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Dengue in Western Terai Region of Nepal

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ABSTRACT

Background: Dengue Fever (DF) is an emerging mosquito-borne disease. It is a nagging public health problem in the low lands of Terai, expanding to new areas of Nepal in recent years.

Methods: A cross-sectional study was conducted to determine anti-Dengue IgM positive rate in Mahendranagar, Dhangadi and Dang between August 2008 and November 2009. Serum samples were collected from 283 patients visiting hospitals with history of fever, headache and suspected DF. The samples were examined by ELISA.

Results: The anti-Dengue IgM positivity was found to be 9.8 %. The positive rate was highest in Mahendranagar (13.3 %) followed by Dhangadi (9.8 %) ($P<0.05$). The Dengue positive cases were higher in female (10.9 %) than males (9.0 %). The positivity was higher in Ethnic group Brahman/Chherti (13.1%) as compared to Janajati (5.6 %). The highest positive cases (10.7 %) were from age group above 50 years. The highest numbers of Dengue positive cases were observed in occupation group of agriculture (18.2 %) ($P>0.05$).

Conclusions: Dengue has substantial expansion in Western and Far Western Terai region of Nepal which was limited to the middle Terai region in the past and mostly infects older people.

Keywords: dengue fever; IgM ELISA; terai region.

INTRODUCTION

Dengue fever (DF) is a vector-borne disease transmitted mainly through the *Aedes* species of infected mosquitos. The four serotypes DENV 1-4 are responsible for the disease.[1] The viral infection can result into dengue fever and more severe forms such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Secondary Dengue Virus Infection (DVI) is accountable for DHF and DSS.[2] The first case of Dengue was reported in 2004. In Nepal, the outbreak occurred following the Indian epidemic of DF/DHF in September-October 2006.[3-6] The occurrence of all the serotypes was reported during 2006 outbreak. [6-8]

There is high threat of Dengue in Nepal as the disease continues spreading in the Terai belt. A severe form of the disease DHF further results in high morbidity and mortality. Therefore, management of DHF must be carried out through recording the immune status of the people.

METHODS

The study was designed as a descriptive cross-sectional process for a period of sixteen months from August 2008 to November 2009 in Western and Far Western Terai region. A total of 283 samples were collected from Mahakali Zonal Hospital (MZH), Mahendranagar; Seti Zonal Hospital (SZH), Dhangadi; and Rapti Zonal Hospital (RZH), Dang. Before collecting blood specimen, consent was obtained from each patient or guardian. Demographic

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information, travel history, etc were recorded through a questionnaire by direct interview with patients or their guardians. A case was included if there was high fever with clinical symptoms suggestive of Dengue infection as specified by WHO.[9] A case was excluded, if routine tests suggested bacterial or any other disease.[9] The sample was kept at Everest International Clinic and Research Center, Kalanki at -20°C until use.

Detection of anti-Dengue IgM antibody by IgM-Capture ELISA

The Elisa Kit (Panbio, Austraila) was used to diagnose dengue. The required number of the wells was determined for the assay. Hundred microliter of diluted serum (1:100) was added into anti-human IgM coated wells. The plate was covered and incubated for 1 hour at 37°C. After incubation, wells were washed with diluted wash buffer. Hundred microliter of dengue antigen-MAb complexes were pipetted into the wells. The plate was covered and incubated for 1 hour at 37°C. The wells were again washed with diluted wash buffer and hundred microliter of Tetramethlylbenzidine which was then pipetted into each well, followed by incubation at room temperature (20-25°C) for 10 minutes. A blue colour was developed. Then, hundred microliter of stop solution was pipetted into all wells. The blue colour was changed to yellow. The absorbance of each well was read within 30 minutes at a wave length of 450 nm with a reference filter of 630 nm by using Multi ELISA Reader Model 2010 (Anthos). The sample was considered as positive if the Panbio Unit exceeded 11 and was negative if it was lesser than 9.

RT-PCR

RNA extraction from each serum sample was done by QIAamp® RNA viral kit (QIAGEN Inc., Valencia, CA), according to the manufacturer's directions.[10] RT-PCR of DV RNA was carried out with DV consensus and serotype-specific primers. RT-PCR products were analyzed by gel electrophoresis on a 2.0% agarose gel (Dotite) containing ethidium bromide (0.5µg/ml). A band on the agarose gel of the correct size when compared to 1 kb DNA ladder was interpreted as a positive result.

Statistical Analysis: Statistical analysis was done using a software SSPS 13.0 version.

