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IC Value: **4.37**

BP Gupta et al. (2013) Int J Appl Sci Biotechnol, Vol 1(4): 224-227
DOI: 10.3126/.ijasbt.v1i4.9135

Research Article

International Journal of Applied Sciences and Biotechnology
ISSN: 2091-2609

SEROPREVALENCE OF DENGUE VIRUS INFECTION IN NEPAL

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Abstract

Dengue Virus infection is an emerging mosquito-borne disease. It is a global health problem and its expanding endemicity towards new territories is a serious concern. Relatively a new disease in Nepalese context, dengue abruptly appeared as massive outbreak in 2010, merely four years after its first introduction. It is a nagging public health problem in the low lands of Terai, expanding to new areas of Nepal in recent years. A cross-sectional study was conducted to determine anti-Dengue IgM positive rate in Lumbini, Dhading and Chitwan district. The study was carried from June 2012 to November 2012. The total number of Serum samples was collected from 275 patients visiting hospitals with history of fever, headache and suspected DF. The samples were examined by ELISA.The anti-Dengue IgM positivity was found to be 29.09 %. The positive rate was highest in Dhading (70.37%) followed by Bharatpur (37.6%) and Lumbini (11.38%). The Dengue positive cases were higher in males (32.5 %) than female (24.8 %). The highest positive cases (41.6%) were from age group less than 15 years. 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 viruses (DENVs), which belong to the genus *Flavivirus*, family *Flaviviridae*, comprise four serotype named dengue virus types 1, 2, 3, and 4 (DENV-1, -2, -3, and -4). Infection with any of these serotypes leads to a broad clinical spectrum, ranging from sub-clinical infection or an influenza-like disease known as dengue fever (DF) to a severe, sometimes fatal disease characterized by haemorrhage and shock, known as dengue haemorrhagic fever/dengue shock syndrome (DHF/ DSS). Dengue virus infection is a global health problem and its expanding endemicity towards new territories is a serious concern. Relatively a new disease in Nepalese context, dengue abruptly appeared as massive outbreak in 2010, merely four years after its first introduction. DENVs are transmitted to humans mainly by the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is the most important arthropod-borne virus in tropical and subtropical countries, with an estimated 50 million infections each year, resulting in 500,000 cases of DHF/DSS and 25,000 deaths.

Like other flaviviruses, dengue virus has a single-stranded, Positive - sense RNA genome of ~10,700 nucleotides, surrounded by a nucleocapsid and covered by a lipid envelope that contains the viral glycoproteins. The RNA genome contains a single open reading frame (ORF) flanked by two untranslated regions (5' and 3'UTRs). The single ORF encodes a precursor polyprotein, which is co- and post-translationally cleavaged resulting in the formation of three structural proteins, Capsid (C), membrane (M), and envelope (E), and seven nonstructural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. There is no specific antiviral therapy or vaccine in clinical use for dengue fever. Medical care is supportive in nature and focuses on monitoring and administration of fluids to prevent dehydration and shock, medications to lower fever and reduce pain, and management of bleeding complications.

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 (WHO 2009).The occurrence of all the serotypes was reported during 2006

outbreak (Pandey et al, 2004). There was an outbreak which was observed in 9 districts of Tarai region in Nepal in 2006 (Pandey et al, 2008). 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.

Materials and Method:

The study was designed as a descriptive cross-sectional. The study was carried from June 2012 to November 2012. The total number of 275 serum samples was collected from Lumbini Zonal Hospital (LZH), Butwal (123) and Dhading District Hospital (DDH), DhadingBesi (27) and Bharatapur Hospital (BH), Chitwan (125). Serum samples were collected from individuals experiencing a febrile illness clinically consistent with dengue infection, selected according to the inclusion and exclusion criteria. A case was included if there was high fever with clinical symptoms suggestive of dengue infection (WHO, 2012). A case was excluded, if routine laboratory testing suggested bacterial or any viral infection other than dengue infection or any other disease (WHO, 2012). Patients' personal details about the symptoms, age, sex etc. were obtained through a questionnaire method by direct interview. The entire test was done at Central Department of Biotechnology, Kirtipur, (CDBT), Kathmandu and Everest International Clinic and Research Center (EICRC), Kalanki, Kathmandu.

Sample collection, storage and transport

The serum samples from suspected cases were collected, stored and transported maintaining the cold chain to EICRC. Aliquots for ELISA were made and stored at 2-8^{o}C until tested.

Laboratory Tests

Detection of anti-dengue IgM-Capture ELISA

All reagents were bringing to room temperature (15-25^{o}C) before use.

Serum Predilution

Positive control, negative control ready for used and patient serum samples were diluted. For this, 10 µl of serum sample was diluted to 1000 µl of serum diluents (1:100).

