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ORIGINAL ARTICLE

Dengue Infection- Prevalence and Seasonal Variation Among Patients Attending a Tertiary Care Hospital at Lower Himalayan Region, India

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Abstract

Background and aims: Dengue is an acute arboviral infection with potential fatal complications. Dengue is an endemic disease worldwide. Around two fifths of the world's population in tropical and subtropical countries are at constant risk of contacting this infection. The case fatality rate in patients with severe dengue infection which consists of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) can be as high as 44%. Since there is no specific treatment or vaccine for dengue till date, prevention and control of the disease mainly depend upon epidemiological surveillance that provide reliable estimates of the disease.**Material and methods:** It is a retrospective based cross-sectional study using secondary data which was maintained in the Microbiology Laboratory registers, of clinically suspected dengue cases reported to various inpatient and out-patient departments of the hospital for a period of two year from June 2017 to May 2019 who were subjected to DENV detect Tm NS1 ELISA and NIV dengue IgM Capture ELISA. A clinical case included a patient with fever and symptoms suggestive of dengue as per WHO guidelines. There were no exclusion criteria for processing of the collected samples.**Results:** A total of 592 serum samples were gathered from patients who were suffering from fever suspected to have dengue infections. Dengue positively was seen in 128(21.6%) patients either by NS1 or IgM ELISA in the present study. The proportion of dengue cases was higher in monsoon season with maximum rate of positivity in the month of October. Paediatric population was the most common affected group.**Conclusion**: The present study concludes that dengue is mainly a disease of monsoon season and also identifies certain vulnerable groups for effective planning of interventions.

Keywords

Dengue, IgM, NS1.

Introduction

Dengue is an acute arboviral infection with potential fatal complications. Dengue is an endemic disease worldwide.[1] According to estimates of the World Health Organisation (WHO), around two fifths of the world's population in tropical and subtropical countries are at constant risk of contacting this infection.[2] In the past decade, the escalation of dengue as a threat to health, finance, and health services has increased substantially.[3] This viral infection has grown 30 folds from the time it was initially reported. It has now expanded and diversified globally causing human sufferings and massive socioeconomic losses.[4]

Dengue is an arboviral infection transmitted primarily by the female Aedes aegypti mosquito and on occasion by

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Aedes albopictus mosquitoes, and is considered the world's most common arthropod-borne viral disease. Currently, there are four distinct but closely related Dengue serotypes, including: DEN 1, DEN 2, DEN 3 and DEN 4, in which recovery of infection by one serotype provides lifelong immunity against only that particular serotype, but confers only partial protection against subsequent infection by another strain.[5] The fifth variant DENV-5 has been isolated in October 2013 which follows sylvatic cycle unlike the other four serotypes which follow the human cycle.[1] Dengue disease in humans produce wide spectrum of clinical features ranging from atypical non severe or nonspecific febrile syndrome to potentially fatal dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), conditioned by age, secondary infection, immunologic status, dengue serotype, and genotype.[3] The case fatality rate in patients with severe dengue infection which consists of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) can be as high as 44%.[6, 7] If intervention occurs early, mortality is less than 6%.[8] Confirmation of Dengue viral infection is most important and essential pre-requisite for managing these complications associated with Dengue viral infection.[9] Diagnostic techniques like isolation of virus by cell culture and nucleic acid detection by reverse transcriptase polymerase chain reaction are time consuming and require costly laboratory setups. Hence in resource limited settings, detection of NS1 antigen and IgM/ IgG antibodies specific to virus remains as important diagnostic parameters.[10]

In addition to the increased number of cases and severity of the disease, there has also been a major change in the geographic range of the disease.[4] Over the years, the incidence which has increased exponentially, the major contributing factors related to the transition of dengue fever from a national to global health concern includes: climatic change, unplanned urbanization, increasing travel and migration across borders, global trade and the expansion of dengue vectors to new geographic regions.[4, 11]

The lower Himalayan region is among the most diverse regions in terms of environmental, socio cultural and economic aspects and covers a wide range of lowlands to highlands. Considering all the facts and since there is no specific treatment or vaccine for dengue till date, prevention and control of the disease mainly depend upon epidemiological surveillance that provide reliable estimates of the disease, thereby guiding in implementation of effective vector-control measures, the present study was carried out to determine the epidemiological determinants and seasonal variation in dengue infection which could help in rendering adequate control measures.

Aims and Objectives

1.To determine seroprevalence of dengue virus among clinically suspected cases.

2.To determine the seasonal variation of dengue infection in the study setting.