RESULTS

Of the total 283 serum samples, 28 (9.8 %) were positive for anti-Dengue IgM antibody. Among three different places, the positive rate was 13.3% in Mahendranagar, 9.8% in Dhangadi and 1.6% in Dang. There was significant association between places and occurrence of disease (Table 1). The anti-Dengue IgM positive rate was 10.7 % at age group above 50, 10.1% at 15-50 years old and 8.8% below 15 years (Table 2). The positive male to female ratio was 1:1 (Table 3). In ethnicity, IgM-positive rate was 13.1% in Brahman/Chherti, 5.6% in Janajati and Teari/Dalits (Table 4). The positive rate was compared based on occupation that included student, agriculture, service holder, businessman, labour and housewife. There were no significant association between occupation of the patients and positive rate (Table 5). Out of 23 serum samples performed, all were negative to RT-PCR.

Table 1. The prevalence of DV by IgM capture ELISA in symptomatic patients

Samples collection site	Total	Positive (n) (%)	P-value
Mahendranagar	142	19 (13.3)	p<0.05
Dhangadi	81	8 (9.8)	
Dang	60	1(1.6)	
Total	283	28 (9.8)	

Table 2. Age wise distribution of total positive cases by IgM Capture ELISA in symptomatic patients

Age (years)	Number	Positive (n) (%)	P-value
Below 15	68	6 (5.8)	p>0.05
15-50	187	19 (10.1)	
Above 50	28	3 (10.7)	
Total	283	28 (9.8)	

Table 3. Sex wise distribution of positive cases

Sex	Number	Positive (n) (%)	P-value
Male	155	14 (9.0)	p>0.05
Female	128	14 (10.9)	
Total	283	28	

Table 4. Ethnic wise distribution of positive cases

Ethnic Group	Total	Positive (n) (%)	P-value
Brahman/Chherti	183	24 (13.1)	p>0.05
Terai/Madhesi	3	0 (0.0)	
Dalits	26	0 (0.0)	
Janajati	71	4 (5.6)	
Total	283	28 (9.8)	

Table 5. Occupation wise distribution of positive cases

Occupational group	Total	Positive (n) (%)	P-value
Student	164	19 (11.5)	p>0.05
Agriculture	57	5 (18.2)	
Service	35	3 (8.5)	
Business	15	0 (0.0)	
Labor	1	0 (0.0)	
House wife	11	1 (9.0)	
Total	283	28 (9.8)	

DISCUSSIONS

The study showed that there is a higher sero-prevalence (9.8%) of Dengue in Western Terai.[11] The number of total Dengue positive cases was significantly higher (67.8%, P<0.05) in Mahendranagar. According to Gupta et al,[12] the increased prevalence of DVI in western Terai might be due to the resident's frequent visits to Indian States where Dengue is prevalent. D.F over the past years occurred mainly at Terai.[6,7] There is limited information available on DVI in Nepal. The sero-positivity of the study was not in accordance with some of the previous findings from Nepal carried out by Sah et al in 2009. The present study result showed less positive rate than Sah et al (30%) which could be due to variation in geographical distribution. However, the present result was in harmony to the study conducted Sherchand et al in 2001.[13] In the present study, anti-Dengue positivity was detected higher in the age group 15-50 which was in accordance with the study of Gupta et al and Osman et al.[12,14]

The disease was prevalent in central and eastern Terai; it has been expanding to western region by and large.[11] Ecological disturbance and demographic changes result in dramatic increase in *Aedes aegypti* mosquito population and Dengue transmission. The movement of indigenous population and travelers, most of them susceptible to Dengue, aid in the spread of DV. The high rate of transmission during outbreak in new geographic areas can result in selective pressure that lead to genetic changes in the pathogen. The new strains of virus may have greater epidemic potential and virulence. Finally, the lack of effective vector control and deterioration in the ability of the public health infrastructure to deal with vector borne diseases contribute to the widespread and increased epidemic activity.[15]

The highest number of positive cases was found among Brahman/Chhetri (13.1%) and in the least among Madhesi and Dalits (0%). Madhesi and Dalits are actually less educated, poor as compared to other groups and have less access to health care facilities than perhaps Brahman/chettri.[16]

In occupation group, people of agriculture background (18.2%) were found more affected than the labour and businessman. The present study showed higher positivity rate in occupation group in agriculture which could be due to the fact that most farmers are involved in outdoor activities and there may be higher chance of being bitten by mosquitoes like *A. aegypti*. This might be the reason that Dengue infection is more prevalent in Terai.

In the present study, RT-PCR was performed in 23 serum samples of febrile patients. The reason for negative PCR result might be due to neutralization of virus by the antibody produced during late collection. It also might be due to degradation of virus because of thawing of temperature during sample transportation and storage. Other explanation might be lack of recent DVI to the febrile patients suspected of Dengue; the fever might be due to any other viral agents.[17]

CONCLUSION

Thus, we can clearly state that Dengue has significant expansion in western and far western Terai region of Nepal which was limited to eastern and middle Terai region in the past. The disease is expanding to new areas, so, the concerned authority should initiate surveillance of Dengue and commence an integrated vector control program to abate from epidemic Dengue in the coming year.

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CONFLICT OF INTERESTS

None declared.

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