Assay Plate

The Elisa Kit was used (Human Gesellschaft for Biochemica and DiagnosticambH, Germany) to diagnose of dengue infection.The required numbers of micro wells were removed from the foil sachet and were inserted into the strip holder. Four micro wells were required for controls: positive control (P) in duplicate and negative control (N) in duplicate. 100 µl diluted patient sample and controls were pipetted into their respective microwells of the assay plate. The plate was covered and incubated for 30 minutes at 37^{o}C. After incubation, wells were washed three times with diluted wash buffer. Hundred microliter of anti-IgM conjugate solution was pipetted into the wells. The plate was covered and incubated for 30 minutes at 37^{o}C. The wells were washed three times with diluted wash buffer and 100 µl of substrate reagent was pipetted into each well. Timing from the first addition, the plate was incubated at room temperature (17-25^{o}C) for 15 minutes. A blue colour was developed. Then 100 µl of stop solution was pipette into all wells in the same sequence and timing as the TMB addition. It was mixed well. 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 620 nm by using Multi ELISA Reader Model 2010 (Anthos, Austria). The test is interpreted either positive or negative on the basis of absorbance with respect to Cut-off value. If absorbance of the sample is greater than cut-off value, the sample is considered positive and if the absorbance of sample is less than cut-off value, the sample is negative.

$$\text{Cut-off value (CoV)} = \text{MNC}+0.35$$

Statistical analysis

The collected data was analyzed to find out hospital wise distribution of the cases. Chi square value and P value was determined to find out whether the findings were statistically significant or not. The collected data were analyzed using Win Pepi software (version 7.9, November 24, 2008) and Statistical package for social science (SPSS) software (version 16.0).

Results

Sex Wise Distribution of Positive DV Cases

Among the 163 male suspected DV cases tested, 53(32.5%) showed positive results for anti-dengue IgM antibody and out of 112 female cases 27 (24.8%) showed positive result for anti-dengue IgM antibody. Statistically there is no significant relationship (p=0.131) between male and female for the occurrence of disease (Table 1).

Table 1: Sex Wise Distribution of Positive Cases

Sex	Total no. of sample	No. of positive sample (%)	% of positives cases in total	Statistics
Male	163	53 (32.5)	19.2	
Female	112	27(24.8)	9.8	χ^2= 2.275
Total	275	80(29.09)		P=0.131

Table 2: Age Wise Distribution of IgM ELISA Positive DV Cases

Age (years)	No. of cases	No. of positive cases (%)	% of positive cases in total	statistics
<15	60	25(41.6)	9.0	χ^2= 7.09
15-50	150	35(23.3)	12.7	P=0.029
>50	65	20(30.7)	7.2	

Table 3: Hospital Wise Distribution of IgM capture ELISA Positive DV cases.

Hospital	No. of sample	No. of positive cases (%)	% of positive cases in total
LZH	123	14 (11.38)	5.0
BH	125	4 7(37.6)	17.0
DDH	27	19(70.37)	6.9
Total	275	80 (29.09)	

LZH:Lumbini Zonal Hospital, Butwal; BH: Bharatapur Hospital; DDH: Dhading District Hospital

Age Wise Distribution of DV Cases

Age wise result for anti-dengue IgM showed higher positive result among the age group 15 to 50 years (23.3 %) which constituted 12.7 % of total cases and least in age group above 50 years (30.7%) which comprised 7.2 % of total cases, statistically, there is significant relationship (p=0.029) between age group and occurrence of disease (Table 2).

Hospital Wise Distribution of DV Cases

Hospital wise distribution of IgM ELISA positive DV cases showed highest in BH (37.6%) which constitutes 17% of total. Lowest number of cases was found from LZH (11.38%) comprising 5.0 % of total samples tested (Table 3)

Discussions

The number of total Dengue positive cases was significantly higher in Dhading. According to Gupta et al 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 in Terai (Pandey et al, 2004).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.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.

The disease was prevalent in central and eastern Terai; it has been expanding to western region by and large.Ecological disturbance and demographic changes result in dramatic increase in *Aedes aegypti* mosquito population and Dengue transmission. The movement of indigenous population and travellers, 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. (Gubler et al, 1997).

In the present study, RT-PCR was performed in 23 serum samples of febrile patients. There is no any PCR positive band is detected.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.

Conclusion

Thus, we can clearly state that Dengue has significant expansion in different 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.

Acknowledgements

We thank Central Department of Biotechnology, TU for support of instrument as well as technical assistance. We would like to thank Dr. Tilak Shrestha, Central Department of Biotechnology for discussion. We would also like to thanks Dr. Roshan Kurmi, and Mr. Binit, Bhawani hospital, Birgunj for their valuable support. Finally, we like to thank all hospitals: Lumbini Zonal Hospital, Butwal; Bharatapur Hospital; Dhading District Hospital.

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