



Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

doi:10.1016/S2222-1808(14)60588-0

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

# The 2011 outbreak of dengue virus infection in Malwa region of Punjab, India—an evaluation of various diagnostic tests

Neerja Jindal\*, Renu Bansal, Nitika Dhuria

Department of Microbiology, GGS Medical College, Faridkot, Punjab, India

## PEER REVIEW

**Peer reviewer**

Yuki Eshita, Ph.D., Associate Professor, Department of Infectious Disease Control, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasamamachi, Yufu-shi, Oita 879-5593, Japan.  
Tel: +81-97-586-5701  
Fax: +81-97-586-5701  
E-mail: yeshita@oita-u.ac.jp

**Comments**

This is a good epidemiological study. The authors evaluated several dengue diagnostic kits including detection of antibody and antigen, as well as decrease of platelet counts as a dengue specific marker. The authors concluded that both tests were needed for the precise diagnose. Also the authors argue why no platelet counts of dengue patient sera collected in this study were statistically different from healthy control sera.  
Details on Page 365

## ABSTRACT

**Objective:** To compare the efficacy of rapid immunochromatographic test (ICT) and ELISA for detection of dengue specific parameters (NS1 antigen and IgM antibodies) and the association of these parameters with thrombocytopenia.

**Methods:** A total of 1787 serum samples were obtained from the same number of clinically suspected cases of dengue during an outbreak (2011) of dengue in the Malwa region of Punjab. All the samples were subjected to rapid ICT, dengue early ELISA and dengue IgM-capture ELISA for detection of NS1 antigen and IgM antibodies. Platelet counts of all the cases positive for dengue infection and 150 cases negative for dengue parameters (control group) were recorded.

**Results:** Rapid ICT for NS1 antigen detection showed same specificity (100%) but lower sensitivity (85.81%) than the dengue early ELISA, while it was 100% and 94.10% respectively for IgM antibody detection. The difference in the platelet counts of cases positive and negative for dengue specific parameters was statically insignificant (standard error of proportions=0.017, Z value=0.84, P value=0.2).

**Conclusions:** The confirmation of serological diagnosis of dengue should always be based on ELISA test and ICT and/or thrombocytopenia should not be used as a stand-alone test.

## KEYWORDS

Dengue, IgM, NS1, Thrombocytopenia, MAC-ELISA, Dengue early ELISA

## 1. Introduction

Dengue is one of the most serious mosquito borne viral diseases prevalent in urban, semi-urban and rural areas in tropical and subtropical countries<sup>[1,2]</sup>. Annually, there are an estimated 100 million dengue virus infections worldwide<sup>[3]</sup>. There is variability in its clinical presentation and the non specific early symptoms mimic other febrile illnesses prevalent in the area. Its laboratory diagnosis is therefore an essential prerequisite for clinical management, surveillance,

control and prevention of the disease.

The precise diagnosis of dengue infection can be achieved through virus isolation, viral RNA detection through RT-PCR or by detecting dengue specific antigen and/or antibodies<sup>[4]</sup>. As the first two methods are time consuming, costly and not within the reach of even most of the tertiary care hospitals, in majority of the cases its diagnosis is based on the detection of dengue specific antibodies and/or NS1 antigen<sup>[3]</sup>. According to the guidelines of National Vector Borne Disease Control Programme (NVBDCP), IgM antibody-

\*Corresponding author: Dr. Neerja Jindal, Department of Microbiology, GGS Medical College, Faridkot, Punjab, India.

Tel: 91-94176-91818

E-mail: neerjarajender@hotmail.com

Article history:

Received 17 May 2014

Received in revised form 20 May, 2nd revised form 24 May, 3rd revised form 31 May 2014

Accepted 17 Jun 2014

Available online 4 Jul 2014

capture ELISA should be considered as diagnostic test for dengue infection<sup>[5]</sup>. However, most of the private hospitals and several diagnostic laboratories are using various rapid immunochromatographic tests (ICT) to detect the dengue specific antibodies and/or antigen. As thrombocytopenia is observed in several patients with dengue virus infection, enumeration of platelet count for demonstration of thrombocytopenia (platelet count<100000/mL) is an accessory test available for diagnosis of dengue infection even in resource poor laboratories<sup>[6]</sup>.

