

Prognostic Value of Rapid Test for Diagnosis of Dengue in Nepalese Patients during 2010 Epidemic

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ABSTRACT

Background

Dengue is an emerging vector borne disease in Nepal and rapid diagnostic test is important for early diagnosis of the disease.

Objectives

The aim of the study was to evaluate the diagnostic accuracy of commonly used rapid immunochromatographic test kit in Nepal during 2010 dengue epidemic and to assess disease burden of dengue.

Methods

A total of 131 acute and nonacute serum samples were collected during recent epidemic of dengue in 2010 from clinically suspected Nepalese patients of different hospitals. Rapid immunochromatographic test kit was used for early diagnosis and enzyme immunosorbent was chosen as a reference assay.

Results

The sensitivity and specificity of rapid test was 70% and 76.54% respectively whereas the prevalence of the disease was 38.17%. The odds ratio for males was 1.8 however; the association with the disease was statistically not significant.

Conclusion

The diagnostic accuracy of rapid immunochromatographic test for dengue diagnosis was low ($k=0.46$). So, it should be substituted by highly sensitive test device for prompt diagnosis and health personnel should consider appropriate timing of sample collection for better performance of rapid test.

KEYWORDS

Dengue, Epidemic, Rapid test

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INTRODUCTION

Dengue virus (DENV) infection causes wide spectrum of diseases characterized with dengue fever (DF) and dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).¹ These diseases are caused by four serotypes of DENV, which are transmitted by infected *Aedes* mosquitoes.^{2,3} Mostly DF is mild while subsequent infection with different serotypes may lead to severe DHF/DSS, and if prompt treatment is not undertaken, the disease may be critical. The clinical features of dengue are nonspecific as symptoms mimic other diseases such as Japanese encephalitis, malaria, leptospirosis and influenza.⁴ Therefore, early diagnosis of patients is very important for proper treatment.

The isolation of DENV in cell culture or detection of viral

RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) is not possible in routine laboratory because of lengthy reactions and expensive reagents.⁵ The enzyme linked immunosorbent assay (ELISA) has been mostly used for diagnosis but it takes several hours to complete and all health systems may not have such sophisticated diagnostic method.⁶ To wrestle in this situation, rapid immunochromatographic test offers a good choice for prompt diagnosis and the test is affordable in all health centers.^{7,8} The rapid diagnostic test (RDT) is imperative especially during dengue outbreak/epidemic for management of patients so that morbidity and mortality can be reduced substantially.

The aim of the study was to determine the prognostic value of commonly used rapid test for dengue diagnosis in Nepalese population and to assess the magnitude of the disease burden during 2010 epidemic of dengue.

METHODS

The study was designed as a descriptive cross-sectional study. The study was carried out between June and November of the year 2010. The total numbers of 131 serum samples (acute and nonacute) were collected from febrile patients with clinical symptoms and suspected of DENV infection during epidemic from Bharatpur hospital, Bharatpur; Dhading district hospital, Dhading and Damauli district hospital, Tanahu.⁹ Acute serum samples were taken from patients having fever in the early five days of DENV infection. Similarly, nonacute samples were collected from patients after five days of infection. Patients that were positive to malaria and typhoid were excluded from the study. The serum samples were stored and transported maintaining reverse cold chain to Everest International Clinic and Research Center (EICRC). The samples were tested by reference assay i.e. IgM capture ELISA followed by rapid test. The entire test was done at EICRC, Kathmandu, Nepal.

Detection of anti-dengue IgM by ELISA (SD, Bioline, Korea)

The required numbers of the wells were determined for the assay. Immediately 100 µl of diluted serum (1:100) was added into wells coated with anti-human IgM. The plate was incubated at 37°C for 60 minutes. Then, the plate was washed five times with diluted wash buffer. A mixture of 100 µl Ag and HRP conjugated MAb was added to the wells. Again the plate was incubated at 37°C for 60 minutes. The plate was then washed several times with diluted wash buffer. One hundred micro liter of TMB was pipetted and added to each well. Incubation was done at 20-25°C for 10 minutes. Finally, 100 µl of stop solution was added and observed the change in color pattern. Within 30 minutes, the absorbance of each well was taken at a wavelength of 450 nm with a reference filter of 620 nm by using ELISA Reader. The test was interpreted either positive or negative on the basis of cut-off value of the sample. The cut-off value

was obtained by addition of 0.3 to average absorbance of negative controls. If absorbance of the sample was greater than cut-off value, then it was considered positive and less than cut-off value as negative.

