapid Immunochromatography (ICT) and ELISA methods for NS1 Ag, IgM and IgG Ab and the results were evaluated for efficacy. RESULTS: Out of 100 adults tested by rapid ICT, 38% were positive for Dengue infection (NS1 antigen, IgM and IgG antibodies) in the serum sample. The same samples were tested by ELISA (Positive for any one of the three parameters) the positivity was found to be 59%. 37 samples positive by ICT were positive by ELISA also. Rapid ICT had a sensitivity of 87.5%, Specificity of 98.8% for NS1 antigen when evaluated against NS1 antigen. ELISA as gold standard. Rapid ICT had a very low sensitivity of 28.6% and 47.1% and an excellent specificity of 98% and 100% for IgM and IgG antibody detection when evaluated against MAC ELISA and GAC ELISA as gold standard respectively.13.6% showed primary infection and 64.4% showed secondary infection. **Conclusion:** Out of the total 100 cases studied, the rapid immunochromatography test has very less sensitivity and specificity is satisfactory. All samples should be subjected to both antigen (NS1) and antibody (IgM and IgG) testing to increase the positivity rate and to prevent the positive cases being missed. Cases with higher degrees of suspicion are to be subjected to diagnostic tests with higher sensitivity & specificity like ELISA and PCR. The commercially available rapid immunochromatographic test device can be used as a screening device during Dengue outbreaks. It should not be used as a standalone device for diagnosis of Dengue. Further molecular studies are essential to know the accurate information of Dengue serotypes which will be helpful in formulating vaccines in future.

KEYWORDS: Dengue, Antigen, Antibody, ELISA.

INTRODUCTION

Dengue is the most rapidly spreading mosquito-borne viral disease in tropical and subtropical regions of the world mainly in man caused by the bite of aedes mosquito.[1] Dengue virus belongs to the broad group Arboviruses, family Flaviviridae, subfamily Flaviviridae and genus Flaviviruses. Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue. It causes high mortality during the early phases of outbreaks.

The dengue virus is found in serum or plasma, circulating blood cells and selected tissues especially those of the immune system, after the onset of illness (2 to 7 days), roughly corresponding to the period of the fever.[2] Dengue diagnosis is achieved either by virus isolation or by viral RNA identification through RT-PCR or by serological detection of dengue specific IgM and IgG antibodies. Virus isolation and RT- PCR are time consuming and costly laboratory methods. Thus, detection of dengue antigen or antibodies is feasible for diagnosing dengue in most cases. Detection of dengue NS1 antigen indicates early dengue infection.

Serological detection of antibodies based on capture ELISA has become the new gold standard for the detection of dengue virus infections.[3] Early diagnosis is useful in triaging patients and have a central role in dengue case management and plays a vital role in forecasting an early warning of an epidemic and in undertaking effective vector control measures. In this context, this study was carried out to detect Dengue specific NS1 antigen, IgM and IgG antibodies by Enzyme Immunosorbent Assay (ELISA) and to detect Dengue NS1 antigen, IgM and IgG antibodies by rapid Immuno-chromatographic (IC) card test. Finally, comparison of two diagnostic tests for early/rapid diagnosis of acute Dengue infection.

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli for one year. A total of 100 serum samples were collected from clinically suspected Dengue cases in adults. Serum samples of patients with acute onset of fever of >4 days, clinically suspicious of Dengue virus infection, those who require hospitalization were included in the study. Whereas patients with any other proven febrile illnesses like Malaria, Typhoid etc. are and children were excluded.

All the results obtained were analysed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. Chi- square test and Fischer Exact test are The differences of above parameters were tested by the parametric tests like 'Z' and 't' and non-parametric like x^2 test, which was applicable wherever. The results of Rapid ICT and ELISA were compared by Mcnemar's x^2 test and confirmed by 'Z' test of proportions. The above statistical procedures were performed by SPSS Statistics 22. The P-Values of less than 0.05 were considered as statistically significant in two – tailed test($P<0.05$).

RESULTS

The selected 100 study subjects were analysed based on age and sex, Of the 100 patients, 59 were males. Of this, 29 (49.2%) were in the age group of 13-22 years and 14 (23.7%) were in the age group of 23-32 years and 9(15.3%),4(6.8%),1(1.7%) and 2(3.4%) were in the age group of 33-42 years,43-52 years,53-62 years and 63-72 years respectively**.**

Out of 100 patients, 41 were females. Of this, 19 (46.3%) were in the age group of 13-22 years and 10 (24.4%) were in the age group of 23-32 years and 6(14.6%), 4(9.8%) and 2(4.9%) were in the age group of 33-42 years, 43-52 years and 53-62 years respectively. Out of 59 ELISA positive cases 35(59.3%) cases belong to urban area and 24(40.7%) to rural area. In ELISA negative cases, 23(58%) cases reside in urban and 18(43.9%) in rural area. The association of cases living in urban and rural area was statistically not significant.

