Comparison of Immuno-chromatography Test with ELISA for Acute Dengue Diagnosis at Tertiary Care Centre

Mihir Kumar Pattanayak[1], Abhishek Gaur[2], Rakesh Kumar[3], Manoj Minz[4], Prasanna Gupta[5]

ABSTRACT

Introduction: In the recent few decades, there had been a dramatic rise in the global incidence of dengue. As the disease is associated with high mortality and morbidity, a rapid and accurate diagnosis is essential for early appropriate management and for prevention of complications. Now days, a variety of rapid diagnostic tests (RDTs) kits and Enzyme Linked Immuno Sorbent Assay (ELISA) based test kits are available. In this present study we have attempted to do a diagnostic test evaluation of rapid ICT with ELISA for detection of NS1 antigen and IgM antibody for acute dengue diagnosis.

Material and Methods: A Cross-Sectional study was carried out in the Department of Microbiology, Govt. Medical College, Raigarh from November 2017 to October 2018. 1200 suspected serum samples were tested for dengue identification by Immuno-chromatography (ICT) based RDT kit (J. Mitra and Co. Pvt.Ltd, India) which detects NS1 antigen, IgM and IgG antibodies. From the Dengue rapid reactive samples test done by ICTs were subjected to ELISA tests for Confirmation NS1 antigen and IgM antibodies.

Results: The Rapid Dengue Test showed a sensitivity and specificity of 98% and 74% for NS1 antigen detection and 76% and 90% for IgM Antibody detection.

Conclusion: Good sensitivity and specificity of rapid diagnostic tests for early detection of dengue was observed. These kits are suitable for early detection of dengue cases, as with high sensitivity and specificity it can help in early screening of patients and can further limit the spread of disease where ELISA facilities are not available.

Keywords: Dengue, Immuno - Chromatography (ICT), ELISA, NS1 Antigen, Sensitivity, Specificity

INTRODUCTION

Dengue viruses are Arboviruses capable of infecting humans, and causing diseases.[1] Dengue virus belongs to the family Flaviviridae, genus Flavivirus. These viruses contain single stranded positive sense RNA.[2] It has four serotypes DEN-1, DEN-2, DEN-3 and DEN- 4 and a new serotype DEN-5, has been recently identified in the year 2013 in Bangkok.[3] Virus transmission in its simplest form involves ingestion of viremic blood by Aedes aegypti mosquito and passage to a second susceptible host.

In the recent few decades, there had been a dramatic rise in the global incidence of dengue. In more than one hundred countries of Africa, America and South East Africa, the disease is now considered to be endemic.[4,5]

The most severely affected regions are those from the Americas, South-East Asia and Western Pacific.

It is estimated by W.H.O (World Health Organization) that annual, there is an incidence of 50–100 million infections globally; and these number can be as high as 390 million due to inadequate notification, surveillance, and under reporting. According to National Vector Borne Disease Control Programme (NVBDCP) a total of 129166 of dengue cases have been detected with 245 deaths in the year 2016 in India, in the year 2017 total no. of cases reported was 188401 with 325 deaths reported and in the year 2018 up to 30th September 40868 with 83 deaths reported.[6]

Dengue fever is a severe flu like illness that affects any age groups person, but rarely causes death. Symptoms of infection are sudden onset of high

Fever (103-106°F), severe headache, backache, intense pain in joints and muscles, retro-orbital pain, nausea and vomiting and a generalized erythematous rash. Rash begins 4-7 days after the mosquito bite and typically lasts 3-10 days.[7]

Severe dengue like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) is a dangerous complication which can lead to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. These similarity in clinical features with other diseases, make confusion to diagnose and recognize it timely. There is no specific treatment for dengue, but early detection and access to proper medical care lowers fatality rates below one per cent and helps in early patient management and immediate application of appropriate vector control methods which can help to prevent the spread and control of the infection.[8,9]

As the disease is associated with high mortality and morbidity, a rapid and accurate diagnosis is essential for early appropriate management and for prevention of complications. Currently the three basic methods used for the diagnosis of dengue virus infection are viral isolation,

[1]Assistant Professor, Department of Microbiology, [2]Associate Professor, Department of Microbiology, [3]Assistant Professor, Department of Microbiology, [4]Professor and Head, Department of Pathology, [5]Prof. and Head, Department of Microbiology, NIMS, Jaipur, Rajasthan, India