There was an outbreak of dengue infection in the Malwa region of Punjab in the year 2011 (June 2011 to October 2011). The retrospective analysis of the outbreak was undertaken to evaluate the performance of rapid immunochromatographic test (to detect dengue specific IgM antibodies and NS1 antigen) vis-a-vis IgM-capture ELISA and dengue early ELISA and the association of platelet counts to early dengue specific parameters (NS1 antigen and IgM antibodies) to establish an accurate and early diagnosis of acute dengue infection.

## 2. Materials and methods

During the 2011 outbreak of dengue infection, a total of 1787 serum samples obtained from clinically suspected cases of dengue infection were tested in Microbiology Department of GGS Medical College and Hospital, Faridkot. Since our laboratory works around the clock, the samples were tested immediately for NS1 antigen and IgM antibodies by rapid visual immunochromatography-based test (Dengue Day 1 test supplied by J Mitra and Co. Pvt. Ltd, New Delhi, India) as per manufacturer's instructions. All the samples were then subjected to Dengue IgM-capture ELISA (National Institute of virology, Pune, India) and Dengue Early ELISA (Panbio, Australia). Platelet counts of all the dengue seropositive cases and 150 dengue seronegative cases which served as controls were recorded. Association of platelet counts with different parameters of dengue infection was evaluated by using standard error of proportions (SEP) test.

## 3. Results

Out of 1787 serum samples tested, 586 (32.79%) were found to be positive for dengue infection. Of these 586, IgM-capture ELISA alone was positive in 445 (75.93%) and NS1 antigen alone in 118 (20.13%) and both the above serological parameters were present in 23 (3.90%).

Dengue specific IgM antibodies were tested by rapid ICT and IgM capture ELISA and comparison of these two tests showed that of the total 1787 samples, 440 were positive and 1319 were negative by both the tests. There were twenty eight samples which were positive by ELISA and negative by ICT and were considered as false negative. No sample was positive by ICT and negative by ELISA test (false positive- Nil). The agreement, sensitivity, specificity, positive predictive

value and negative predictive values between the two tests were 98.43%, 94.10%, 100%, 100% and 97.92%, respectively (Table 1).

**Table 1**

Dengue specific IgM antibody detection and NS1 antigen detection.

Parameter tested	Agreement <sup>a</sup>	Sensitivity <sup>b</sup>	Specificity <sup>c</sup>	PPV <sup>d</sup>	NPV <sup>e</sup>
IgM antibody	(440+1319)×100	440×100	1319×100	440×100	1319×100
	1787	440+28	1319+0	440+0	1319+28
	98.43%	94.10%	100%	100%	97.92%
NS1 antigen	(121+1646)×100	121×100	1646×100	121×100	1646×100
	1787	121+20	1646+0	121+0	1646+20
	98.88%	85.81%	100%	100%	98.79%

<sup>a</sup>: (Number of samples positive by both methods+number of samples negative by both methods)/total number of samples×100; <sup>b</sup>: True positive/(true positive+false negative)×100; <sup>c</sup>: True negative/(true negative+false positive)×100; <sup>d</sup>: True positive/(true positive+false positive)×100; <sup>e</sup>: True negative/(true negative+false negative)×100.

NS1 antigen was also tested by using rapid ICT and Dengue Early ELISA and their comparative evaluation showed that 121 samples were positive while 1646 were negative by both the tests. Twenty samples were positive alone by ELISA and were considered as false negative. No sample was positive by ICT and negative by ELISA (false positive- Nil). The agreement, sensitivity, specificity, positive predictive value and negative predictive values between the two tests were 98.88%, 85.81%, 100%, 100% and 98.79% respectively (Table 1).

Platelet counts of all the dengue positive cases was recorded and comparison of counts between the following groups was studied.

Group a: The patients positive for dengue infection (586) and control group (150).