Among our study group 55% was associated with vomiting, 55% had headache, 73 % had myalgia, 19% had bleeding manifestations, 11% had abdominal pain and 13% presented with URI.

In our study population hematocrit was found to be elevated in 37% and 31% presented with leucopenia and 11% had liver enzymes elevated. The ultrasonography findings shows that 13% had ascites, 9% had pleural effusion, 9% with GB wall edema and 8% presented with hepatomegaly.

All the 100 samples were tested by both rapid ICT and ELISA for dengue parameters (NS1 antigen, IgM and IgG antibodies). Out of this ELISA was positive for 59% of samples while 38% were positive by ICT as shown in table 1.

Table 1: Detection of Dengue cases by rapid ICT and ELISA in detection of dengue.

Test	Sample tested	Positive		Negative	
		Cases	%	Cases	%
ELISA	**100**	**59**	**59%**	**41**	**41%**
ICT		**38**	**38%**	**62**	**62%**

Detection of dengue positive cases by rapid immunochromatographic card test was evaluated for its sensitivity and specificity against ELISA for (NS1 antigen, IgM and IgG antibodies) as reference test. From the below table, sensitivity of rapid ICT was 62.7% when evaluated against ELISA, a reference test. Specificity of ICT was 97.6% compared to ELISA and positive and negative predictive value were 97.4% and 64.5% respectively.

Table 2: Comparison of ICT and ELISA.

ICT	ELISA	
	Positive	Negative
Positive	37	1
Negative	22	40
Total	59	41

The below table 3 shows that among 38 ICT positive cases, 9 (23.7%) showed positive for NS1 only, 4 (10.5%) for IgM only, 12 (31.6%) for IgG only, 3 (7.9%) for NS1+IgM+IgG, 1 (2.6%) for NS1+IgM, 2 (5.3%) for NS1+IgG and 7 (18.4%) were positive for IgM+IgG.

Table 3: Detection of positive cases by ICT.

Parameters	ICT	ELISA
NS1 only	9	2
IgM only	4	5
IgG only	12	8
NS1+IgM+IgG	3	13
NS1+IgM	1	1
NS1+IgG	2	0
IgM+IgG	7	30
Total	38	59

The above table 7 (figure 7) shows that among 59 ELISA positive cases, 2 (3.4%) showed positive for NS1 only, 5 (8.5%) for IgM only, 8 (13.6%) for IgG only, 13 (22%) for NS1+IgM+IgG, 1 (1.7%) for NS1+IgM, 0 (0%) for NS1+IgG and 30 (50.8%) were positive for IgM+IgG.

ICT was able to differentiate 14(36.7%) and 21(55.6%) as primary and secondary infection respectively. Among the 59 ELISA positive cases 8(13.6%) showed primary infection and 38(64.4%) showed secondary infection. 3(7.9%) of ICT positive cases and 13(22%) of ELISA positive cases showed positive for all the three parameters (NS1+IgM+IgG) which indicates that the patient may be either in the late stages of primary infection or early stages of secondary infection.

Moving on to individual antigen analysis sensitivity of rapid ICT was 87.5% when evaluated against NS1 antigen ELISA, a reference test. Specificity was 98.8% compared to ELISA and positive and negative predictive value were 93.3% and 97.6% respectively.

Similarly sensitivity of rapid ICT was 28.6% when evaluated against IgM Capture ELISA, a reference test. Specificity was 98% compared to ELISA and positive and negative predictive value were 93.3% and 58.8% respectively and sensitivity of rapid ICT was 47.1% when evaluated against IgG Capture ELISA, a reference test. Specificity was 100% compared to ELISA and positive and negative predictive value were 100% and 64.5% respectively.

We also evaluated other parameters related to positive cases, Sex wise distribution of dengue positive cases in the above table shows that males are more affected than the females (fig 12). Out of 59 positive cases detected by ELISA, 35(59.3%) were male and 24(40.8%) were female. Majority of positive cases are in the age group of 13-22 years Out of 59 dengue positive cases shown by ELISA, 33 (55.9%) were in the age group of 13-22 years. This is followed by a higher incidence in the age group of 23-32 years (25.4%).

DISSCUSSION

Dengue is one of the major re-emerging viral infections. For the past decades there has been a significant raise in the geographic spread, number of cases and disease severity. An early diagnosis of the disease aids in effective management and prevents further complications like DHF and DSS. With this background. the present study attempts to evaluate the rapid immunochromatography test against ELISA in diagnosing acute dengue infection.

