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Original Article

Microbiology Section

Never Ending Challenge of Dengue - Current Scenario in the Hilly State of North India

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ABSTRACT

Introduction: Dengue is an arthropod borne (Arbovirus) viral infection. It is the most common arthropod borne virus found in India. It is transmitted principally by vector *Aedes aegypti* followed by *Aedes albopictus*. Due to cold weather Dengue is not endemic in Himachal Pradesh, however the number of cases are now on an increase. Also, there is paucity of published studies from this state on dengue.

Aim: To estimate the current scenario of Dengue in this hilly state of North India in patients visiting our hospital. We also compared two tests i.e. NS1 antigen capture ELISA test and Dengue IgM Capture ELISA test for diagnosis of dengue.

Materials and Methods: All cases with fever and suspected dengue infection were included in the study. Total 225 blood samples were collected from these patients. NS1 antigen and IgM detection were performed for diagnosis.

Results: Out of total 225 suspected cases 73 cases (32.4%) were diagnosed with dengue infection. Overall NS 1 antigen ELISA was a more sensitive test as compared to IgM capture ELISA.

Conclusion: Rapid tests must be performed early in the course of disease to establish diagnosis and initiate symptomatic treatment. Multiple tests done together enhance chances of dengue detection and are recommended. Vector control is the main key to control dengue spread in future.

Keywords: Arbovirus, *Aedes aegypti*, NS1 antigen

INTRODUCTION

Dengue is an arthropod borne (Arbovirus) viral infection. Dengue virus belongs to genus Flavivirus. It is an enveloped single stranded positive sense RNA virus. It is the most common arthropod borne virus found in India. It has five serotypes, DEN 1 to 5 [1]. It is transmitted principally by vector *Aedes aegypti* followed by *Aedes albopictus*. Dengue is prevalent all over India [2].

There are an estimated 50 million cases of dengue annually worldwide, with 4 lakhs cases of dengue haemorrhagic fever (DHF) [3]. Risk of DHF is 0.2% during first infection and is tenfold higher during infection with a second dengue virus serotype [3]. Mortality rate of DHF is about 5% in most countries [4].

The total number of serologically confirmed dengue cases from all over India in 2010 were 28,066 and the predominant serotype found was dengue-1 [5]. In 2014, 36,484 cases were reported, much less than 75,808 cases reported in 2013 [1]. Total number of people down with the vector-borne fever in Delhi this year was a menacing 10,683 by Oct 13, 2015 [6]. Himachal Pradesh reported 3 cases in the year 2010 followed by zero in 2011, 73 in 2012 and 89 in 2013 [7].

Clinical symptoms appear 4-7 days after an infective mosquito bite. Prodromal symptoms are malaise, chills and headache. Fever may return to normal after 5-6 days or subside on third day and rise again at 5-8th day (Saddle back form). Myalgia and deep bone pain give it a name of break bone fever and are characteristic. Rash may be present along with lymphadenopathy. Classic dengue fever is usually self-limiting. A severe entity dengue haemorrhagic fever occurs usually after reinfection with a different serotype. Dengue shock syndrome, is more severe with features of shock and hemo concentration [1,3]. Infection provides lifelong immunity against that serotype however only short duration cross-protection is present between serotypes.

Various methods to diagnose dengue virus infection are virus isolation, RNA detection by RT-PCR, IgM capture ELISA and IgG ELISA. In primary infection IgM becomes positive on day 5 of fever to day 90. IgG becomes positive on 14-21 day and then slowly increases. Virus isolation is done in cell culture of infant mouse brain and is gold standard [8]. Diagnosis is also done by detection of NS 1 antigen (Non

structural antigen 1) be ELISA. It becomes positive on day 1 of fever to day 18 [1,3,8].

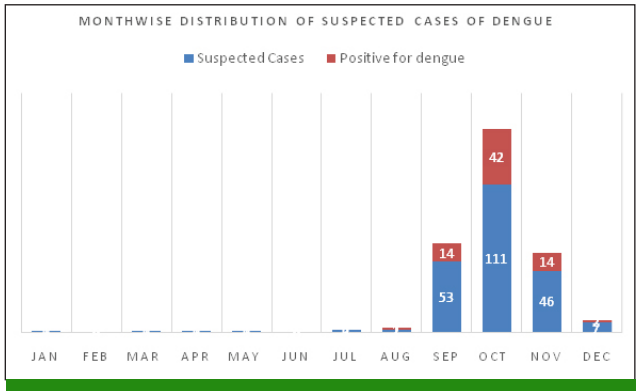
Studies regarding prevalence of dengue from Himachal are limited. Hence, we planned this study to estimate the current scenario of dengue from this hilly state.

