Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Evaluation of Rapid Immunochromatographic Tests in Comparison to ELISA for Detection of NS1, IgM and IgG Antibodies for Early Detection of Dengue Infection in Pediatric Patients.

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Received: May 2019
Accepted: May 2019

ABSTRACT

Background: Dengue fever is one of the most common arthropod-borne viral disease seen in humans. It is one of the major public health problems in developing countries including India. The signs and symptoms of dengue fever may range from moderate fever to thrombocytopenia, hemorrhagic manifestation and shock. Severe untreated cases may even prove to be fatal. The diagnosis of dengue fever may be made on the basis of detection NS1 antigen or IgM and IgG antibodies. This study was aimed at analyzing the sensitivity and specificity of RICT kit with ELISA for NS1 antigen and IgM, IgG detection so as to explore its suitability for regular use in any modest resource constrain laboratory or primary health center as a bedside test. **Methods:** This was a prospective cohort study conducted from August 2018 to Jan 2019 in a tertiary care private and teaching hospital. Informed written consent was taken from parents or caretakers of patients. The institutional ethical committee approved the study. Samples were collected during the acute phase of illness i.e. 1-5 days of fever. The samples were grouped into 2 categories according to the days of fever - 1-5days and 5-15 days. The NS1 Antigen, IgG and IgM antibodies tests were done by ELISA and ICT in all the cases. EDTA blood samples were collected & the platelet count was done. SSPE 21.0 software was used for statistical purpose. **Results:** A total of 200 blood samples were studied. Out of the 200 studied sample 132 (66%) belonged to male patients and remaining 68 (34%) belonged to female patients. The M: F ratio was found to be 1:0.51. Most common age group of the patients was between 4-10 years (66%) followed by 15-18 (14.5%) and 11-15 years (10.5%). The least common age group was found to be less than 4 years of age (9%). 102 (51%) patients were having platelet count of less than 1 lac/mm3. The analysis of blood samples for NS1 antigen positivity showed that out of 200 sample NS1 was positive in 51 (25.5%) patients by ELISA and 49 (24.5%) patients by ICT. IgG/IgM ELISA was positive in 81 (40.5%) samples whereas IgG/IgM ICT was positive in 79 (39.50%) samples. Presence of either NS1 antigen or IgG/IgM antibodies was positive in 122 (61%) by ELISA and 119 (59.50%) by ICT. The comparison of NS1 ELISA and NS1 ICT showed that the results were comparable for both the tests with no statistically significant difference (P>0.05). Similarly, there was no statistically significant difference in IgG/IgM ELISA and IgG/IgM ICT and a combination of NS1 and IgG/IgM by ELISA and ICT (P>0.05). **Conclusion:** Performance of rapid diagnostic tests to detect the presence of Dengue NS1 antigen & IgM & IgG antibodies to dengue virus in comparison to ELISA in present specimen was found to be satisfactory. Even though RDT is treated as screening test places where other advanced diagnostics are not available RDT can be used for the diagnosis of Dengue virus infection.

Keywords: Dengue, Serological tests, Rapid diagnostic tests, ELISA, Diagnosis.

INTRODUCTION

Dengue fever is one of the most common arthropod-borne viral disease seen in humans. The dengue virus (DENV) belongs to the Flavivirus family and has four serotypes (DENV1-4), which are clinically indistinguishable. Its mode of transmission is by mosquito bite and the Aedes mosquitoes are vector for their transmission.[1] Latest estimates suggest 390 million infections of dengue occur each year, of which 100 million result in symptomatic disease. Infection with DENV results in varying degrees of pathological conditions, ranging from mild asymptomatic dengue fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which may turn fatal.[2] The severe form of dengue namely dengue hemorrhagic fever and dengue shock syndrome are usually seen in individuals who are infected by different serotypes and different time period. Presence of prior

