Original Research Article

Comparative analysis of NS1 antigen card test and ELISA of clinically suspected dengue fever cases in a tertiary care hospital, Mumbai

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ABSTRACT

Background: Dengue, mosquito borne acute febrile viral illness has evolved as a global life threatening public health concern in South east Asia affecting around 2.5 billion individuals in more than 100 countries.
Aim: The study was conducted to compare commercially available NS1 Ag card test with IgM Capture ELISA.
Materials and Methods: A total of 521 clinically suspected cases of dengue were tested by Rapid Card test and Enzyme Linked Immunosorbent Assay (ELISA).
Results: Confirmed dengue positive cases by these methods were 228.A total of 222 cases were found to be positive by Card test. Out of these 222 positive samples, 61 (27.5%) were positive for NS1 antigen only. A total of 123 cases were positive for IgM by ELISA (99 for IgM only and 24 for IgM + IgG) as compared to 117 cases by rapid card test. Thus six cases (2.6%) of IgM positivity were missed by the rapid card test.
Conclusion: Immunochromatographic testing (ICT) can prove to be a very useful tool for especially in resource poor settings, such as in the peripheral health care center. On the other hand, in tertiary care hospitals, where a large number of samples are to be tested and the technical expertise is available, ELISA can be very effective in diagnosis of dengue infection.

1. Introduction

Dengue is caused by a positive stranded RNA (Ribo Nucleic Acid) virus of the Flaviviridae family with four distinct serotypes (Dengue Virus 1 to 4), that are closely related antigenically. Recovery from one type of infection provides lifelong immunity against that serotype, but confers only partial and transient protection against subsequent infection by the other three serotypes. It has been suggested that sequential infection increases the risk of more serious disease, resulting in DHF. [1]

The number of dengue cases reported to WHO increased over 8 folds over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 4.2 million in 2019. [2]

Treated DHF/dengue shock syndrome (DSS) is associated with a one percent mortality rate, while, mortality rate among untreated cases rises up to 20%. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for four to five days, so many laboratories are nowadays performing Non-Structural 1 dengue antigen (NS1 Ag) detection test, and it is showing promising results.

Dengue vaccine development has been in progress for several decades, however the complex pathology of the illness, the need to control four virus serotypes simultaneously and insufficient investment by vaccine developers have hampered progress of vaccines. The vaccine should provide protective immunity to the four serotypes to avoid the ADE (Antibody dependent enhancement) phenomenon. [3] Once the vaccine is

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developed, a lot of challenges (significant practical, logistic, and scientific) need to be addressed, before these vaccines can be widely and safely used on the vulnerable populations.[4]

There is also no effective anti-viral therapeutics in the market. Supportive therapy, such as fluid replacement is the only treatment available for severe forms of the disease.[5] In absence of specific treatment and vaccine for dengue fever (DF); vector control is the only method by which spread of dengue can be prevented. As effective control and preventive programmes depend upon improved surveillance data, need for continuous sero-epidemiological surveillance for the timely formulation and implementation of effective dengue control programme.[6]

Early laboratory diagnosis of dengue virus infection is important, as it can allow early intervention and better prognosis, particularly if the disease is diagnosed prior to the defervescent phase, when the risk for DHF due to plasma leakage, as well the evolution to shock, is higher and this is routinely done by serological tests.

2. Materials and Methods

This study was carried out in a tertiary care hospital, Mumbai over a period of 16 months from February 2010 to June 2011. It included patients clinically suspected of dengue admitted in the wards. Five milliliter of blood received from all suspected cases. Serum was separated and tested for Dengue serology by Immunochromatography (ICT) based RDT lateral flow assay (Advantage Dengue NS1 Ag & Ab Combi Card test, Jai Mitra & co. Ltd., New Delhi) for presence of IgM and IgG antibody and NS1 Ag as per instructions of manufacturer.

All the serum samples were tested for IgM (PanBio dengue IgM capture ELISA test) and IgG antibodies (PanBio dengue IgG capture ELISA test). Procedure for ELISA test was carried out as per the instructions of manufacturer.

3. Result

A total of 521 clinically suspected cases of dengue were tested for the presence of NS1antigen and IgM, IgG antibodies by the rapid immunochromatography card test. They were also subjected to capture ELISA for IgM and IgG antibodies. 228 cases were confirmed to be dengue positive by these methods.

Table 1: Total no. of serologically positive cases by rapid, ELISA and by both the tests

Rapid test	ELISA test	Rapid +ELISA
222	171	228

A total of 123 cases were positive for IgM by ELISA as compared to 117 cases by rapid card test.

Table 2: Serologically positive cases diagnosed by rapid tests:

NS1 Ag	Antibody	IgM	IgG	IgM + IgG	NS1 +IgM	NS1+IgG	NS1 +IgM+IgG	Total samples Positive
61	161	72	40	17	25	04	03	222
27.5%	72.5%	32.4%	18.0%	7.7%	11.3%	1.8%	1.3%	100%

IgM positivity was seen in 32.4% of cases followed by NS1 positivity in 27.5% cases.

Table 3: Serologically positive cases diagnosed by ELISA test

IgM	IgG	IgM+IgG	Total samples Positive
99	48	24	171
57.9%	28.1%	14.0%	100%

IgM positivity rate was 57.9% by ELISA test.

