Dengue Sero-prevalence among clinically suspected patients: A study at a tertiary care hospital in central part of Nepal.

Suman Gautam[1]*, Roshan Sapkota[1]

[1]Department of Laboratory Medicine, Chitwan Medical College, Bharatpur, Chitwan, Nepal.

*Corresponding author: Suman Gautam, Chitwan medical college,Tribhuwan University,Nepal, Email: aachut21@gmail.com

ABSTRACT

Background: Dengue virus (DENV) is a single stranded positive-sense RNA virus which belongs to the Flaviviridae family genus Flavivirus. Dengue is a arthropod vector borne disease transmitted by Aedes species of the daytime-biting *Stegomyia* family mosquitoes, mainly *Aedes aegypti* and less frequently *Aedes albopictus* between natural hosts humans by one of five closely related but antigenically distinct virus serotypes DEN-1 to DEN-5.

Objectives: The study was undertaken to find out the prevalence of Dengue virus infection amongst the patients with clinical symptoms.

Methods: A descriptive, cross-sectional study was conducted over one year period in CMCTH from January to December 2016.

Results: Anti-dengue IgM positivity was found to be 14.5% (115/793 cases). The highest number of dengue cases was observed in 21-30 years followed by 31-40 years with greater predilection in males than in females. The positivity of anti-dengue IgM antibody according to the month is highest in October followed by November.

Conclusion: The incidence of dengue is also being moving towards the new areas, hilly region from terai. The higher prevalence of dengue positivity among suspected cases suggests early diagnosis and vector control program should be commenced to forecast any future outbreaks.

Keywords: Dengue virus, central Nepal, IgM capture ELISA, Aedes, Sero-prevalence

1. Introduction

Dengue virus (DENV), (+ve) ssRNA virus belongs to genus Flavivirus, family Flaviviridae.[1] Five DENV serotypes viz DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5 responsible for the

disease[2], transmitted to human by *Aedes* species of the daytime-biting *Stegomyia* family mosquitoes, mainly *Aedes aegypti* and less frequently *Aedes albopictus* characterized by acute febrile illness.[3,4] The clinical presentation of DENV infection range from an asymptomatic to mild febrile illness i.e. dengue fever (DF) to most severe life threatening forms Dengue Hemorrhagic fever (DHF).[5] Moreover, secondary infection with another serotype is considered to be a major risk factor for developing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[6]

Infection with one serotype provides congruent lifelong immunity to the same serotype but infection with different serotypes lead to severe form of disease condition i.e. DHF and DSS.[7,8]

The prevalence of dengue viral infection has been increasing dramatically in the recent years around the world. DV Infection is the major global public health problem that is responsible for illness of millions people and thousands of deaths in the tropical countries worldwide every year and is expanding endemicity towards new territories.[9]

Nepal is bordered by India in the eastern, western and southern belts that is one of the countries with higher risk and so is more vulnerable to worse consequences of DENV Infection. As with other vector borne diseases, outbreak of DF is related with increasing temperature, travel and frequent movement of people which is common due to open border between Nepal and India. DF was first reported in foreign visitor in Chitwan district of Nepal in 2004.[10] Nepal had experienced first dengue outbreak in November 2006 with 23 confirmed cases most of which had travel history to India[11], followed by massive outbreak in 2010 mearly after four years of its first appearance.[12] The evidence of major dengue fever cases in terai belt and the prevalence of A. aegypti in this region imply the expanding occurrence of dengue viral infection and its vulnerability to dengue outbreaks.[13]

Although there is high risk of dengue in Nepal, there are only few studies has been done on the prevalence of the dengue. So the aim of this study was to determine the Sero-prevalence of the dengue virus infection in the chitwan and adjoining regions, for providing valuable information on the epidemiology of the disease for the implementation of the dengue control program and management of the disease.

2. Materials and Methods

2.1 Study Design

A descriptive, cross-sectional study was carried out over a one year period from January 2016 to December 2016 in Immunology department of Department of Microbiology, Chitwan Medical College Teaching Hospital (CMCTH). Blood specimen was collected from all the patients

visiting CMCTH suspected of having Dengue virus infection. A total of 793 samples were collected and processed for anti-dengue IgM by IgM capture ELISA.

2.2 Sample collection and storage

All the patients suspected of DENV Infection during examination at hospital by clinicians justified by two or more symptoms i.e. retro-orbital pain, muscle and joint pain, loss of appetite, vomiting, diarrhea, abdominal pain, metallic taste in mouth, leucopenia and thrombocytopenia in complete blood cell count, haemorrhagic manifestations.

About 2 to 3 ml of blood was collected from each patients suspected with dengue virus infection using strict aseptic precautions and serum was prepared using standard microbiological methods. Aliquots of serum for ELISA were prepared and stored at 2-8ºC until tested.