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immunity to other serotype is a risk factor for development of DHF and DSS. Although dengue serotype 2 is the most prevalent serotype over the past 50 years, serotypes 3 and 4 have appeared in some epidemics.[3]

The patients usually give history of residing in an endemic area or there can be recent history of travelling to an endemic area. After an incubation period of 4-14 days patient may present with symptoms such as fever, chills, rash and severe body ache. In pediatric patients many of the children with dengue are initially diagnosed as measles because of presence of maculopapular rash which may be seen in dengue as well as measles. The other clinical features may include headache, orbital pain, hemorrhagic manifestation and in severe cases hypovolemic shock may occur due to depleted intravascular volume of blood.[4]

Early diagnosis is crucial for appropriate management of the patients. Four-fold rise in titers of IgG or IgM to one or more dengue viruses may suggest the diagnosis. The other method of diagnosis includes detection of viral genomic sequences in serum, PCR on CSF and detection of NSI antigen. Viral isolation is the gold standard for diagnosis and serotyping of dengue virus infection, but this method is time consuming and requires sophisticated laboratory Enzyme immunoassays are gaining popularity for early diagnosis of Dengue.[5] This study aims at analyzing the sensitivity and specificity of RICT kit with ELISA for NS1 antigen and IgM, IgG detection so as to explore its suitability for regular use in any modest resource constrain laboratory or primary health center as a bedside test. A rapid and accurate diagnosis of dengue in the acute phase of illness is important for initiation of therapy as well as for early enhancement of epidemic control measures especially in low endemic areas. Detection of specific IgM antibody by ELISA forms the mainstay for diagnosis. However, IgM antibodies develop after 4 to 5 days of infection.[6]

Molecular diagnosis such as RT- PCR requires experienced personnel and specialized laboratory equipment. As an alternative the detection of NS1 antigen of dengue virus has been identified as highly conserved glycoprotein expressed on either membrane bound or secreted form.[7]

With this background in mind we conducted this prospective study to detect NS1antigen and IgM, IgG antibody positivity among the study population and to compare ICT with ELISA for detection of dengue antigen (NS1) as well as antibodies (IgG, IgM).

MATERIALS AND METHODS

This was a prospective cohort study conducted over a period from August 2018 to Jan 2019 in a tertiary care private and teaching hospital. Informed written consent was taken from caretakers of all the pediatric patients who have been included in this study. The institutional ethical committee approved the study.

Cases: Majority of the samples were collected during the acute phase of illness i.e. 1-5 days of fever. The samples were grouped into 2 categories according to the days of fever - 1-5 days and 5-15 days. Serum was separated from all the samples and subjected for NS1 Ag, IgM and IgG antibodies detection on kits provided by J. Mitra & co, New Delhi. WHO criteria for clinical diagnosis of dengue was followed.[10] Brief demographic details of our patients were added in the form of a separate table. As NS1 and IgM ELISA was not possible for all samples in our resource constraint laboratory, 200 samples were selected, based on their onset of clinical symptoms (fever, rash, bleeding manifestation, arthralgia) for further confirmation of NS1 and IgM ELISA the samples were sent to other equipped laboratory. These 200 were further confirmed using ELISA technique for NS1 Ag by Panbio Australia and IgM by NIV, Pune in an attempt to detect NS1 antigen and IgM detection by ELISA. Platelet count of 200 samples were also done. Statistical analysis was done using SSPE 21.0 software. P lead than 0.05 was taken as statistically significant.

Control: Twenty blood samples from healthy individuals were collected as control& subjected for detection of both NS1 Ag and dengue specific IgM. NS1 Ag assay Detection of IgM and IgG test was performed using NS1Ag MICROLISA (J. Mitra& Co, New Delhi) test kit as per manufacturer's instruction.

Platelet Count: EDTA blood samples were collected & the platelet count was done in the CCL, Department of Pathology, and interpreted as normal, when the count was between 1, 50, 000- 4, 50, 000 and DHF, when the count was ‹ 1, 00, 000/ cmm (WHO cutoff for platelet count for DHF).