MATERIALS AND METHODS

This was a cross sectional laboratory based study, conducted in the Department of Microbiology of our 500 bedded rural tertiary care Medical College. All patients presenting in various clinical departments with fever and suspected diagnosis of dengue during one year period from January 2015 to December 2015 were included in the study. Serum samples from these patients were sent to the Department of Microbiology for testing. NS1 antigen capture ELISA test (Panbio diagnostics) and Dengue IgM Capture ELISA (Panbio diagnostics) test were performed on this samples. A total of 225 blood samples was taken. Any repeat samples were excluded. Patient details were noted from requisition forms. As it was a laboratory based study with no direct patient interaction and data was collected from records patient consent was not taken.

RESULTS

Among 225 patients included in the study 144 were males and 81 were females. 98 patients were outdoor patients and 127 were indoor admitted patients. From 127 indoor patients 120 were admitted in Medicine ward, 5 in Intensive care unit, 6 in Paediatric Ward and 6 in Skin ward. Out of total 225 suspected cases 73 cases (32.4%) were diagnosed with dengue infection. Very few cases were suspected during months of January to August 2015 and none were positive for dengue. Cases started increasing from September to October and decreased in November 2015 with a sharp decline in December 2015 [Table/Fig-1]. Age wise distribution of cases is given in [Table/Fig-2]. Among the 73



[Table/Fig-1]: Month wise distribution of suspected cases of dengue infection.

Age (years)	Cases positive for dengue	Suspected cases
0-10	1 (0.45%)	3
11-20	8 (3.56%)	24
21-30	36 (16%)	96
31-40	17 (7.56%)	54
41-50	6 (2.67%)	26
>50	5 (2.23%)	22
Total	73 (32.4%)	225

[Table/Fig-2]: Age wise distribution of suspected cases of dengue infection (n=225).

cases positive for dengue infection 62 were positive by both NS1 Ag –ELISA and IgM capture ELISA, seven were positive only by NS1 Ag- ELISA and four were positive only by IgM capture ELISA.

DISCUSSION

Dengue fever has been referred as "water poison" associated with flying insects in a Chinese Medical encyclopedia (265-420 AD [9]. The word dengue means "cramp like seizure" [9]. Four dengue serotypes (DENV 1-4) are circulating in Asia, Africa and America [10]. Dengue infection is endemic in many parts of India. In India, the first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780.

Change in prevalent serotype is the major factor responsible for the outbreak of dengue cases. The predominant circulating serotypes of dengue virus in Delhi in 2015 were DEN-2 and DEN-4. These serotypes are considered deadly as compared to DEN-1 and DEN-3 [11]. DEN-2 is the more virulent among the four serotypes of dengue and symptoms are more severe [4].

32.4% cases were positive for dengue infection in our study. Gupta E et al., reported 44.56% (811) positive cases from 1820 samples tested during 2003-2005 from Delhi, North India. Maximum cases were between 21-30 years and peak was observed during October [12]. In a study from Pune, Maharashtra 48.45% samples were positive for dengue from 2005-2010 with peak cases in October and 21 to 30 year age group maximum affected [2].

Ahmed et al., in a study from Delhi reported maximum dengue cases (524; 30.8% of 1,700) from the age group 21-30 years in the year 2010 [5]. Maximum dengue cases in our study were also in the age group of 21 – 30 yrs (36 out of 73; 49.3%). This age group belongs to the most economically productive age group and hence the effect of dengue can be seen. Peak number of dengue cases was observed in the month of October (42 out of 73). Number of cases started increasing in September (14 out of 73). This trend paralleled

that from other parts of the country.

