Serological study of dengue virus infection in *Terai* region, Nepal

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ABSTRACT

A cross-sectional study was conducted to determine dengue virus IgM-positive rate in *Terai* region, Nepal from August to December 2007. Serum samples were collected from 183 symptomatic cases. The samples were examined for dengue virus specific IgM using particle agglutination test. Of 183 serum samples, 55 (30.0%) had positive for dengue IgM antibody. The positive rate was highest (50.0%) in Biratnagar, and lowest (19.6%) in Chitwan male to female ratio was 2:1 in IgM-positive populations. IgM-positive rate was 29.0% at ages 21-30, 25.4% at ages 11-20 and 23.6% at ages 0-10, but 10.9% at ages 31-40, and ages over 40. There was not significant association between occupation of the patients and positive rate among farmer, labour, service, business and student.

Keywords: Dengue virus, Particle agglutination test, *Terai* region.

INTRODUCTION

Dengue virus (DENV) belongs to the family *Flaviviridae*, the genus *Flavivirus*. There are four serotypes, dengue virus types 1, 2, 3, and 4. Humans are natural hosts of DENV, and DENV is maintained between mosquitoes and humans in nature. *Aedes aegypti* and *Aedes albopictus* are the main vectors of DENV. DENV infection can be asymptomatic or causes two forms of illness, dengue fever (DF) and dengue hemorrhagic fever (DHF). DF is a self-limited febrile illness, while DHF is a life-threatening, often fatal illness.[1]

DF/DHF is a major public health problem that is responsible for millions of cases of illness and thousands of deaths in tropical countries worldwide every year.[2,3] There was a great concern of the disease in *Terai* region of Nepal after the outbreak in Indian.[4,6] DENV infection was recorded since 90's in Nepal and the first case of dengue was reported in 2004.[5] Then a confirmed outbreak was observed in nine districts of *Terai* region in Nepal in 2006.[6] These reports suggest that DENV has been circulating in Nepal for the past several years.

At present, diagnosis and management of dengue and Japanese encephalitis and other infectious diseases is based on patient's clinical symptoms due to the lack of limited diagnostic facility in Nepal.[7] The threat of the dengue virus is emerging as the disease caused high morbidity in neighboring countries.[4] Although there is high risk of dengue in

Nepal, there have been only few studies of the sero-prevalence to DENV. DENV infection can be serologically inferred by detecting immunoglobulin M (IgM). IgM-capture enzyme linked immunosorbent assay (ELISA) is used as a standard method for detecting IgM.[8] Recently, the particle agglutination (PA) assay has been developed for detecting DENV IgM.[9] In the present study we applied the PA assay to serum samples collected from febrile patients clinically suspected to be dengue, in *Terai* region.

This study would contribute to determine the epidemiological situation of dengue and to estimating the accuracy of dengue diagnosis in Nepal. This study provides useful information for dengue control and management of the cases in Nepal.

MATERIALS AND METHODS

Serum samples: The present study was conducted cross-sectional for a period of five months from August to December, 2007 in Eastern and Central *Terai* region, Nepal. Before collecting blood specimen, demographic and sociological information was recorded and informed consent was obtained from each patient or guardian. The study population comprised of individuals from all the age groups, attending outpatient and inpatient from different dengue endemic region. The study was carried out in hospitals of Chitwan, Hetauda, Birgunj and Biratnagar. Blood samples were collected from a total of 183 individuals experiencing a febrile illness clinically consistent with DENV infection. The samples were then kept on the ice compartment of the refrigerator, brought to Everest International Clinic and Research Center and immediately were stored at -70^{0}C in deep freeze.

Table-1: Positive rates of DENV IgM positive in symptomatic patients.

S.N	Sample collected site	Total	Positive	%	P-value
1	Chitwan	66	13	19.6	P<0.05
2	Birgunj	60	15	25.0	
3	Hetauda	17	7	41.1	
4	Biratnagar	40	20	50.0	
	Total	183	55	30.0	

Detection of anti-DENV IgM antibody by the particle agglutination kit: The samples were diluted with serum dilution buffer at 1:100 and added in anti-human IgM-coated V bottom 96-well microplates and react for 30 min at room temperature (RT). The serum samples were then removed from the wells and were washed thrice with wash buffer. One hundred microlitre of the hydroxyapatite-coated nylon (Ha-Ny) beads slurry (For IgM detection red colored beads slurry) was added into wells and allowed them to settle for one hour at RT. When the Ha-Ny beads formed a button pattern at the bottom of well, the reaction was defined as negative. Adhesion of Ha-Ny beads on the wall of the well was defined as positive reactions.