Treatment of Dengue patients included bed rest, rehydration and other supportive measures. Platelet concentrates were administered only for those patients with very low platelet counts and seriously ill with hemorrhagic complications.

Inclusion criteria

- Pediatric patients (up to 18 years of age).
- Patients who have given informed consent to be part of the study.
- Patients suspected for viral fever with temperature more than 37.5C with or without rashes and a positive tourniquet test followed by lab investigations such as low platelet counts.

Exclusion criteria

- Age above 18 years
- Those febrile patients with normal platelet count/hematocrit level or those who were positive

for malaria after peripheral smear examination were excluded from the study.

- Those who refused consent.
- Patients having thrombocytopenia of causes other than dengue such as idiopathic thrombocytopenic purpura and aplastic anemia etc.

RESULTS

In this study 200 samples from pediatric patients were selected, based on their onset of clinical symptoms (fever, rash, bleeding manifestation, arthralgia). These 200 samples were further confirmed using ELISA technique for NS1 Ag by Panbio Australia and IgM by NIV, Pune in an attempt to detect NS1 antigen and IgM detection by ELISA. Out of the 200 studied sample 132 (66%) belonged to boys and remaining 68 (34%) belonged to girls. The M: F ratio was found to be 1:0.51.

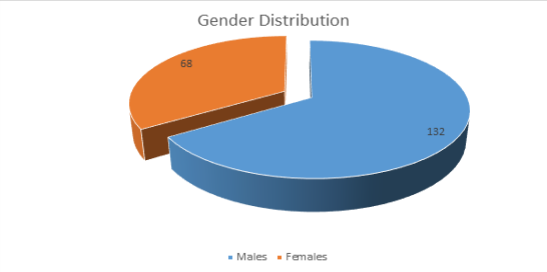

Figure 1: Gender Distribution of the Studied cases.

The analysis of age groups of the patients showed that most common age group of the patients was between 4-10 years (66%) followed by up to 15-18 years (14.5%) and 11-15 years (10.5%). The least common age group was found to be between less than 4 years of age (9 %).

Table 1: Age groups of the studied cases.

Age Group	No of patients	Percentage
Less than 4 yrs.	18	9 %
4-10 years	132	66 %
11-15 years	21	10.5 %
>15 yrs-18 yrs.	29	14.5 %
Total	200	100%

The analysis of cases on the basis of duration of fever showed that there was history of fever for 1-5 days in 119 (59.5%) patients where as in 81 (40.5%) patients the fever was present for more than 5 days and up to 15 days.

Table 2: Duration of Fever in studied cases.

Duration of Fever	No Of Patients	Percentage
1-5 Days	119	59.5 %
6-15 Days	81	40.5%
Total	200	100%

Out of 200 patients 102 (51 %) patients were having platelet count of less than 1 lac/mm3. Platelet count was between 1-2.5 lac/mm3 in 78 (39%) patients and 20 (10 %) patients were found to have a platelet count above 2.5 lac/mm3.

Table 3: Platelet count in studied cases.

Platelet Count	No Of Patients	Percentage
< 100000 /cu mm	102	51 %
100000-250000	78	39 %
> 250000	20	10 %
Total	200	100%

Isolated NS1 positivity was seen in 51 (25.5%) patients when done by ELISA and 49 (24.5%) patients when done by immunochromatography test.

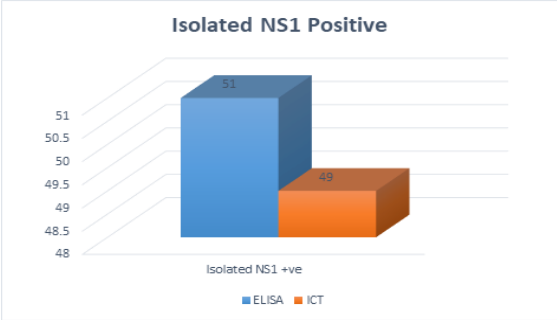

Figure 2: Isolated NS1 positivity by ELISA and ICT.