NS1 antigen is a marker of acute active infection only. Once anti-NS1 IgG antibodies are produced NS1 is no longer detectable in serum. It becomes positive on day one of infection. 69 cases were positive by NS1 Ag ELISA in our study out of a total positive of 73. Only four cases that were positive with IgM capture ELISA could not be detected by NS1 Ag ELISA. Reason for this could be samples taken later in the course of infection when NS 1 Ag levels are comparatively lower. Overall it was a more sensitive test than IgM capture ELISA.

IgM antibodies become positive from 5th day onwards. In our study 66 cases were IgM positive out of total 73 positive cases. Seven cases were negative and the reason could be sample taken earlier in the course of infection when IgM is not yet detectable in serum.

Laboratory diagnosis of dengue is important because of a wide range of clinical presentations. Although the treatment for dengue is mainly symptomatic and supportive. No specific antiviral drugs are available but early initiation of supportive therapy will decrease mortality. Virus isolation is time consuming and facilities for RT-PCR are not available in routine laboratories. We need rapid and easy to perform test for early identification of dengue infection.

NS1 Antigen ELISA is a good test for early detection of dengue infection. It is also specific for dengue virus and hence overcomes the disadvantage offered by cross reactivity among various flavi viruses. IgM capture ELISA done in addition enhances the case detection rate. It should also be supplemented with IgG antibody detection so that additional number of cases missed by these two tests can be detected. A single test will not only miss a number of cases but also make diagnosis difficult in cases of secondary infections. Serological diagnosis is challenged by cross reactivity of IgG antibodies of other flavivirus and multiple tests will help establish diagnosis in these cases.

LIMITATIONS

Limitations of our study include not performing IgG ELISA hence, few cases must have been left out. Additionally serotype identification could not be done but considering the trend in North India it is presumed that the serotype must be DEN-2 and DEN-4.

Control measures for dengue infection include effective vector control as vaccines are still in trial phase and no specific treatment is available. Several larvicidal substances like DDT, malathione, temephos etc., are used however, resistance to these agents is an emerging concern. People must be made aware regarding spread and control of dengue infection. Active involvement of public will help reduce the menace of dengue.

CONCLUSION

In India, vector control is the key to dengue prevention. Political will and public awareness will go a long way in keeping dengue under check. Also dedicated research must be conducted to prepare a suitable vaccine as no specific antiviral treatment is available for dengue. Early diagnosis by rapid tests will help in reducing mortality by initiating supportive treatment earliest.

REFERENCES

[1] Arboviruses. In: Apurba SS, Sandhya BK, Anand J, editors. Essentials of Medical Microbiology. 1st ed. New Delhi: *Jaypee Brother's medical publishers*. 2016;p485-92.

[2] Cecilia D. Current status of dengue and chikungunya in India. WHO South-East Asia. *Journal of Public Health*. 2014;3:22-27.

[3] Arthropod-Borne & Rodent Borne Viral Diseases. In: Brooks GF, Butel JS, Carroll KC, Morse SA, editors. Jawetz, Melnick, &Adelberg's Medical Microbiology. 24th ed. New York: Mc Graw Hill; 2007. p511-24.

[4] Perappadan BS. AIIMS identifies dengue strain circulating in Delhi [Internet]. Cited 2015 Sep. Available from www.thehindu.com.

[5] Ahmed NH, Broor S. Dengue fever outbreak in Delhi, North India: a clinico-epidemiological study. *Indian J Community Med*. 2015;40:135-38.

[6] With 10,683 cases in Delhi, 2015 dengue outbreak worst in last 20 years [Internet]. Delhi:PTI. Updated 2015 Oct. Available from www.indianexpress.com

[7] Annual Report, National Programmes under NRHM [Internet]. Chapter 6.p88. Available from www.mohfw.nic.in

[8] Ahmed NH, Broor S. Comparison of NS1 antigen detection ELISA, real-time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase. *J Vector Borne Dis*. 2014;51:194-99.

[9] Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. *Indian J Med Res*. 2012;136:373-79.

[10] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ et al. Dengue: a continuing global threat. *Nat Rev Microbiol*. 2010;8(12 Suppl):S7-16. doi: 10.1038/nrmicro2460.

[11] 'Deadly' dengue serotypes circulating in Delhi this year:AIIMS [Internet]. New Delhi:PTI. Cited 2015 Sep. Available from www.dnaindia.com

[12] Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, North India. *Virology Journal*. 2006;3:92.

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