2.3 Laboratory Test

Detection of anti dengue-IgM by capture ELISA (SD, Bioline, Korea)

Anti- dengue IgM, was detected using a commercially available IgM capture ELISA kit (SD Bioline, standard Diagnostic Inc., South Korea). The test results were interpreted either positive or negative according to manufacturer's instructions. The sensitivity and specificity of detection quoted by the manufacturer was 96.4% and 98.9% respectively, as evaluated by the US centers for Disease control and prevention by comparison to a 'gold standard' haemagglutination inhibition test.[14] The negative control provided by the manufacturer was anti-dengue IgG/IgM antibody-negative human serum maintained in proclin 300 preservative. This diagnostic kit provided qualitative detection of IgM antibodies specific to DENV in human serum, dependent on the principle. A 96-well microtitre plate was pre-coated with mouse monoclonal anti-human IgM antibodies in each well. During a first incubation, any anti-dengue IgM antibody present in the patient serum was bound to mouse monoclonal anti-human IgM antibodies. This was then bound to a complex of dengue antigen and mouse monoclonal anti-dengue horseradish Peroxidase conjugate. Following further incubation, all unbound material was removed by aspiration and washing. The enzyme activity found in the wells was directly proportional to the dengue IgM antibody concentration in patient serum, as evidenced by incubating the solid phase with the substrate solution Tetramethylbenzidine. Colorimetric reading was performed using a spectrophotometer at 450 nm.

Interpretation of the results

The test results was recorded and interpreted as either positive or negative on the basis of absorbance with respect to cut-off value determined as the mean absorbance of the negative controls + 0.300. If absorbance of the sample will be greater than cut-off value, the sample was considered positive and if the absorbance of sample will be less than cut-off value, the sample was negative for anti dengue-IgM.

2.3 Data Analysis Procedure

Data was entered, cleaned and analyzed using Statistical Package for Social Science version 20 (SPSS v. 20) and data presented in tables. P value was calculated using t test for the determination of the statistically significance of the data.

2.5 Ethical consideration

An informed consent was taken from the individual patient and ethical clearance was obtained from the Institutional Review Committee (IRC) of Chitwan Medical College Teaching Hospital.

3. Results

Among 793 suspected DENV cases, sample was collected and processed for IgM ELISA during study period. Out of 793 samples, 115 (14.50%) were found to be positive for anti-dengue IgM antibodies.

3.1 Age and Gender wise distribution

In the study population, highest number 187(23.58%) belonged to the 20-30 years age group whereas lowest number 10(1.26%) to above 80 years age group. Gender wise, among total suspected cases 51.58% (409/793) were males and 48.42% (384/793) were females. Among a total 115 anti-dengue IgM antibodies positive cases, higher positive rate was seen in the 20-30 years age group followed by 30-40 years age group 27.83% and 26.10% respectively. In contrast, there was not a single positive case in the age group 80-90 years. Gender wise, positive case of males was observed to be 15.89% which constituted 8.2% of total cases and 13.02% were females which comprised 6.30% of total cases. This gender bias, a male to female ratio of 1.3:1 was statistically significant (p value < 0.5) (Table 1& 2).

Table 1. Age and Gender wise distribution of dengue cases

Age Group	Total suspected cases (n= 793)			Positive cases (n=115)		
Years	Male	Female	Total (%)	Male	Female	Total (%)
0-10	26	14	40(5.04)	1	2	3(2.61)
10-20	72	54	126(15.90)	11	6	17(14.78)
20-30	84	103	187(23.58)	16	16	32(27.83)
30-40	68	77	145(18.28)	18	12	30(26.10)
40-50	55	57	112(14.12)	10	10	20(17.39)
50-60	53	39	92(11.60)	4	2	6(5.22)
60-70	25	29	54(6.81)	4	2	6(5.22)
70-80	19	8	27(3.4)	1	0	1(0.86)
80-90	7	3	10(1.26)	0	0	0
Total	409	384	793	65	50	115

Table 2: Gender wise distribution of cases

Sex	Total no. of sample	No. of positive sample (%)	% of positive cases in total	Male:Female among positive cases
Male	409	65 (15.89%)	8.20	
				1.3:1
Female	384	50 (13.02%)	6.30	
Total	793	115 (14.50%)		

3.2 Monthly distribution of positive cases

Month-wise suspected cases of DENV shows highest number of cases in October followed by November whereas lowest in the month of January. The positivity of anti-dengue IgM antibody according to the month was also observed highest in October 50.43% followed by November 32.17%, in contrast no positive cases were observed over the period of 5 month (Table 3).