Corresponding author: Prasanna Gupta, Professor and Head, Department of Microbiology, NIMS, Jaipur, Rajasthan, India

How to cite this article: Mihir Kumar Pattanayak, Abhishek Gaur, Rakesh Kumar, Manoj Minz, Prasanna Gupta. Comparison of immuno-chromatography test with ELISA for acute dengue diagnosis at tertiary care centre. International Journal of Contemporary Medical Research 2019;6(2):B1-B4.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.2.2

detection of the viral genomic sequence by a nucleic acid amplification technology assay (Reverse transcription polymerase chain reaction (RT-PCR)), and detection of dengue virus-specific IgM antibodies by the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA and/or the rapid dengue immune-chromatographic test (ICT).[10] However viral nucleic acid amplification technology assay need a specialised laboratory with trained personnel which are not available in hospital setting.

As per Indian national guidelines, a dengue patient is labelled as a "probable case" if he satisfies the clinical criteria during dengue outbreak or positive non ELISA based immuno-chromatography tests (ICT) such as NS1 antigen (Ag) ICT / IgM ICT.[11] A case is labelled as 'confirmed' when NS1 Ag / Ig M is positive by ELISA or detection of viral nucleic acids PCR or by culture and isolation of Dengue virus. Hence, for the routine diagnosis in peripheral hospitals we can use either rapid ICT or ELISA for the detection of NS1 Ag, IgM antibodies. But the disadvantage with rapid ICTs is their reliability. Recently, there are many reports on the sensitivity and specificity of the rapid diagnostic tests that are used to detect NS1 Ag and the IgM antibody. So the aim of our study is to assess and evaluate the performance of rapid ICTs in comparison with ELISA based tests in terms of sensitivity and specificity.

MATERIAL AND METHODS

The present study was conducted study from November 2017 to October 2018 in the Department of Microbiology, Govt. Medical College, Raigarh; as it was "Dengue Sentinel Surveillance Centre" notified by Director of Health Services, Chhattisgarh. A specially designed, semi-structured questionnaire form was used to collect the data on the demographic factors such as age, sex, and residence, in addition to the data on the history of the illness, the possible risk factors and the results of the investigations 1200 Blood samples of suspected dengue patients were collected from OPD and IPD in the Central Laboratory of the institution were collected under aseptic precautions and serum was separated and stored for further analysis. The serum samples were tested for dengue identification by ICT based RDT kit (J. Mitra and Co. Pvt.Ltd, India) which detects NS1 antigen, IgM and IgG antibodies. From the Dengue rapid reactive samples test done by ICTs were subjected to ELISA tests (Dengue NS1 Ag Microlisa (J.Mitra and Co. Pvt.Ltd, India and IgM capture ELISA (NIV Pune, India)) for Confirmation. All the tests were performed strictly adhering to the kit manufacturer's instruction.

RESULTS

In our study, a total of 1200 clinically suspected dengue patient were enlisted and their serum samples were tested. Out of these 375 samples were rapid test reactive by ICT based test. Among these, predominance of male patient having 63.34% (245), whereas female patient having 34.66% (130) were observed. Maximum numbers of the cases who were rapid ICT test reactive between the ages of 16-45 years of age. Among these reactive patient samples, many sample showed reactivity for one or more markers like NS1Ag, IgMAb or IgG Ab. Majority of NS1Ag only 291were (77.60%), followed by IgMAb only were 48 (12.80%) and combination of NS1Ag and IgM Ab were 23 (6.13%). In comparison of ELISA based test showed positivity, NS1Ag only were 221 (73.67%) followed by combination of NS1Ag and IgM Ab were 70 (23.33%) and IgMAb only 10 (3%). Table-5 shows comparison between ICT based RDT with ELISA based test of NS1 antigen with sensitivity and specificity are 98% and 74% respectively whereas table-6 shows comparison between ICT based RDT with ELISA based test of IgM antibody with sensitivity and specificity are 76% and 90% respectively.

