

# Use of Particle Agglutination in the Diagnosis of Dengue Virus Infection in Western Nepal

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## Abstract

Dengue is an emerging vector borne viral disease in Nepal. The study was conducted to detect dengue virus specific IgM and to analyze demographic distribution of positive cases. This is a cross-sectional study carried out in western region of Nepal from August-October 2007. A total of 116 serum samples were collected from suspected dengue virus infected patients visiting hospitals and investigated by particle agglutination. Out of 116 sera, 12 (10.34%) had a positive dengue virus specific IgM. The positivity in age group below 15 years old was 23.08%, 7.32% in 15-50 years age group and 14.29% above 50 years age group. The ratio of positivity in male and female was 1:1. However, there were no statistical significance in the positivity with age ( $P=0.27$ ) and sex ( $P=0.91$ ). Thus, dengue has been established in western region of Nepal and diagnosis of the disease can be done by particle agglutination assay. The particle agglutination should be suggested for the diagnosis of dengue in all health centers of Nepal. Awareness programmes on prevention of dengue and surveillance of the disease should be conducted to reduce morbidity and mortality during possible outbreak of it.

**Key words:** dengue, immunoglobulin M, particle agglutination, western Nepal

## Introduction

Dengue virus (DENV) causes an acute febrile illness that is transmitted from human to human by mosquitoes, predominantly *Aedes aegypti*. The DENV serotypes DEN-1 to DEN-4, of the genus Flavivirus, are responsible for the disease. The disease presents as dengue fever (DF) and a more severe forms dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Usually, DF is self limiting featured by mild to high fever, severe headache with pain behind the eyes, muscle and joint pain, rash and vomiting, however DHF resulting from secondary infection is characterized by hemorrhagic events and circulatory failure along with fever. Infection with one serotype results life long immunity but there is no cross protection against other serotypes (Gubler 1998). The

disease has been a public health concern in Nepal after the first outbreak of dengue in 2006. The diagnosis of the disease is a major problem in most health centers due to poor laboratory facility. In such situation, particle agglutination (PA) is an alternative for dengue diagnosis as it does not require special laboratory set up.

The PA is merely an agglutination reaction which is performed with the help of a kit. The kit consists of IgM antibody-coated microplate and purified DENV antigen coated Ha-Hy (hydroxyapatite-coated nylon) beads. The microplate captures human IgM antibodies from the serum and addition of the beads bind to IgM molecules, which are captured on the microplate giving

positive test. If the test sample does not contain anti-virus specific antibody, the beads sediment at the bottom of the well signifying the reaction is negative. Sensitivity and specificity of the kit are 98.4 % and 97.5 % respectively (Yamamoto *et al.* 2000). We used PA assay to detect IgM antibody for the diagnosis of dengue in this study.

## Methodology

This is a cross-sectional study conducted from August to November in 2007. A total number of 116 serum samples were collected by purposive sampling method during postmonsoon from Mahakali Zonal Hospital, Mahendranagar, Bheri Zonal Hospital, Nepalgunj and Lumbini Zonal Hospital, Butwal. A case was included if there was high fever with clinical symptoms and suspected of DENV infection according to WHO guidelines (WHO 1997).

The blood samples (5 ml from adult and 3 ml from children) were collected from each suspected case in sterile, clean, dry and labeled test tube. The collected blood in test tube was allowed to clot at room temperature. Then, the blood was centrifuged at 5000 rpm for 5 minutes and the serum was separated. The serum was then collected in vial and stored at 4°C for a week. The samples were transported to Everest International Clinic and Research Center (EICRC), Kathmandu maintaining reverse cold chain. Aliquots