Group b: Patients having dengue specific IgM antibodies by IgM capture ELISA test (445+23) and patients positive for NS1 antigen by Dengue Early ELISA (118+23).

Group c: Patients positive for NS1 antigen alone (118) by Dengue Early ELISA and those having IgM antibodies along with NS1 antigen (23).

Statically the differences were found to be insignificant with *P* values=0.20, 0.43 and 0.44 in Groups a,b,c respectively (Table 2).

**Table 2**

Comparison of association of platelet counts in groups a, b and c.

Group	Total	Platelet count<100000/mL (%)	<i>P</i> value
Dengue Positive	586	569 (97.0%)	0.20
Dengue Negative	150	143 (95.3%)	
IgM Positive	468	453 (96.7%)	0.43
NS1 Positive	141	137 (97.1%)	
NS1 Positive alone	118	114 (96.6%)	0.44
IgM & NS1 both positive	23	22 (95.7%)	

Group a: Dengue positive and dengue negative patients; Group b: Dengue patients positive for IgM antibodies and positive for NS1 antigen; Group c: Dengue patients positive for NS1 antigen alone and positive for both IgM antibodies and NS1 antigen.

## 4. Discussion

Of the 586 serum samples found to be positive for dengue infection in the present study, 468 (79.86%) showed positive

results for the the anti-dengue IgM antibodies which is the mainstay of the diagnosis of primary dengue infection. However, dengue specific antibodies appear around 5th day of fever in primary infection and by 3rd day in secondary infection<sup>[7]</sup>, thus creating a window period during which these antibodies are undetectable and unable to diagnose the early acute dengue infection.

Of late, tests for detection of non-structural protein (NS1) are available for the diagnosis of dengue infection. NS1 antigen has been shown to be a highly specific marker of this infection as it possesses not only group specific but also type specific determinants and can diagnose dengue from day 1 of the fever<sup>[2,4]</sup>. In our study, NS1 antigen alone was positive in 118 (20.13%) serum samples. We would have missed these 118 cases had we not included this test in our study. Arya *et al.*, 2011 reported that testing for NS1 assisted in the diagnosis of an additional 22.4% cases of primary dengue infection in the early phase of illness<sup>[8]</sup>. Kulkarni *et al.*, 2011 observed positivity of NS1 antigen in 30% of their cases and suggested that the test must be conducted in all cases of fever in both endemic and non-endemic areas<sup>[1]</sup>. NS1 positive cases are also viraemic and can transmit the infection if bitten by a mosquito<sup>[8]</sup>.

Many rapid ICT devices for detecting antibodies to dengue virus are commercially available and numerous studies have evaluated their performance in comparison to ELISA test. In our study, their sensitivity and specificity for IgM antibodies detection were 94.10% and 100% respectively. This is similar to findings of Moorthy *et al.* and Jayasimha *et al.* who have also reported better sensitivity of IgM-capture ELISA in comparison to rapid ICT<sup>[9,10]</sup>. On the other hand, some studies have reported comparable sensitivity and specificity for both the ELISA and rapid ICT<sup>[11]</sup>. The difference in the sensitivity and specificity of ELISA and rapid tests could be because of different principles of assay, different antigens and conjugates used in various commercial devices<sup>[10]</sup>.

Efficacy of the available tests for diagnosis of NS1 antigen was also studied. We observed that the rapid ICT had same specificity (100%) but lower sensitivity (85.81%) than the Dengue Early ELISA. Shrivastava *et al.* reported sensitivity and specificity of 62% and 100% respectively for rapid ICT<sup>[3]</sup>. Dengue Early ELISA has the added advantage of giving good detection rate upto 7 day of illness. Therefore the test could also be considered concurrently with an assay for dengue specific IgM antibodies in cases with a history of fever of more than 6 d<sup>[12]</sup>.