The present study shows that out of 100 suspected cases, males are found to be predominant than the females in the ratio of 1.4: 1. Male preponderance was also observed in similar studies conducted by Manisha Patankar *et al,*[4] and Gargi Ghosh *et al.*[5]

The suspected cases who were seeking medical attention belongs to the age group of 13-22 years in the present study. This result is similar to the study conducted by Gargi Ghosh *et al,*[5] *D*emographic characteristics like age distribution and gender differences are important for the successful planning of public health programmes and effective control of communicable diseases.

The current study shows that among the 100 cases studied presented with fever associated with vomiting had headache (55%), 73 % had myalgia, 19% had bleeding manifestations, 11% had abdominal pain and 13% presented with URI The lab and USG findings shows that haematocrit was found to be elevated in 37% and 31% presented with leucopenia and 11% had liver enzymes elevated, 13% had ascites, 9% had pleural effusion, 9% with GB wall edema and 8% presented with hepatomegaly which was similar to NP Singh *et al,*[6] and in contrast to Ghargi ghosh *et al.*[5]

The positivity rate of rapid ICT was 38% while it was 59% by ELISA. In the present study Dengue viral infection accounts for 59% of hospitalization in adults with acute febrile illness. The prevalence is comparable to a study conducted by Chakravarti *et al* in 2011.[7]

Vijayakumar et *al,*[8] in his five year study conducted at Christian Medical College, Vellore observed that the prevalence of dengue infection was unusually high in 1999 (43.5%) and in 2003 (55.1%) whereas in the intervening years the percentage was just above 25%.

In contrast there was a high prevalence of dengue infection (65.2%) conducted by Siraj A. Khan *et al,*[9] in a hilly region of Andhra Pradesh. Dengue epidemics have been reported in various parts of Tamilnadu including Tirunelveli district The present result shows an increase in the dengue virus activity in this region.

The sensitivity of rapid ICT was 62.7% when evaluated against ELISA,[9] a reference test. Specificity of ICT was 97.6% compared to ELISA and positive and negative predictive value were 97.4% and 64.5% respectively. The sensitivity calculated in present study correlates well with the sensitivity of dengue rapid test found in a study conducted by Pramiladevi *et al,*[10] (68.75%) but with low specificity than the present study. Similar study by

Scott R. Fry et *al*,[11] showed sensitivity and specificity as 69.2% and 96% respectively.

Hence all the samples negative by ICT have to be confirmed by a more sensitive test like ELISA or PCR.

The present study shows that among 38 ICT positive cases, 9 (23.7%) showed positive for NS1 only, 4 (10.5%) for IgM only, 12 (31.6%) for IgG only, 3 (7.9%) for NS1+IgM+IgG, 1 (2.6%) for NS1+IgM, 2 (5.3%) for NS1+IgG.

In contrast, a study by Bala *et al*,[12] showed that out of 78 ICT positive cases 26 cases (33.33%) was positive for NS1 antigen only, 7 cases (8.97%) positive for IgG + IgM, 7 cases (8.97%) and 27cases (34.6%) were positive for NS1 +IgG and NS1+IgM+IgG respectively.

Results reported in another comparative study by Gargi Ghosh *et al* showed that of 320 samples positive for one or more dengue parameters by ICT, 165 (51.56%) were positive for NS1 only, 73 (22.81%) were positive for only IgM, and 39 (12.2%) were positive for IgG only 3 (0.93%) were positive for NS1 + IgM + IgG, 33 (10.31%) and 7 (2.19%) for NS1 + IgM and IgM + IgG respectively.

Overall, there was a significant increase in the outcome when the single sample is subjected to a combination of NS1 antigen, IgM and IgG antibody detection compared to the outcome from using each individual marker.

In the present study, distribution of positive cases by NS1 antigen ELISA,MAC and GAC ELISA shows that among 59 ELISA positive cases, 2 (3.4%) showed positive for NS1 only, 5 (8.5%) for IgM only, 8 (13.6%) for IgG only, 13 (22%) for NS1+IgM+IgG, 1 (1.7%) for NS1+IgM, 0 (0%) for NS1+IgG and 30 (50.8%) were positive for IgM+IgG.

In a similar study by Chakravarti *et al*,[7] in 2011 observed that there is a gradual increase in positivity toward the end of the acute phase of the disease when combining NS1 antigen ELISA which was detectable in patient sera from Day 1 onwards and dengue IgM ELISA that was detected from Day 3 onward.

The present study also shows an increase in positivity when a single serum from the patient was subjected to a combination of NS1 antigen ELISA, MAC and GAC ELISA rather than subjecting to a single parameter detection where one or more biomarkers are likely to be missed

The present study shows that the distribution of primary and secondary infections among dengue positive cases. ICT differentiates 14 (36.7%) and 21(55.6%) as primary and secondary infection respectively. Among the 59 ELISA positive cases 8(13.6%) showed primary infection and 38(64.4%) showed secondary infection. 3(7.9%) of ICT positive cases and 13(22%) of ELISA positive cases showed positive for all the three parameters NS1+IgM+IgG.