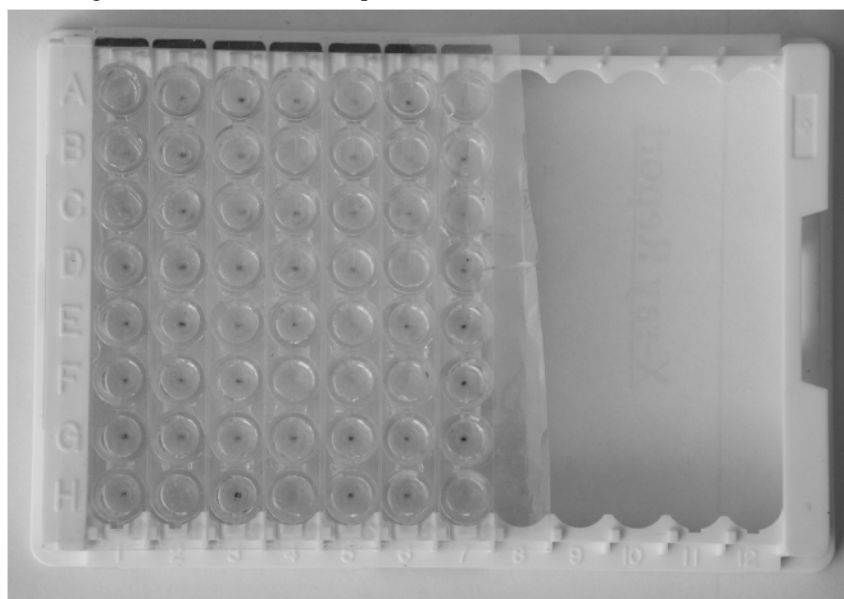
were made for analysis and refrigerated at 2-8°C until tested.

Sera were tested for anti-dengue IgM with PA assay kit (Pentax, Japan). The diluted serum (1:100) was added to the wells and incubated for 30 minutes at room temperature (20-25°C). After washing the wells, dengue virus antigen-coated beads slurry was added and allowed to settle for an hour. The result was interpreted on the basis of beads agglutination pattern (Fig. 1).

Patients' personal details about the symptoms and age, sex, etc were obtained through a questionnaire method by direct interview after obtaining the consent. Data analysis was done with the help of chi square test using Win pepi software.

## Result and Discussion

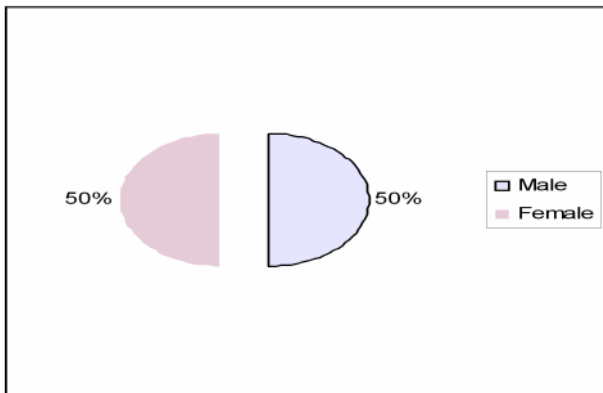
A total of 116 study subjects were included in the research for detection of anti-dengue IgM by PA (Table 1). Out of the total subjects, 12 (10.34%) had positive anti-dengue IgM test. The number of positive dengue serology for males and females were six each (Fig. 2). The percentage of positive cases were 23.08 (3/13) in the age group below 15 years old. Similarly, 7.32% (6/82) of positive cases were from 15- 50 years old and 14.29% (3/21) from above 50 years old (Fig.3). The association of DENV infection with sex (P=0.91) and age (P=0.27) was not statistically significant.



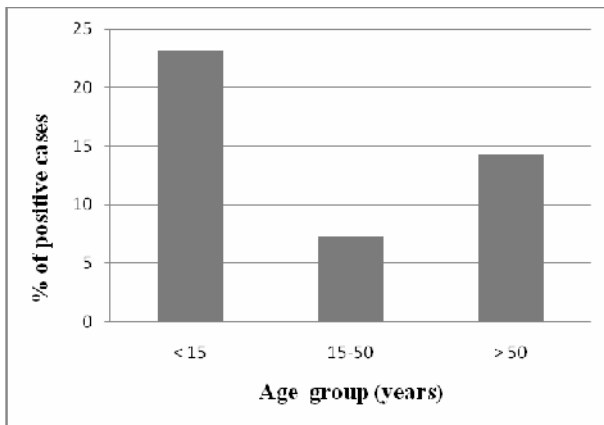
**Fig. 1.** Image showing beads agglutination pattern

