J Nepal Health Res Counc 2009 Apr;7(14):29-32

Serodiagnosis of Dengue by Particle Agglutination Assay

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ABSTRACT

Background: Dengue is an emerging public health problem in Tarai Region of Nepal. The most affected are among the poorest populations living in remote, rural areas and urban slums who have even no access for medical treatment. This study was carried out with the objective of the determining the serodiagnosis of dengue in symptomatic individuals and comparing it to IgM ELISA.

Methods: One hundred eighty three samples were collected from the suspected cases having clinical symptoms of dengue in the Tarai region of Nepal during September to October 2007. Particle Agglutination (PA) assays was performed and it was compared with IgM capture ELISA.

Results: Out of the total of 183 serum samples that 55(30%) samples were positive by PA assay. When compared with IgM-capture ELISA, a 50 of 55 were positive (sensitivity of 98% and specificity of 96%; a positive prediction value of 90% and negative prediction value of 99%).

Conclusions: The finding of this study showed that dengue is firmly established in low endemic Tarai region of Nepal. The sensitivity and specificity of PA assay is acceptably high and will be useful in rural areas of Nepal.

Key words: Dengue, Tarai region, Particle agglutination test, IgM capture ELISA

INTRODUCTION

Dengue Viruses (DV) are transmitted to humans by infected mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus*[1]. There are four serotypes, dengue virus types 1, 2, 3, and 4.[2] The secondary infection by a different dengue serotype in patients harboring pre-existing, heterologous dengue antibodies is a risk factor.[3,4] However there is evidence that primary infection of dengue can cause severe manifestation leading to DHF/DSS indicating important of viral virulence suggested that molecular differences in dengue is also risk factor for manifesting.[5]

DF is an emerging disease affecting Nepal since 2004.[6] There was an concern of the disease in Nepal after the Indian and Pakistani epidemic of DF/DHF, which claimed more than 100 deaths and more increasingly several thousand cases in the year 2006.[7] There was an outbreak which was observed in 9 districts of Tarai region in Nepal in 2006.[8] At present, diagnosis and management of dengue, JE and other infectious diseases in Nepal is based on patient's clinical symptoms due to lack of limited diagnostic facility.[9] The laboratory diagnosis of dengue therefore, usually relies on serology. The simple and rapid ELISA methods have been adapted to detect antibodies against DENV.[10] The antigen capturing ELISA

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for virus detection is the most useful procedure currently available and it is widely recommended for Virological Surveillance.[11] Now a days Particle Agglutination (PA) system, which does not require specific laboratory facilities, has been developed for detecting DEV IgM.[12] In the present study we validated the PA assay in more serum samples. We applied the PA system to serum samples collected from febrile patients clinically suspected to suffer from dengue, in Tarai region of Nepal. We compared the results with those obtained by IgM-capture ELISA.

METHODS

One hundred and eighty-three blood samples were collected from the suspected patients having clinical symptoms of dengue suspected on the basis of fever, severe headache and rashes in Dengue-epidemic areas. The specimen was collected from hospitals of Chitwan, Hetauda, Birgung and Biratnagar in Tarai region of Nepal in 2007. Before collecting blood specimen, demographic information was recorded and informed consent was obtained from each patient or guardian. The samples were kept in the ice compartment of the refrigerator, brought to Everest International Clinic and Research Center in Kathmandu and they were stored at -70^{0}C in deep freeze until use.

The ELISA Kit is used to this report (PanBio Dengue Duo, Australia) to diagnose of dengue infection. The required number of the well are determined for the assay. Ten µl antigens were mixed with 2.5 ml antigen diluent. Then required volume of dilute antigen was removed from the mixture. The equal volume of Monoclonal antibody tracer were added to diluent antigen and mixed it left in a room that temperature between 20-25 ^{0}C. Immediately one hundred µl of diluted serum (1:100) were added into wells contained anti-human IgM plate. The plates were incubated at 37^{0}C for 30 min. The plates were washed six times with diluted wash buffer. One hundred µl of Ag-Mab tracer were mixed to plate. Again incubated at 37^{0}C for 30 min. The plates were washed six times with diluted wash buffer. One hundred µl Tetramethylbenzidine was Pipete and added in each well. This time incubation was done at 20-25^{0}C for only 10 min. Finally one hundred µl of stop solution was added and the color developed was closely observed. With in 30 min the absorbance of each well were taken at a wavelength of 450 nm as a reference to wave length by using Microplate ELISA Reader Model 700 (Cam Tech USA). If Panbio Unit exceeds 11, then Sample was considered as positive one and lesser as 9 were considered as negative one.

The samples were diluted with serum dilution buffer at 1:100. The human IgM (Red coloured plate) capture micro plates that were cut by a cutter for a number of strips needed and removed the seal from the strip. In addition two wells were required for positive and negative controls. The strips were washed thrice with wash buffer and taped on the paper towel to remove excess liquid from them.

The fifty µl of diluted samples were added to the wells. Samples were added immediately after washing strips to prevent surface of the wells from drying. Otherwise the wells may lose the ability to capture antibodies if dried completely and the wells were incubated for 30 min at room temperature. The strip was washed thrice with wash buffer and taped on paper towels to remove any liquid as previously. The Dengue antigen coated bead slurry (red colored) was gently mixed. The one hundred µl of the beads slurry was added into wells and allowed to settle for one hour at room temperature. When the Ha-Ny beads formed a button pattern at the bottom of well, the reaction was defined as negative. In contrast to this adhesion of Ha-Ny beads on the wall of the well was taken as positivity. Statistical analysis was done using a software SSPS 11.5 version.