Association of the platelet counts to the dengue specific serological markers has been studied by some workers<sup>[1,8]</sup>. Kulkarni *et al.* reported that in case of fever, thrombocytopenia was more consistently present in dengue positive rather than dengue negative cases<sup>[1]</sup>. However, in the present study the difference in the platelet counts of cases positive and negative for dengue specific parameters was statistically insignificant ( $P=0.2$ ). This could be because thrombocytopenia is not associated with dengue fever alone. It could be the result of viral infections other than dengue prevalent in that area or it could be drug induced thrombocytopenia, collagen vascular disease or idiopathic

thrombocytopenia<sup>[13]</sup>. Moreover, it is not possible to rule out dengue infection in cases negative for all the dengue serological parameters without testing for viral replication by cell culture and/or molecular detection of dengue viral genome (RT-PCR), the facilities for which are not available even in our tertiary care hospital. In our study, 1. association of the platelet counts in dengue cases positive for IgM antibodies to those positive for NS1 antigen and 2. association of the platelet counts in dengue patients positive for NS1 antigen alone to those positive for both IgM antibodies and NS1 antigen, were also found to be statistically insignificant ( $P=0.43$  and  $0.44$ ). However, Kulkarni *et al.*, 2011 reported that thrombocytopenia was more consistently associated whenever NS1 was detected compared to IgM antibody detection and it gave excellent association when both NS1 and IgM were positive compared to NS1 positivity alone<sup>[1]</sup>. The wide variation in the platelet counts of the dengue patients is natural since the mechanism of dengue related thrombocytopenia and coagulopathy is complex<sup>[14]</sup>.

There are certain limitations of the present study as the study was conducted during an outbreak of dengue infection. Ours is a referral centre and most of the cases have reported to us from peripheral areas. A large number of these patients have received treatment before reaching our hospital and the precise day of fever at the time of conducting the test was not clear. Another limitation was the absence of gold standard test for authentication of the results. Facilities for molecular test and cell culture are not available even in very advanced laboratories.

Therefore it is concluded that for early and accurate diagnosis of dengue infection, the patients of febrile illness should be made aware to report early to the health care facilities. NS1 antigen assay alone or used in combination with MAC-ELISA could improve the diagnostic algorithm of dengue infection which could contribute significantly in the treatment and control of dengue infection. Considering the moderate performance and high cost of ICT devices, we recommend that ICT should not be used as a stand-alone test. The confirmation of serological diagnosis should always be based on ELISA test. As thrombocytopenia could also be the result of other viral fevers prevalent in the area, the patients having thrombocytopenia should always be tested for specific parameters of dengue. This would help prevent the spread of unnecessary panic in the community and to know the exact extent and nature of the outbreak to take effective control measures.

### Conflict of interest statement

We declare that we have no conflict of interest.

---

### Comments

#### Background

Dengue fever is one of the most serious mosquito borne viral diseases prevalent in tropical and subtropical countries.

Its laboratory diagnosis is an essential prerequisite for clinical management, surveillance, control and prevention of the disease. The precise diagnosis of dengue infection can be achieved through virus isolation, viral RNA detection or by detecting dengue specific antigen and/or antibodies.

### Research frontiers

There was an outbreak of dengue fever in the Malwa region of Punjab in the year 2011. The analysis of the outbreak was undertaken to evaluate the performance of rapid ICT (to detect dengue specific IgM antibodies and NS1 antigen) vis-a-vis IgM-capture ELISA and Dengue Early ELISA and the association of platelet counts to early dengue specific parameters (NS1 antigen and IgM antibodies) to establish an accurate and early diagnosis of acute dengue infection.

### Related reports

NS1 antigen shows a highly specific marker of this infection and can diagnose dengue from day 1 of the fever. Arya *et al.*, 2011 reported that testing for NS1 antigen assisted in the diagnosis of an additional 22.4% cases of primary dengue infection in the early phase of illness. Kulkarni *et al.*, 2011 observed positivity of NS1 antigen in 30% of their cases and suggested that the test must be conducted in all cases of fever. Moorthy *et al.* and Jayasimha *et al.* also reported better sensitivity of IgM-capture ELISA in comparison to rapid ICT.