Sex	N=375	Percentage (%)
Male	245	63.34
Female	130	36.66

Table-1: Sex distribution dengue probable cases

Age groups	N=375	Percentage (%)
1-15	23	6.2
16-45	285	76
46-80	67	17.8

Table-2: Age distribution Dengue probable cases

Dengue specific parameters	Tests	Percentage (%)
NS1 Ag only	291	77.60
IgM Ab only	48	12.80
NS1 Ag+ IgM Ab	23	6.13
NS1 Ag+IgM Ab+IgG Ab	4	1.06
IgM Ab+IgG Ab	4	1.06
IgG Only	2	0.53
NS1 Ag+ IgG Ab	3	0.80

Table-3: Dengue Probable cases [RDT, n= 375]

Dengue specific parameters	No. of Tests	Percentage (%)
NS1 Ag only	221	73.67%
IgM Ab only	10	3.00%
NS1 Ag+ IgM Ab	70	23.33%

Table-4: Dengue Confirm cases [ELISA, n= 300]

Test	No. of Positive	No. of Negative	Total
Rapid Test	321	54	375
ELISA	291	84	375
Sensitivity = 98 %, Specificity= 74 %, Positive predictive Value = 91%, Negative predictive value = 94%			

Table-5: NS1Ag Results of RDT Test in comparison to ELISA

Test	No. of Positive	No. of Negative	Total
Rapid Test	79	296	375
ELIA	80	295	375
Sensitivity = 76%, Specificity = 90%, Positive predictive Value = 70%, Negative predictive value = 93%			

Table-6: IgM Ab Results of Rapid Test in comparison to ELISA

DISCUSSION

It has been observed in tropical countries that most of the febrile diseases have similar signs and symptoms which can often resembles those of dengue, making it difficult to diagnose without laboratory confirmation, thus increasing the disease burden.[12]

In our study, majority of patients were predominantly males 245 (63.34%) than females 130 (36.66%), with a male to female ratio of 1.72:1. Our observation was similar and well corroborated with earlier studies.[13-16] All age groups people can infected by dengue viruses. In our study highest prevalence was seen in the age groups between 16-45 years and with male preponderance which is seen in other studies also.[17,15,18]

We also observed that 375 dengue rapid reactive cases, 321(85.6%) were either NS1 antigen alone or in combination with antibodies. 291 (77.6%) cases were exclusively NS1 antigen reactive. In comparison with ELISA based test, out of 300 dengue positive cases, 291(97%) were either NS1 antigen alone or in combination with antibodies. 221 (73.67%) cases were exclusively NS1 antigen positive. NS1 Ag circulates at high level in blood. Hence positive NS1 antigen test in a patient indicates acute phase of illness. Dengue specific IgM can be detected in blood only after 3 to 5 days of illness; hence it cannot be used as an early diagnostic marker.[19]

Many studies conducted in various hospital have obtained a wide range of sensitivity (48.5 to 98.7%) and specificity (71.42 to 100%) of ICT based RDTs compared with ELISA, similarly in the our study, we found a sensitivity and specificity of 98 % and 74% respectively for NS1 antigen detection and 76% and 90% respectively for IgM Antibody detection. As our study indicates that NS1 antigen is highly sensitive, it is an effective tool for early dengue infection. The positive predictive value of rapid ICT for NS1 Ag was high (91 %). This indicates that the probability of patient having acute dengue infection if the tests are positive is almost same as the ELISA based tests. This study finding corroborates with other studies, which have shown the PPV of rapid ICTs to be more than 85%.[20-22] So the ICT based RDTs had a major advantage due to easy to perform, less technical effort and can be done within few minutes.[23] Whereas ELISA is more costly and to perform, lab needs to be equipped with instruments like ELISA reader and washer.

In comparison to ELISA, main advantage of the ICT based RDT is that a single sample can be run without waiting for the samples to be gathered and processed. Another major advantage is that combination test kits in which there is provision for performing both NSI antigen, IgM and IgG antibodies tests at one go are available.[24] Lacking of lab infrastructure in rural and remote areas, ICT based RDT can play a major role in diagnostic and in patient management of acute dengue infection. The sensitivity and specificity of various kits may vary and this need to keep in mind while performing tests. But initial validation requires with ELISA will help for proper investigations.

CONCLUSION

In our study we found a good sensitivity and specificity of rapid diagnostic tests for early detection of dengue primary infection. These kits are suitable for early screening of patients and can further limit the spread of disease. These tests are easy to execute, and also need no professionalism or sophisticated equipment's and can help in an early detecting of cases, there by accelerating an early diagnosis, especially in centres where facilities for ELISA are not available. Therefore studies like this will contribute significantly to the clinical management and can reduce morbidity and mortality in dengue infection.