RT-PCR: RNA extraction from (140µl) of each serum samples was done by QIAamp® RNA viral kit (QIAGEN Inc., Valencia, CA), according to the manufacturer's directions.[10] RT-PCR of DEN virus RNA was carried out with DENV consensus and serotype-specific primers. Dengue RNA was reverse-transcribed into cDNA. Modifications to the procedure were as follows. Three microliters of total RNA were used in the ready-to-go RT-PCR beads kit (Amersham Biosciences), and the reaction included the forward and reverse specific primers of 0.5 µl of DC, DEN 1, DEN 2, DEN 3, and DEN 4. Forty six microlitre of PCR graded water was added to make final volume of fifty microlitres in the Ready to go RT-PCR bead. RT was carried for denaturation at 95^{0}C for 1 min, annealing 55^{0}C for 1min, extension 72^{0}C for 1 min and final extension for 7 min for 35 cycles RT-PCR products were analyzed by gel electrophoresis on a 2.0% agarose gel (Dotite) containing ethidium bromide (0.5 µg/ml). A band on the agarose gel of the correct size was interpreted as a positive result.

Statistical Analysis: Statistical analysis was done using a software SSPS 11.5 version.

Table-2: Age and sex wise distribution of total positive cases by PA test in symptomatic patients**.**

Age Group (yrs)	Male		Female		Total	
	n	%	n	%	N	%
0-10	9	23.6	4	23.5	13	23.6
11-20	8	21.0	6	35.2	14	25.4
21-30	10	26.3	6	35.2	16	29.0
31-40	6	15.7	0	0	6	10.9
Above 41	5	13.1	1	5.8	6	10.9
Total	38	100.0	17	100.0	55	100.0

RESULTS

Of a total of 183 serum samples, 55 were positive (30.0%) for DENV IgM. Among the four different places (Fig.1), the positive rate was 50.0% in Biratnagar, 41.1% in Hetauda, 25.0% in Birgunj and 19.6% in Chitwan (Tabel-1). IgM-positive rate was 29.0% at ages 21-30, 25.4% at ages 11-20 and 23.6% at ages 0-10, but 10.9% at ages 31-40, and ages over 40 shown in Table-2. The male to female ratio was 2:1 in IgM-positive populations. IgM-positive rate was compared based on the occupation: farmer, labour, service, business and student. There was not significant association between occupation of the patients and positive rate shown in Table-3.

DISCUSSION

Our study showed that there is a considerably higher prevalence (30.0%) of dengue infection. The number of positive cases was significantly higher (50.0%, P<0.05) in Biratnagar. The increased prevalence of dengue in *Terai* region of Nepal might be due to cross frequent traveling to Indian state of Bihar where dengue is a common problem. DF occurred mainly in *Terai* region of during past few years in Nepal.[5,6] The study conducted in Southwestern region of Nepal showed that dengue antibody positive rate was 10.4%.[11] However, in Nepal dengue has emerged as a new disease since 2004.[4] In the present study, DENV IgM was detected higher in the age group 21-30. Our finding was supported by Gupta *et al*[12] and Rahman *et al*.[13] As reported by Neeraja *et al,*[14] there is higher seropositive in male than female 2:1. Ours study also showed higher prevalence of dengue

Table-3: The No of IgM positive cases according to their Occupations**.**

Occupation	n	Positive no.	%	P-value
Farmer	90	24	26.6	
Laborer	25	8	32.0	
Service	19	5	26.3	P>0.05
Business	16	4	25.0	
Student	33	14	42.4	
Total	183	55	30.0	

Fig.1. A map indicating the *Terai* region and also four hospitals

IgM antibody in male than female (P<0.01) because males have relatively higher exposure to outside environment than female.

IgM-positive rate appeared to be higher among students followed by labour and farmer. The vector is active at day time, particularly at shade of tree or near by buildings. The students are prone to get bite because they play near school where the sun is not so bright and it is vulraneble place. The other groups labour and farmer are also active in their work and takes a rest at shade of tree.

The development of the PA assay, which does not require specific equipments and is relatively economical, would be beneficial for rural areas with limited facilities and where the trained personnel are not available.[9] Serially collected paired serum sample could provide more valuable information. Cross-reactivity of anti-flaviviral IgG has well documented; however, IgM is known to be specific.[7,8] Hence, this assay system is useful especially in the rural areas of Nepal to support the clinically diagnosis, management, and epidemiological studies of dengue.

The present studies indicate that dengue is firmly established in *Terai* region, Nepal. This strongly suggests a need for a continuous, surveillance and monitoring of DF/DHF should be strengthening with respect of increased incidence of dengue infection in *Terai* region of Nepal.

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