Rapid immunochromatographic test (SD, Bioline, Korea)

The kit and specimen were allowed to keep in room temperature. The test device from foil pouch was obtained and 5 µl of serum was added to square well marked “S” using capillary pipette. Few drops (3-4) of assay diluents were added to round shaped well and the result was read within 20 minutes. The result was interpreted negative if the control line was only visible on the test device. The test was IgM positive when the control line (C) and IgM line (M) were visible on the device. The result was considered invalid if control line failed to appear.

Analysis

Diagnostic accuracy was calculated using RDT result and compared with the outcome of reference assay ELISA. A 2 × 2 table was constructed in which the result of reference assay was cross tabulated with the index RDT to define true positive, false positive, false negative and true negative values. Likewise sensitivity, specificity, negative predictive value and positive predictive value were also determined. The kappa statistic was used as a measure of agreement between RDT and the reference assay. The demographic information was obtained by direct interview through questionnaire and consent was taken during collection of serum samples. The collected data was analyzed using Win Papi and SPSS version 16 software.

RESULTS

Among 131 febrile patients with median age 35.5 years old (range 1-68), 50 were positive to anti-dengue IgM by ELISA while the rest was negative (Table 1). The immunochromatographic test detected anti-dengue IgM in only 35 patients out of 50 that were positive by enzyme immunoassay. The remaining 15 patients were negative by rapid test. The number of patients that were negative by enzyme immunoassay but positive by rapid test was 19 (Table 2). The sensitivity of rapid immunochromatographic test was 70% and specificity was 76.54%. The kappa value of the test was 0.46 (Table 4).

Table 1. Diagnosis of patients with DENV infection by ELISA.

Patient demographics	Acute serum samples* (%)	Nonacute serum samples# (%)	No. of infected patients (%)
Age (years)	Male: female ratio [Odds ratio, male; 95% CI]		
1-68	1.94:1(33/75:17/56) [1.8, 1.15-3.72] P=0.622	30/103 (29.13)	20/28 (71.43)
			50/131 (38.17)

*Samples collected from patients with fever during early five days of infection

Samples collected from patients after five days of infection

Table 2. Comparison between rapid immunochromatographic test and ELISA for the diagnosis of DENV infection.

RDT	ELISA		Total patients (n)
	Positive (n)	Negative (n)	
Positive	35	19	54
Negative	15	62	77
Total	50	81	131

DISCUSSION

The rapid diagnosis of dengue is indispensable during dengue epidemic for proper management of the patients. Diagnosis depends on the accuracy of the test to discriminate DF from other complaints.¹⁰ The diagnostic accuracy is essential because the clinical symptoms of dengue are similar with other febrile illness. Moreover, the early diagnosis is helpful for prognosis of severe forms of dengue such as DHF/DSS and their prompt and appropriate treatment.¹¹ The RDT of dengue can be achieved by immunochromatographic method. Despite its presumptive diagnosis, it is a useful tool for diagnosis of primary and secondary dengue infection.¹² Various commercially available rapid diagnostic kits are used and these vary in their sensitivity and specificity. The evaluation of such test kits have not been done earlier and we evaluated the most commonly used rapid diagnostic device during epidemic of dengue in 2010. The study was concentrated to assess the test ability to detect IgM so, the results of the IgG detection bands were not taken into consideration.