The primary infection is indicated by the presence of NS1 antigen alone or IgM alone and presence of NS1+IgM whereas the secondary infection is indicated by the presence of IgG alone and NS1+IgG as NS1 appears early in both primary and secondary infection. Sultana N *et al*,[12] and Hossain *et al*,[13] also reported in a similar study where secondary infections are more prevalent than the primary infections.

Secondary Dengue infection is often associated with severe form of the disease thus detection of dengue secondary cases at the early stage of infection is of major importance to avoid fatal out comes. The rapid ICT provides just a hint about the primary or secondary infection but lacks in giving quantitative information.

The sensitivity of ICT was low (87.5%) while the specificity was 98.8% when evaluated against NS1 antigen ELISA. Similar low sensitivity was reported by Philippe Dussart *et al*,[14] which showed 81.5% and but had a specificity of 100%. Based on the study by Hang *et al*.[15] 2009, false-negative results may occur most probably due to the formation of immune complexes of NS1 antigen with IgG, especially in secondary infections, where there is non- availablility of target antigen to the monoclonal antibody from ELISA.

The sensitivity of ICT was very low (28.6%) while the specificity was 98.8% when evaluated against MAC ELISA. Similar low sensitivity and high specificity was reported by Hunsperger *et al*,[16] and Stuart D. Blacksell *et al*.[17] Similar studies done by observed that during a secondary infection, about 20% of patients do not have IgM at detectable levels. Vasquez S *et al*.[18] stated that different kits used for IgM detection have variablility in their sensitivity and specificity. The sensitivity and specificity of IgM based assays is strongly influenced by the quality of the antigen used and can vary greatly between commercially available products**.**

The sensitivity of ICT was very low (54.9%) while the specificity was 98 % when evaluated against GAC ELISA. Similar low sensitivity and high specificity was reported by Hasan *et al*. IgG is a less reliable marker in the diagnosis of dengue infection. Both clinical and sub-clinical infections produce IgG that persists for several years which affects the interpretation of test results. It is highly likely that IgG levels could be higher in endemic areas because of bites from infected mosquitoes.

The present study shows that out of 59 ELISA positive cases 35(59.3%) cases belong to urban area and 24(40.7%) to rural area. In ELISA negative cases, 23(58%) cases reside in urban and 18(43.9%) in rural area.

Sex wise distribution of dengue positive cases in the present study shows that males are more affected than the females. Out of 59 positive cases detected by ELISA, 35(59.3%) were male and 24(40.8%) were female in the ratio of 1.5:1. Manisha Patankar *et al*,[4] *also* showed similar results where males are more predominant than the females. In contrast, a study by Paramasivan *et al*,[19] reported that females are more predominant than the males. High prevalence amongst males is probably due to more outdoor activities by males in comparison to females which results in more exposure to day biting mosquitoes.

The present study shows that the majority of positive cases are in the age group of 13-22 years. Out of 59 dengue positive cases shown by ELISA, 33 (55.9%) were in the age group of active adults 13-22 years. Manisha Patankar *et al* also reported the same results in which active adults forms the majority of the positive cases. As active adults are doing more outdoor work activities, there are more chances of them being infected.

Sharma et *al*,[20] proposed that exposure to multiple serotypes over a period of time results in immunity development, i.e., multitypic immunity in adults. The shift in the age preponderance partly may be due to the accumulation of multitypic immunity in the adult population. It is suggested that over a period of time, as the co-circulation of multiple dengue serotypes increases in a particular geographic area, adults have a lower chance of remaining susceptible to infection. This results in the young population susceptible to dengue infection. Therefore, monotypically immune individuals are more likely to be from younger age groups.

CONCLUSION

The rapid immunochromatography test has very less sensitivity and specificity is satisfactory. The PPV is satisfactory but NPV is less satisfactory. The rapid test can be used as a screening tool in situations of outbreaks but not as a diagnostic test. All samples should be subjected to both antigen (NS1) and antibody (IgM and IgG) testing to increase the positivity rate and to prevent missing of positive cases. Cases with higher degrees of suspicion are to be subjected to diagnostic tests with higher sensitivity & specificity like ELISA and PCR. The commercially available rapid immunochromatographic test device can be used as a screening device during dengue outbreaks. It should not be used as a standalone device for diagnosis of dengue. It is recommended that highly suspicious cases should be subjected to tests with higher degree of accuracy. Further molecular studies are essential to know the accurate information of Dengue serotypes which will be helpful in formulating vaccines in future.

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