The analysis of blood samples for antibody positivity by ELISA showed that out of 200 sample dengue antibodies (either IgG, IgM or both) were positive in 81 samples (40.50%) by ELISA.

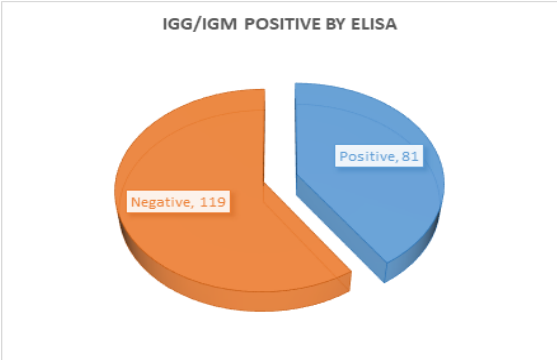

Figure 3: IgG, IgM positivity by ELISA.

The analysis of blood samples for antibody positivity by immunochromatography test showed that IgG, IgM or both were positive in 79 (39.50%) samples when done by immunochromatography test.

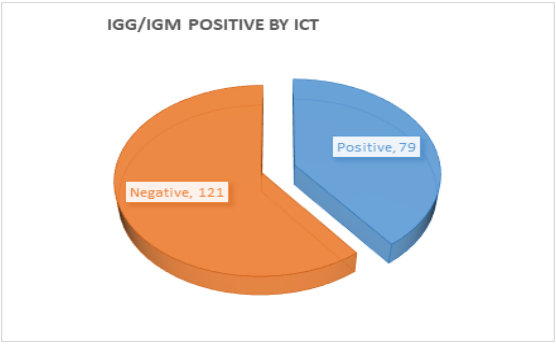

Figure 4: IgG, IgM positivity by ICT.

Based on a combination of NS1 and antibody tests (IgG or IgM or both) by ELISA 122 (61%) patients were found to be positive for dengue infection. In these cases, presence of either antigen or antibody or both was taken as positive.

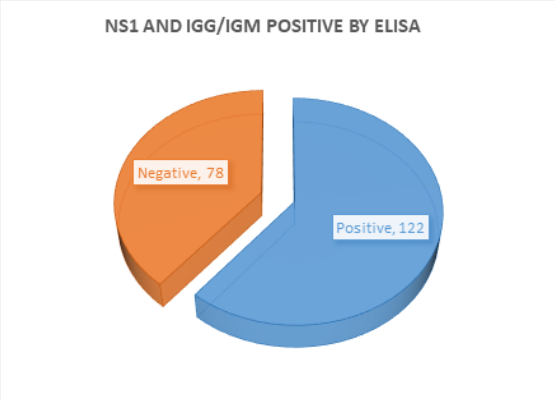

Figure 5: NS1 and/or IgG, IgM positivity By ELISA.

119 (59.50%) patients were found to be positive when combination of NS1 and antibody tests (IgG or IgM or both) was done by immunochromatography test. In these cases presence of either antigen or antibody or both was taken as positive.

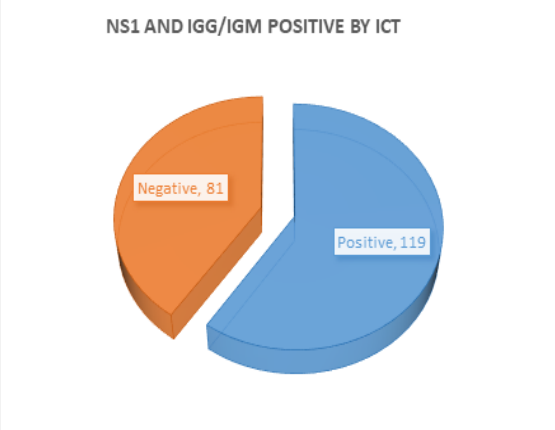

Figure 6: NS1 and/or IgG, IgM positivity By ICT.