RESULTS

The 183 serum samples collected from the febrile patients with the clinical symptoms of dengue, in DF-epidemic area of the Tarai region were tested for D-specific IgM by two serological methods namely IgM-capture PA and ELISA. Fifty-five (30%) of the 183 sample were IgM positive by the PA (Tab 1). Fifty (90%) out of the fifty-five PA IgM-positive samples were also IgM positive by IgM capture ELISA (Table 2). This PA assay has the Sensitivity of 98% and Specificity of 96%, a positive prediction value of 90% and negative prediction value of 99% in comparison with IgM-capture ELISA. Five samples were positive by PA assay, but negative by IgM captured ELISA. One hundred twenty seven cases were Dengue IgM negative by both IgM-captured ELISA and PA. These results suggest that there is high level of compatibility between the PA assay and IgM-captured ELISA.

Table 1. The Prevalence of IgM Cases by PA tests in symptomatic Patients.

S.N	Sample collected site	PA test		
		Total	Positive	Percent
1	Chitwan	66	13	19.6
2	Birgunj	60	15	25
3	Hetauda	17	7	41.1
4	Biratnagar	40	20	50
	Total	183	55	30.05

Table 2. Comparison of the Results between PA Assay and IgM-capture ELISA

		IgM-captured ELISA		
		Positive	Negative	Total
Particle agglutination assay	Positive	50	5	55
	Negative	1	127	128
Total		51	132	183

Sensitivity was 98% and Specificity was 96%.

DISCUSSION

Dengue is a major public health problem that is responsible for millions of cases of illness and thousands of deaths in tropical countries every year.[13] The increasing importance of Dengue and DHF in Asia, South America, and the Caribbean countries underlines the importance of early detection in controlling the spread of the disease.[13] Dengue occurred mainly in the Tarai region of Nepal during past few years. In Nepal, sporadic cases were noticed in foreigners in Nineties and the first case of DF was reported in the year 2004.[6] In previous report by Scherchanda *et al.*, the prevalence of dengue antibodies in the Southwestern region of Nepal was 10.4%.[14] Then, a confirmed outbreak i.e eleven cases, which was observed in nine districts of Tarai region in Nepal in 2006.[8] This report suggests that dengue virus have been circulating in Nepal for several years. A study by Epidemiology and Disease control Division, Department of Health Service of Nepal on *A.aegypti* between September and December 2006, indicated that *A. aegypti* has been introduced in the country and that its densities were high enough for successful transmission.[15]

At present, Dengue is diagnosed based on patient's clinical symptoms: high fever, headache, muscle and joint pain. Dengue infections are being misdiagnosed for other related infections and its importance is underestimated. Despite the available clinical guidelines, dengue can be misdiagnosed due to limited information on it among medical community. Given the lack of specificity of the symptoms of dengue, clinicians can confuse dengue with other similar infections, such as influenza, enterococus, chikungunya, viral haemorrhagic fevers, leptospirosis, malaria, or typhoid.[16] Moreover, dengue infection as an undifferentiated febrile illness may represent a large proportion of all the symptomatic cases of dengue.[17] Although there is currently no effective antiviral treatment; rapid diagnosis of Dengue to detect the antibody provides the opportunity to treat Dengue Infection.

The laboratory diagnosis of Dengue usually relies on serology. This is simple and rapid, ELISA methods which have been adapted to detect antibodies against DENV.[10] ELISA based methods using specific MABs can also lead to definite diagnosis. The antigen capture ELISA for virus detection is the most useful procedure currently available and it is widely recommended for virological surveillance.[11] However, IgM- capture ELISA requires relatively more sophisticated equipments, and highly trained personnel and is more useful in the referral diagnostic centers in developing countries.

The development of PA assay, which does not require such specific equipment and is relatively economical too and therefore, be beneficial for the rural areas with limited facilities and where trained personnel are not available. We did the PA assay of serum samples collected from Tarai region of Nepal. The sensitivity and specificity of the PA assay is high and will be useful in rural areas of Nepal. The discrepancies between the PA and IgM-combo ELISA need to evaluate further considering the patients histories in-depth. It may not be specific compare to IgM ELISA leading to false positive result for other flavivirus or other infectious disease. Serially collected serum samples could provide more valuable information. Although cross-reactivity of anti-flaviviral IgG has been well documented. IgM is known to be specific antibodies to dengue and is the antibody assay by ELISA in the study.[18] The data suggest that the PA assay for DV is quick, easy to perform and specific. This assay system is useful especially in the rural areas of Nepal to support the clinical diagnosis, management, and epidemiological studies of Dengue.

CONCLUSION

The findings of this study showed that dengue is firmly established in low endemic Tarai region of Nepal. The sensitivity and specificity of PA assay is acceptably high and determined to be useful in rural areas of Nepal.

ACKNOWLEDGEMENTS

We thank Nepal Health Research Council, WHO Regional Office for South East Asia for financial support and Pentax Co. Ltd, Japan for their supplies of PA kits, reagents as well as technical assistance. We would like to thank Dr. Durga D. Joshi for providing laboratory facilities, advice from Dr. Shingo Inone, Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan and all the staff of Everest International Clinic and Research Center for their technical support and all the participating hospitals: Bharatpur Hospital; Narayani Sub-regional Hospital and Koshi Zonal Hospital.

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