Immunoglobulin M (IgM) capture ELISA (SD, Bioline, Korea) was chosen as a gold standard for confirmatory diagnosis of DENV infection. This enzyme immunoassay revealed 97.6% sensitivity along with specificity of 86.6%. The overall performance was graded perfect as given by kappa value of 0.85 so, the kappa value of 0.81 or more is regarded almost perfect for serological diagnosis.¹³ Therefore, rapid immunochromatographic test was evaluated with reference to ELISA. Firstly, serum samples were tested with IgM capture ELISA then, dengue positive and negative

samples were analyzed by immunochromatographic method. The sensitivity of the rapid test was 70% while its specificity was 76.54%. The kappa value (0.46) for RDT was not in agreement with the reference assay.

The result was similar to the study conducted by World Health Organization (WHO) to evaluate the commercially available four rapid test kits in which one of them was SD, Bioline. The sensitivity and specificity of the test was 60.9% and 90% respectively with kappa value of 0.5.¹³ But, the result was not in accordance with the earlier study performed in which SD, Bioline had sensitivity and specificity of 10.6% and 99% respectively.¹⁴ Similarly, the result was also different from another study done where SD, Bioline showed sensitivity of 21.8% and 98.85% specificity.⁴ In addition to these, the findings of the present study were in contrast with other studies done in Thailand (sensitivity 79% and 95% specificity) and Pakistan (sensitivity 48% and 100% specificity).^{5, 15}

The variation in sensitivity and specificity of rapid test kit among different studies laid a big question. Nevertheless, the possible justification might be due to regional differences of enrolled patients and the untimely collection of specimen after the onset of the disease.⁵ The other confounding factors for the result might be storage temperature of the test kit and prevalence of other febrile endemic diseases. The collection of blood sample at appropriate time is important for serological diagnosis of DENV infection. The dengue IgM antibody is produced at about five days after the disease onset. Therefore, samples collected at the early stage of acute infection may provide a false negative result and one should be encouraged for second test at the time of discharge to confirm DENV infection. Likewise, storage conditions can influence the performance of dengue RDT. The sensitivity of rapid immunochromatographic test kit decreases when stored at 35°C but there is no loss at 4°C.⁴ The test kits might be exposed to high temperature and humidity during transportation, which could directly influence the test performance.¹³

Table 3. Characteristics of RDT.

Manufacturer	Country	Assay principle	Target antibody	Specimen	Vol. of sample	Time for result	Sensitivity	Specificity
Standard Diagnostics	Kyonggi-do, Korea	Lateral flow	IgM or IgG	Serum or plasma	5 µl	15-20 min	91.2%	90%

Table 4. Efficacy of rapid test compared with ELISA.

Sensitivity (CI)	Specificity (CI)	Positive predictive value (CI)	Negative predictive value (CI)	False positive (CI)	False negative (CI)	Accuracy (CI)	Ka (CI)
70	76.54	64.81	80.52	23.46	30	76	0.46
(55.5-81.5)	(56.2-80.8)	(51.5-76.1)	(71-87)	(15.9-33.5)	(19.2-43.8)	(66.1-80.5)	(0.45-0.47)

^a Except k value, all are in percentage, CI is 95%

The performance of the rapid test is important for early diagnosis of acute dengue patients. The claim of manufacturer regarding the performance characteristics (91.2% sensitivity; specificity 90%) did not comply with our result. The test device we employed was not highly sensitive (70%) to detect cases while the specificity was slightly high (76.54%). The false-positive reactivity of rapid test might be due to occurrence of prevalent diseases such as malaria, leptospirosis, brucellosis and even influenza where dengue is endemic.⁴ The test can be encouraged in a condition when there is low prevalence of those diseases. Clearly, this underscores the urgent need of investigation for proficient use of RDT in the diagnosis of dengue.

The prevalence of dengue disease was 38.17% and this was estimated by IgM capture ELISA. The burden of the disease was high as compared to the previous studies.^{16, 17} The percentage of males (44) infected with DENV was high as compared to females (30.36) which was in harmony with other studies.^{17, 18} The odds ratio for males was 1.8 however, the association between the disease and sex was statistically not significant ($P>0.05$). Certainly, the disease has been recognized as a public health problem since 2006 outbreak however; surveillance of the disease is still missing.

The number of dengue cases has been gradually increasing each year and mortality has occurred in 2010 epidemic. It is high time that preparedness for management of patients should be undertaken at the earliest for potential dengue epidemic.