**Table 1.** Distribution of the subjects by age and sex

Samples collected	116
Male: female ratio	1.07:1 (60:56)
Age (years)	
< 15 (child)	13
15-50 (adult)	82
> 50 (old)	21



**Fig. 2.** Sexwise distribution of patients with anti-dengue IgM



**Fig. 3.** Agewise distribution of patients with anti-dengue IgM

The exact year about the introduction of dengue in Nepal is not clear, but sporadic cases were reported in foreigners visiting Nepal in the late 1980s and 1990s (Kurane *et al.* 2000). Since then, the first case of DF was reported in the country in a Japanese volunteer in the year 2004 (Pandey *et al.* 2004). The outbreak of DF

was documented in November 2006 in several locations of Terai region of Nepal followed by 2010 epidemic, which claimed hundreds of hospitalization and few deaths (STIDH 2010). All the serotypes of DENV have been found in Nepal indicating the possibility of severe form of the disease (WHO/SEARO 2006). Dengue is a big threat for health as the incidence of the disease has increased 30-fold with increasing geographic expansion to new countries as well as from urban to rural settings (WHO 2008).

The anti-dengue IgM positivity was 10.34% in western region. The result was similar to the study conducted by Sherchand *et al.* (2001). The positivity to dengue indicates the circulation of DENV in the region. All the study areas are close to the borders of India, where dengue is endemic. The movement of people and the use of modern transportation across the border increase the transmission of the disease. The rate of transmission is pronounced in post monsoon period as the distribution of *Ae. aegypti* larval indices is highest during this period (Chakravorti & Kumaria 2005). Heavy rainfall results decrease in temperature and subsequently relative humidity is increased. This favorable situation along with abundant stocks of fresh water reservoir generated due to rain, develop optimum conditions for mass breeding and propagation of vector as well as transmission of the virus.

The dengue positive cases were clustered in the age group below 15 years old. The result was not in accordance with the data obtained during first outbreak of dengue in Nepal (WHO/SEARO 2006) and the study carried out by Gupta *et al.* (2006). This might be due to exposure of children to DENV as a result of day time transmission in the schools and outdoor activities of the children. The discrepancy of positive cases in different age groups might be due to disproportionate collection of samples in each age group. However, age was an independent predictor for DENV infection ( $P>0.05$ ).

Out of 12 positive cases, male and female patients were six each comprising 50% of the total positive cases. The finding was similar to the result obtained by Dominguez (1997), where 51% were males and 47% were females infected with DENV. However, the association between sex and the disease was not statistically significant ( $P>0.05$ ).

This study was based on PA test, which also detects cross reacting antibodies to other flavivirus such as Japanese encephalitis (JE) virus. However, subjects with previous JE immunizations were excluded from the study. Therefore, false positive results because of cross reactivity were minimal.

The diagnosis of dengue can be done by culture and molecular techniques but, these methods are not feasible in health centers of Nepal. The isolation of DENV in cell culture and detection of viral RNA by reverse transcriptase-polymerase chain reaction is not possible in routine laboratories because of long reaction time and high costs (Kittigul & Suankeow 2002). Enzyme linked immunosorbent assay (ELISA) has been widely used for the diagnosis of DENV infection but this method is also expensive and takes several hours to complete (Lam & Devine 1998). Therefore, PA could be the choice for dengue diagnosis because it is easy to perform and relatively inexpensive as compared to ELISA. Moreover, the result can be interpreted in a fairly short time. The reliability of PA has been trusted as it has sensitivity of 98% and specificity of 96% whereas positive prediction value of 90% and 99% negative prediction value during evaluation of the assay with gold standard ELISA (Sah *et al.* 2009).

Clearly, dengue was evident in western region of Nepal and PA was used as a serological tool to detect IgM antibody for the diagnosis of DENV infection. The PA should be recommended in all health centers for dengue diagnosis in developing countries like Nepal. Besides, awareness programmes on prevention of dengue and surveillance of the disease should be conducted to reduce morbidity and mortality during possible outbreak of dengue.

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