### Innovations & breakthroughs

During the 2011 dengue outbreak, 586 serum samples out of 1787 were positive. 468 (79.86%) were detected by IgM-capture ELISA test. One hundred and eighteen (20.13%) samples except for 468 were detected by NS1 antigen test. The difference in the platelet counts of cases positive and negative for dengue specific parameters was statically insignificant (SEP=0.017, Z value=0.84, P value=0.2).

### Applications

Dengue early ELISA has the added advantage of giving good detection rate upto 7 day of illness. Therefore the test could also be considered concurrently with an assay for dengue specific IgM antibodies in cases with a history of fever of more than 6 days.

### Peer review

This is a good epidemiological study. The authors evaluated several dengue diagnostic kits including detection of antibody and antigen, as well as decrease of platelet counts as a dengue specific marker. The authors concluded that both tests were needed for the precise diagnose. Also the authors argue why no platelet counts of dengue patient sera collected in this study were statistically different from healthy control sera.

## References

- [1] Kulkarni RD, Patil SS, Ajantha GS, Upadhyaya AK, Kalabhavi AS, Shubhada RM, et al. Association of platelet count and serological markers of dengue infection—importance of NS1 antigen. *Indian J Med Microbiol* 2011; **29**: 359–362.
- [2] Datta S, Wattal C. Dengue NS1 antigen detection: a useful tool in early diagnosis of dengue virus infection. *Indian J Med Microbiol* 2010; **28**: 107–110.
- [3] Shrivastava A, Dash PK, Tripathi NK, Sahni AK, Gopalan N, Lakshmana Rao PV. Evaluation of a commercial dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. *Indian J Med Microbiol* 2011; **29**: 51–55.
- [4] Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, Mohamad M, et al. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J Virol Methods* 2007; **140**: 75–79.
- [5] Joshi PL. *Guidelines for clinical management of dengue fever, dengue haemorrhagic fever and dengue shock syndrome*. Delhi, India: Directorate of National Vector Borne Disease Control Programme; 2008, p. 11–12.
- [6] World Health Organization. *Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control*. 2nd edition. Geneva, Switzerland: WHO; 1997, p. 12–23.
- [7] Peeling RW, Arstob H, Pelegrino JL, Buchy P, Cardoso MJ, Devi S, et al. Evaluation of diagnostic tests: dengue. *Nat Rev Microbiol* 2010; **8**: S30–S38.
- [8] Arya SC, Agarwal N, Parikh SC, Agarwal S. Simultaneous detection of dengue NS1 antigen, IgM plus IgG and platelet enumeration during an outbreak. *Sultan Qaboos Univ Med J* 2011; **11**: 470–476.
- [9] Moorthy M, Chandu S, Selvaraj K, Abraham AM. Evaluation of a rapid immunochromatographic device for the detection of IgM and IgG antibodies to dengue viruses (DENV) in a tertiary care hospital in South India. *Indian J Med Microbiol* 2009; **27**: 254–256.
- [10] Jayasimha VL, Thippeswamy MTR, Yogesh Babu KV, Vinodkumar CS, Niranjan HP, Raghkumar KG, et al. Dengue: seroprevalence, comparison of rapid test with ELISA. *Natl J Basic Med Sc* 2012; **3**: 57–60.
- [11] Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M, Ager AL. Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the panbio rapid immunochromatographic test for diagnosis of dengue fever in Jamaica. *J Clin Microbiol* 1999; **37**: 1600–1601.
- [12] Kumarasamy V, Chua SK, Hassan Z, Wahab AH, Chem YK, Mohamad M, et al. Evaluating the sensitivity of a commercial dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. *Singapore Med J* 2007; **48**: 669–673.
- [13] Peters CJ. Infections caused by arthropod and rodent-borne viruses. In: Fauci AS, editor. *Harrison's principles of internal medicine*. 17th ed. New York: McGraw-Hill Medical Publishing Division; 2008, p. 1226–39.
- [14] Arya SC, Agarwal N. Evaluation of automated blood count analyzers for utility in resource poor laboratories. *Clin Chim Acta* 2009; **401**: 187.