CONCLUSION

The rapid immunochromatographic test was evaluated against the gold standard ELISA and the overall performance of the rapid test was not good indicating low prognostic value so, the alternative to SD, Bionline with high accuracy should be explored for early diagnosis of DENV infection. Health personnel should consider appropriate timing of sample collection for better performance of RDT.

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REFERENCES

- Schreiber KV. An investigation of relationships between climate and dengue using a water budgeting techniques. *J Int Biometeorol* 2001;45:81-89.
- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998;11:480-496.
- Groen J, Koraka P, Velzing J, Copra C, Osterhaus AD. Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies. *Clini Diagn Lab Immuno* 2000;7:867-871.
- Blacksell SD, Neuton PN, Bell D, Kelly J, Mammen MP Jr, Vaughn DW, et al. The comparative accuracy of 8 commercial rapid immunochromatographic assays for the diagnosis of acute dengue virus infection. *Clin Infect Dis* 2006;42:1127-1134.
- Kittigul L, Suankeow K. Use of rapid immunochromatographic test for early diagnosis of dengue virus infection. *Eur J Clin Microbiol Infect Dis* 2002;21:224-226.
- Lam SK, Devine PL. Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgG antibodies produced during dengue infection. *J Clin Diagn Virol* 1998;10:75-81.
- Chakravarti A, Parthasarathy P, Chakravarti A. Evaluation of a rapid immunochromatographic test in the diagnosis of dengue fever. *Indian J Patho Microb* 2003;46(1):127-128.
- Cuzzubbo AJ, Endy TP, Nisalak A, Kalayanarooj S, Vaughn DW, Ogata SA, et al. Use of recombinant envelope proteins for serological diagnosis of dengue virus infection in an immunochromatographic assay. *Clini Diagn Lab Immuno* 2001;8(6):1150-1155.
- World health organization. Dengue hemorrhagic fever: diagnosis, treatment and control 1997 2nd Ed. Geneva, World Health Organization.
- Vaughn DW, Nisalak A, Kalayanarooj S, Solomon T, Dung NM, Cuzzubbo A, et al. Evaluation of rapid immunochromatographic test for diagnosis of dengue virus infection. *J Clin Microbiol* 1998;36(1):234-238.
- Berry N, Chakravarti A, Gur R, Mathur MD. Serological investigation of a febrile outbreak in Delhi, India, using a rapid immunochromatographic test. *J Clin Microbiol* 1998;36:2793-2796.
- Sekaran SD, Lan EC, Subramaniam G. Comparison of Five Serological Diagnostic Assays for the Detection of IgM and IgG Antibodies to Dengue Virus. *African J Microbiol Resea* 2008;2:141-147.
- World health organization. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Diagnostics Evaluation Series No. 3 2009 [cited 2010 July 27]. Available from <http://apps.who.int/tdr/publications/tdr-research-publications/diagnostics-evaluation-3/pdf/diagnostics-evaluation-3.pdf>
- Nga TT, Thai KT, Phuong HL, Giao PT, Hung LQ, Binh TQ, et al. Evaluation of two rapid immunochromatographic assays for diagnosis of dengue among vietnamese febrile patients. *Clin Vacc Immunol* 2007;14(6):799-801.
- Yusuf NW, Kausar N, Akbar R, Iqbal N. Comparison of diagnostic efficacy of rapid diagnostic devices for dengue virus infection—a pilot study. *J Ayub Med Coll Abbottabad* 2008;20(4):26-28.
- Sherchand JB, Pandey BD, Haruki K, Jimba M. Sero-diagnosis of Japanese encephalitis and dengue virus infection from clinically suspected patients of Nepal. *J Inst. Med* 2001;23:81-85.
- Sah OP, Subedi S, Morita K, Inone I, Kurane I, Pandey BD. Serological study of dengue virus infection in terai region. *Nepal Med Coll J* 2009;11(2):104-106.
- Dominguez NN. Current DF/DHF prevention and control programme in the Philippines. *Dengue Bull, WHO*; 1997.