Table 4: Comparison of IgM antibody by rapid card test and ELISA test

Rapid card test	ELISA
117	123
51.30%	53.90%

(Chi square=123.9 (df=1), p < 0.001, is significant)

Table 5: Comparison ofIgG antibody by rapid card test and ELISA test

Rapid card test	ELISA
64	72
28.10%	31.60%

(Chi square=159.2 (df=1), p < 0.001, is significant)

A total of 72 cases were positive for IgG by ELISA as compared to 64 cases by rapid card test

4. Discussion

A total of 222 samples were positive by card test. Out of these 222 positive samples, 61 (27.5%) were positive for NS1 antigen only. Results of the present study were consistent with the results (30%) of Kulkarni et al. (2011).[7] Datta (2010)[8] and Srivastava (2011)[9] have shown NS1 positivity in 23.3% & 16% of the cases respectively. Considering very high specificity of NS1, it can be stated that in the absence of NS1 antigen detection, 27.5% of the cases would have been missed in the present study. In addition, NS1 was also positive in combinations with IgM (11.3%), IgG (1.8%) and IgM + IgG (1.3%). In the present study NS1 in combination (IgM, IgG and IgM+IgG) was positive in 14.4% of cases. Kulkarni et al[7] had shown positivity in 11.3% of combination cases of NS1 Ag + IgM and NS1 Ag + IgG. Tricou et al.,[10] recently compared the performance of two other commercial NS1 rapid tests and identified that combined NS1 and IgM/IgG testing increased the overall test sensitivity without reducing specificity. 72 (32.4%) were positive for IgM only, while 40 (18%) were positive for IgG only. Kulkarni et al[7] in their study on association of platelet counts and serological markers have recorded a IgM positivity of 50%, IgG positivity of 3% and IgM +IgG positivity of 6%. In the present study IgM +IgG positivity rate was found to be 7.7%. Thus the findings of the present study for IgM only and IgM +IgG correlated with that of Kulkarni et al,[7] the present study recorded a high positivity rate for IgG only. This can be attributed to the presence of IgG antibody due to past exposure to dengue infection. At the same time, even past subclinical infections can lead to a detectable IgG Ab in the blood, which may persist for several years subsequently.

A total of 123 cases were positive for IgM by ELISA (99 for IgM only and 24 for IgM + IgG) as compared to 117 cases by rapid card test. Thus six cases (2.6%) of IgM positivity were missed by the rapid card test. The literature states that ELISA is more sensitive than rapid card test. Hence more cases were detected by the ELISA test in the present study. Similar observation was also made in the case of IgG ELISA, 72 cases were positive, while only 64 cases were positive by the rapid card test. Thus 8 cases (3.5%) were missed by the rapid card test. In a study by Aikat et al.,[11] rapid tests revealed a prevalence rate of dengue infection to be 17.7% compared to 18.3% with ELISA indicating a slightly lower accuracy for rapid test results.

5. Conclusion

Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases). Most of the cases coming to our hospital were referred from various places and had received a few days treatment before reaching this hospital. The precise day of fever at the time of conducting the test could not be obtained in large number of cases. In spite of this, NS1 was positive in 61 cases. During the early stages of the disease antigen detection holds promise as a useful tool to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis. Clearly, the emphasis must be on disease prevention if the trend of emergent disease is to be reversed. Effective disease prevention programs depends on effective vector control measures and sustained community involvement.

6. Source of Funding

7. Conflict of Interest

The authors declare that there is no conflict of interest.

8. Acknowledgment

References

1. Gubler DJ, Sather GE. Laboratory diagnosis of dengue and dengue hemorrhagic fever. In: Homma A, Cunha J, editors. Proceedings of the International Symposium on Yellow Fever and Dengue; 1988. p. 291–322.
2. Dengue in South East Region, 2010. A report by World Health Organization, (Available on WHO website). Accessed February 25, 2010.
3. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. *Clin Microbiol Rev*. 1998;11(3):480–96. doi:10.1128/cmr.11.3.480.

4. Chaturvedi UC, Shrivastava R. Dengue haemorrhagic fever: A global challenge. *Indian J Med Microbiol*. 2004;22(1):5–6.

5. Garg P, Nagpal J, Khairnar P, Seneviratne SL. Economic burden of dengue infections in India. *Trans R Soc Trop Med Hyg*. 2008;102(6):570–7. doi:10.1016/j.trstmh.2008.02.015.

6. Guzman MG, Kouri G. Dengue diagnosis, advances and challenges. *Int J Infect Dis*. 2004;8(2):69–80.

7. Kulkarni RD, Ajantha GS, Kalabhavi AS, Shetty PC, Patil SS, Upadhya AK, et al. Association of platelet count and serological markers of dengue infection- importance of NS1 antigen. *Indian J Med Microbiol*. 2011;29(4):359–62. doi:10.4103/0255-0857.90159.

8. Dutta AK, Biswas A, Baruah K, Dhariwal AC. National guidelines for diagnosis and management of dengue fever/ dengue hemorrhagic fever and dengue shock syndrome. *J Indian Med Assoc*. 2011;109(1):30–5.

9. Srivastava A, Dash PK, Tripathi NK, Sahani AK, Gopalan N, Rao PV. Evaluation of commercial NS1enzyme-linked immunosorbent assay for early detection of dengue infection. *Indian J Med Microbiol*. 2011;29:51–5.

10. Tricou V, Vu HT, Quynh NV, Nguyen CV, Tran HT. Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. *BMC Infect Dis*. 2010;10:142.

11. Aikat A, Sarkar A, Tudu N, Moitra S, Pal PP, Sarkar TK. Diagnostic dilemma of dengue fever:how much reliability are there in rapidity. *J Indian Med Assoc*. 2011;109(8):543–5.

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