The comparison of NS1 positivity by ELISA and ICT showed that out of 200 samples which were tested 51 (25.5%) samples tested positive for NS1 by ELISA whereas 49 (24.5%) samples tested positive for NS1 by ICT. The results were found to be comparable and there was no statistically significant difference in these 2 methods of NS1 detection (P=0.817).

Table 4: Comparison of NS1 by ICT and ELISA

	NS1 ELISA +ve	NS1 ELISA -ve	Total
NS1 ICT +ve	49	0	49
NS1 ICT -Ve	2	149	151
Total	51	149	200
P= 0.817 (Not Significant) 95% CI- 0.6029 to 1.4909			

The comparison of IgG or IgM positivity by ELISA and ICT showed that out of 200 samples which were tested 81 (25.5%) samples tested positive for either IgG, IgM or both by ELISA whereas 79 (39.5%) samples tested positive for IgG, IgM or both by ICT. The results were found to be comparable and there was no statistically significant difference in these 2 methods for IgG, IgM detection (P=0.959).

Table 5: Comparison of IgM/IgG by ICT and ELISA

	IgM/IgG ELISA +ve	IgM/IgG ELISA -ve	TOTAL
IgM/IgG ICT +ve	79	0	79
Igm/IgG ICT -ve	2	119	121
Total	81	119	200
P= 0.959 (Not Significant) 95% CI- 0.6429 to 1.4311			

The analysis of blood samples for presence of either NS1 antigen or IgG, IgM dengue or a combination of antigen and antibodies showed that out of 200 samples 122 were positive either for antigen (NS1) or dengue antibodies (IgG or IgM or Both) by ELISA whereas 119 samples were positive either for antigen (NS1) or dengue antibodies (IgG or IgM or Both) by ICT. The results were found to be comparable and there was no statistically significant difference in these 2 methods for combination of NS1 and IgG, IgM antibodies (P=0.959).

Table 6: Comparison of NS1 and IgM/IgG by ELISA and ICT.

	NS1 and IgM/IgG ELISA +ve	NS1 and IgM/IgG ELISA -ve	TOTAL
NS1 and IgM/IgG ICT +ve	119	0	119
NS1 and IgM/IgG ICT -ve	3	78	81
Total	122	78	200
P= 0.977 (Not Significant) 95% CI- 0.6525 to 1.4640			

DISCUSSION

Over the last two decades Dengue has emerged as the major vector-borne viral infection worldwide. It is transmitted by the female Aedes mosquito to human beings. Dengue may manifest as a spectrum of clinical syndromes i.e., from dengue fever to severe dengue. The World Health Organization (WHO) Dengue guidelines have identified a number of warning signs to aid in identifying the severity of disease in endemic areas. It is important to be vigilant as the prediction of which patients will progress to severe disease remains challenging.[8]

In India there are cyclic epidemics of dengue over the years. Dengue infection is one of the leading causes of hospitalization and death among children in the country. Concurrent infection in some patients with multiple serotypes of dengue resulted from co-circulation of several serotypes of the virus in India. Overall, India alone possess 34% (about 33 million

infections) of the total global threat of dengue leading to hyper-endemicity, prevailing mostly in urban areas. However, the wide spread problem of under reporting of dengue cases from India has come into focus very recently and the real burden of dengue in the country is heavily ignored.[9]

Overall, the factors for the global spread of dengue infection include vector and host factors. Aedes mosquito vector has adapted itself in urban areas in many parts of the world through dissemination on cargo ships, globalization, and increase in breeding sites through rapid and often poorly planned urbanization of cities.[10] Because of changes in climatic conditions, travel, socioeconomic status, trade, and viral characteristics the incidence and prevalence of dengue infection are expected to rise in future.[11] These factors in addition to ineffective vector control programs and lack of antiviral therapy or vaccines has led to recognize dengue as a public health threat for two-thirds of the world's population.[12]

Dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Virus detection and serological conversion have been the main targets of diagnostic assessment, however cross-reactivity of antibody responses among the flaviviruses has been a confounding issue in providing a differential diagnosis.[13] Furthermore, there is no single, definitive diagnostic biomarker that is present across the entire period of patient presentation, particularly in those experiencing a secondary dengue infection.[14]

Nevertheless, the development and commercialization of point-of-care combination tests capable of detecting markers of infection present during different stages of infection (viral nonstructural protein 1 and immunoglobulin M) has greatly simplified laboratory-based dengue diagnosis.[15] The dengue virus NS1 RDT showed 99.2% sensitivity and 96.0% specificity when analyzed using dengue virus NS1 ELISA as standard. The specificity and sensitivity of the RDT when compared with real time reverse transcriptase polymerase chain reaction (qRT-PCR) was 93.6% and 91.1%, respectively. The serotype specific evaluation showed more than 90% sensitivity and specificity for DENV-1, 2, and 3. The RDT is a good diagnostic tool in difficult to reach rural and tribal areas.[16] (Shukla, Singh et al. 2017) The NS1/IgM RDT (dengue day 1) showed high sensitivity throughout the acute phase of illness, in primary and secondary infections, in different severity groups, and detected all 4 dengue serotypes, including coinfections. This NS1/IgM RDT is a useful point-of-care assay for rapid and reliable diagnosis of acute dengue and an excellent surveillance tool in our battle against dengue.[17] Diagnosis of dengue based on clinical features alone is difficult. Rapid diagnostic tests for dengue may need to be routinely used patients presenting with sepsis and septic shock in tropical countries. This approach could improve diagnosis and management of those patients.[18]

Primary dengue virus infection is characterized by elevations in specific NS1 antigen levels 0 to 9 days after the onset of symptoms; this generally persists up to 15 days. Earlier diagnosis of Dengue reduces risk of complication such as DHF or DSS, especially in countries where dengue is endemic. Primary infection with dengue virus results in a self-limiting disease characterized by mild to high fever lasting 3 to 7 days, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting.[19] Secondary infection is the more common form of the disease in many parts of Southeast Asia and South America. IgM antibodies are not detectable until 5-10 days in case of primary dengue infection and until 4-5 days in secondary y infection after the onset of illness. IgG appear after 14 days and persist for life in case of primary infection and rise within 1-2 days after the onset of symptoms in secondary infection. This form of the disease is more serious and can result in DHF and DSS where the fatality rate can be as high as 40%. Early diagnosis of DSS is particularly important, as patients may die within 12 to 24 hours if appropriate treatment is not administered.[20]

CONCLUSION

Performance of rapid immunochromatographic tests (ICT) to detects the presence of Dengue NS1 antigen & IgM & IgG antibodies to dengue virus in comparison to Elisa in present specimen is satisfactory. The results by ICT and ELISA were found to be statistically comparable. Even though ICT are treated as screening test places where other advanced diagnostics tests like Nucleic acid detection, isolation in cell culture, antigen detection, Viral isolation and RNA detection, RTPCR and serological test like hemagglutination inhibition test, more specific alternative diagnosis are not available ICT can be used for the diagnosis of Dengue virus infection.

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How to cite this article: Ahirrao MH, Ahirrao BM, Dravid NV, Shah AD. Evaluation of Rapid Immunochromatographic Tests in Comparison to ELISA for Detection of NS1, IgM and IgG Antibodies for Early Detection of Dengue Infection in Pediatric Patients. Ann. Int. Med. Den. Res. 2019; 5(4):PE01-PE06.

Source of Support: Nil, **Conflict of Interest:** None declared