REFERENCES

1. Park K. Park's Textbook of Preventive Medicine.24th edition. Jabalpur: m/s Banarsidas Bhanot; 2017. p. 261-262.
2. K Surrender.Textbook of Microbiology. 1st Edition. Jaypee brothers Medical Publisher (p) Ltd 2012. p-581-582.
3. Sastry AS. Bhat SK. Aroboviruses 1st Edition. Jaypee Essentials of Medical Microbiology First edition. Jaypee Brothers Medical Publishers (P) Ltd: 2016. p 489- 491.
4. Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res. 1999; 53:35-70.
5. World Health Organization. Handbook of the World Health Organization. Geneva: WHO. (Dengue haemorrhagic fever: diagnosis, treatment and control). 2000, 1-84.
6. NVBDCP (National Vector Borne Disease Control Programme. Directorate General of health services. Minister of health and family welfare).Available from http://nvbdcp.gov.in/den-cd.html.
7. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control: New edi, Geneva: World Health Organization; 2009. Available at https://www.ncbi.nlm.nih.gov/books/NBK132015/.
8. W.H.O. Int. Media Centre Factsheets. [cited on 10th November 2017] Available from http://www.who.int/mediacentre/factsheets/fs117/en /
9. Parkash O, Hanim Shueb R. Diagnosis of Dengue Infection Using Conventional and Biosensor Based Techniques. Ploss A, ed. Viruses. 2015; 7:5410- 5427.
10. Datta S, Wattal C. Dengue NS1 antigen detection: A useful tool in early diagnosis of dengue virus infection. Indian J Med Microbiology 2010;28:107-10.
11. National Guidelines for Clinical Management of Dengue Fever, Dec 2014. http://www.nvbdcp.gov.in/iec.html.
12. Capeding MR, Chua MN, Hadinegoro SR, Hussain IIHM, Nallusamy R, Pitisuttithum P, et al. Dengue and Other Common Causes of Acute Febrile Illness in Asia: An Active Surveillance Study in Children. PLos Negl Trop. Dis 2013;7:e2331
13. Kulkarni SK. Trend and pattern of dengue cases admitted in a tertiary care centre. Sch J App Med Sci. 2016; 4:649-52.
14. Raju BJ, Rajaram G. Prevalence of dengue fever and

dengue hemorrhagic fever in government general hospital tirupati. Int J Res Health Sci 2013; 1:23-27.

15. Dash PK, Sharma S, Srivastava A, Santhosh SR, Parida MM, Neeraja M, et al. Emergence of dengue virus type 4 (genotype I) in India. Epidemiol Infect 201; 139:857-6.
16. Neeraja M, Lakshmi V, Teja VD, Umabala P, Subbalakshmi MV. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol 2006; 24:280-282.
17. Gupta, Ekta, Lalit Dar, Geetanjali Kapoor, and Shobha Broor. 2006. The Changing Epidemiology of Dengue in Delhi, India. Virol. J., 2006;3:1–5.
18. Sarkar, Arindam, Debjani Taraphdar, and Shyamalendu Chatterjee. 2012. Molecular Typing of Dengue Virus Circulating in Kolkata, India in 2010. J. Trop. Med., 960329.
19. Ingale SV, Upadhaye AJ, Upadhaye JJ, Int. J Res Med Sci. 2018;6:812-816.
20. Pal, Subhamoy, Allison L. Dauner, Mitra Indrani et al. Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. PloS one 2014;9: e113411.
21. Groen, Jan, et al. Evaluation of Six Immunoassays for Detection of Dengue Virus-Specific Immunoglobulin M and G Antibodies. Clin. Diag. Lab. Immunol., 2000;7: 867–71.
22. Shih, Hsin-I et al. Applications of a Rapid and Sensitive Dengue DUO Rapid Immunochromatographic Test Kit as a Diagnostic Strategy during a Dengue Type 2 Epidemic in an Urban City. PloS one 2016;11: e0158437.
23. Chaterji, Shera et al. Evaluation of the NS1 Rapid Test and the WHO Dengue Classification Schemes for Use as Bedside Diagnosis of Acute Dengue Fever in Adults. The American J. Trop. Med. Hygiene 2011;84:224–28.
24. Mitra, Shubhanker, et al. Comparative Evaluation of Validity and Cost-Benefit Analysis of Rapid Diagnostic Test (RDT) Kits in Diagnosis of Dengue Infection Using Composite Reference Criteria: A Cross-Sectional Study from South India." J. Vector Borne Dis. 2016;53: 30–36.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 26-12-2018; **Accepted:** 19-01-2019; **Published:** 30-01-2019