Table 3. Monthly distribution of dengue cases along with number of positive cases

Month	Total cases (n=793)	Positive cases (n=115)
January	6	0
February	15	0
March	15	1(0.87%)
April	19	0
May	18	1(0.87%)
June	19	0
July	52	0
August	71	1(0.87%)
September	88	16(13.91%)
October	322	58(50.43%)
November	147	37(32.17%)
December	21	1(0.87%)
Total	793	115

4. Discussion

Dengue is an important emerging disease of the tropical and sub-tropical regions of the world. In common with other vector-borne diseases, dengue requires conductive predisposing conditions for endemicity and outbreaks. The countries of South East Asia share such common features as large human populations, rapid urbanization, development activities and monsoon rains. Urban populations now constitute the reservoir and travelers are the predominant factor for the dissemination of viruses between countries.[15]

Nepal had encountered sporadic dengue cases since 90's in foreigners and the first case of DF was reported in Nepal in the year 2004[16] and report suggested that dengue infections are being misdiagnosed for other related infections and its importance is underestimated. The exact dengue burden in the country is under reported due to the lack of proper knowledge about it in the society as well as the majority of health staff and lack of proper laboratory facilities.[13]

Present study was based on IgM capture ELISA for the laboratory diagnosis which is the most widely used serological test for dengue diagnosis. It has been found to be as reliable, useful and inexpensive indicator of primary infection and becomes the frontline diagnostic test in situation where speed is required or the demonstration of rising titer is not possible.[17] The technique has sensitivity and specificity of approximately 96.4% and 98.9% respectively. IgM capture ELISA can be applied to the Sero-diagnosis of dengue virus infection when both Japanese encephalitis (JE) and dengue virus co-exist because ELISA was highly specific as only 10.7% dengue virus infection cross reacted with JE antigen.[18] Cross-reactivity with other circulating flaviviruses does not seem to be problem as the subjects with previous JE immunization were excluded from the study, thus the chances of false positive results due to cross reactivity is minimal.

In this study, 14.5% were serologically positive out of 793 suspected cases of DENV infection amongst them most of the cases were from Chitwan followed by Nawalparasi and its surrounding areas i.e central region as well as western region of Nepal. The result suggests the increasing trend of dengue infection in this area based on previous studies and concurs with result 13.7% from Bangladesh in 2002[19], 12.7% by A. poudel et al.[13] whereas 19.7% has been reported from India in 2011[20] and 19.31% from Nepal[21], this higher prevalence rate may be due to endemic nature of the country India and the district bordering with India and open border of Nepal and India.

The age-wise distribution of Sero-positive cases shows highest prevalence rate in the age group of 21-30 (27.83%) followed by 30-40 (26.10%). The prevalence at the age group 21-30 is supported by the observation from Nepal, India and Bangladesh.[22-25]

There was higher Sero-prevalence rate in male than female i.e 1.3:1 among the positive cases. This gender bias towards males over female is supported by observation from Nepal, India and Bangladesh.[22,26,1,27,28] This biased on male is due to relatively higher exposure to outside environment through exposed to farming and other outdoor activities.

The seasonality of disease outbreak incorporate the factors such as rainfall, humidity, type of land, density of mosquito, mosquito bite rate, air temperature.[29,30] In this study, dengue case were reported all year round but the preponderance of positive cases was reported highest in the October followed by November but in contrast no positive cases was reported in the 5 months January, February, April, June, July. At the time of October and November movement and transmigration of the people is high due to crop harvesting, important festivals like Dashain, Tihar, Chhhat, Loshar, new year which contributes for the external pursuits.

As this study was conducted at the principal tertiary care center in the main city of chitwan to which people travelled considerable distance from different nearby places terai as well as hilly region for the treatment so we consider that our finding are illustrative of suspected dengue cases in chitwan and nearby areas along with high rate of positivity.

5. Conclusion

This descriptive study has revealed the emergence of dengue and its firm establishment in the chitwan and its nearby terai as well as hill region of Nepal. Anti-IgM capture ELISA, was used in the study is a most genuine and routinely used method for the diagnosis of Dengue cases in Nepal and developing countries. 14.5% of total suspected cases positive for anti-dengue IgM antibody suggest for early diagnosis of the disease and proper management. Hence, in the developing countries like Nepal Anti-IgM capture ELISA has become the most popular, reliable, applicable and cost effective technique for the rapid diagnosis of dengue virus cases. The public awareness should be raised and people of all the age group should be educated about the mode of disease transmission and prevention. Artificial water collection should be discouraged and cleaning program should be conducted to prevent the breeding of vector and enhanced vector control program should be commenced to forecast any future outbreaks.

Competing Interests

Acknowledgments

Data Availability

The data used to support the finding of this study are restricted by the Institutional Review Committee (ICR) of CMCTH in order to protect patient privacy. Data are available from corresponding author and Dr. Sanjay Ray Yadav, Lab coordinator of Laboratory department CMCTH for researcher who meet the criteria for access to confidential data.

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