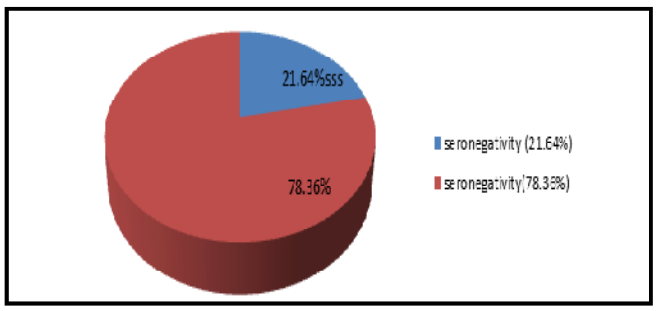
3.To study the demographic profile of dengue positive patients.

Material and Methods

The present cross-sectional study was conducted in the Department of Microbiology at a tertiary care Government Medical College and Hospital, Kathua using secondary data which was maintained in the Microbiology Laboratory registers, of clinically suspected dengue cases reported to various inpatient and out-patient departments of the hospital for a period of two year from June 2017 to May 2019. A clinical case included a patient with fever and symptoms suggestive of dengue as per WHO guidelines.[12] There were no exclusion criteria for processing of the collected samples. About 2 ml to 3 ml of blood was collected from each patient using strict aseptic precautions. Serum was separated by centrifuging samples at 3000 rpm for five minutes and tested immediately. In case of delay in processing, sera were stored at a temperature of 2°C-8°C. Depending upon the duration of illness, a total of 592 sera were collected and serologically tested, for demonstration of NS1 antigen (fever =/<5 days) or anti DV IgM antibodies (fever >5 days). NS1 antigen detection was done using DENV Detect NS1 ELISA kit, by In Bios International Inc. USA and IgM anti dengue antibodies were detected by NIV dengue IgM capture kits by NIV, Pune, India. The procedures were performed as per the instructions provided by the respective kits and are as follows:

a)DENV detect tM NS1 ELISA: A 50 microl of undiluted serum was added directly to each antibody coated well containing 50 microl sample diluent for each sample. The assay plate was incubated at 37°C for one hour and then washed. To each well, 100 micro l of conjugate solution was added and it was then incubated for 30 minutes. The microwells were washed thereafter and 100 microl of TetraMethyl-Benzidine/Hydrogen peroxide (TMB/H 202) substrate solution was added to these well. After 20 minutes of incubation at room temperature, 50 microl stop solution was added to each well and the colour density of the residue i.e., Optical Density (OD) was read within one minute at the wavelength of 450 nm. The cut-off

Fig. 1 Seroprevalence of Dengue Infection



Negative Control x 3.0). Patients with positive NS1 antigen and anti dengue IgM were considered positive cases for dengue viral infection.

Statistical Analysis

The data was collected, tabulated and analysed. Pearson's Chi-square test was used as test of significance. A value of $p<0.05$ was considered significant.

Results

A total of 592 serum samples were gathered from patients who were suffering from fever suspected to have dengue infections. Dengue positively was seen in 128(21.6%)

Table I. Gender Wise Distribution of Dengue Suspects and Positives

Gender	Suspects	Positive	Rate of positivity
	No (%)	No (%)	(%)
Male	374 (63.17)	77 (60.15)	20.58
Female	218 (36.83)	51 (39.85)	23.40
Total	592 (100)	128 (100)	21.62
	x^2= 0.64 , p value= 0.42		
	Level of Significant =0.05		

Table II. Age Wise Distribution of Samples of Dengue Suspects and Positives

Age in years	Suspects	Positives	Rate of positivity
	No (%)	No (%)	%
<15	162 (27.36)	51 (39.85)	31.48
16-30	250 (42.23)	50 (39.06)	20
31-45	119 (20.10)	16 (12.5)	13.44
46-60	45 (7.60)	8 (6.25)	17.78
>60	16 (2.70)	3 (2.34)	18.75
Total	592 (100)	128 (100)	
	x^2= 14.84 , p value=0.005		
	Level of Significant= 0.05		

value was calculated based on the average OD value obtained from control sample.

b)NIV dengue IgM Capture ELISA: To the microwells coated with anti human IgM antibodies, 50 microl of 1:100 diluted serum and controls were added to ELISA plate. The assay plate was incubated at 37°C for one hour and then washed. About 50 microl of DEN antigen conjugate solution complex was added to the assay plate and was incubated for one hour. Now, 50 microl of antidengue monoclonal antibody was added to each well, followed by incubation for 1 hour and all the wells were washed. A 50 microl of avidin-HRP was added to each well and again incubated for 30 minutes. A 100 microl TetraMethyl-Benzidine/ Hydrogen peroxide (TMB/H202) substrate solution was added to each well, after washing and incubated for 10 minute at room temperature. The OD was read within 30 minute, of addition of 100 microl stop solution, at the wavelength of 450 nm. The cut-off value was calculated using the formula (Mean Absorbance

patients either by NS1 or IgM ELISA in the present study (*Fig1*)

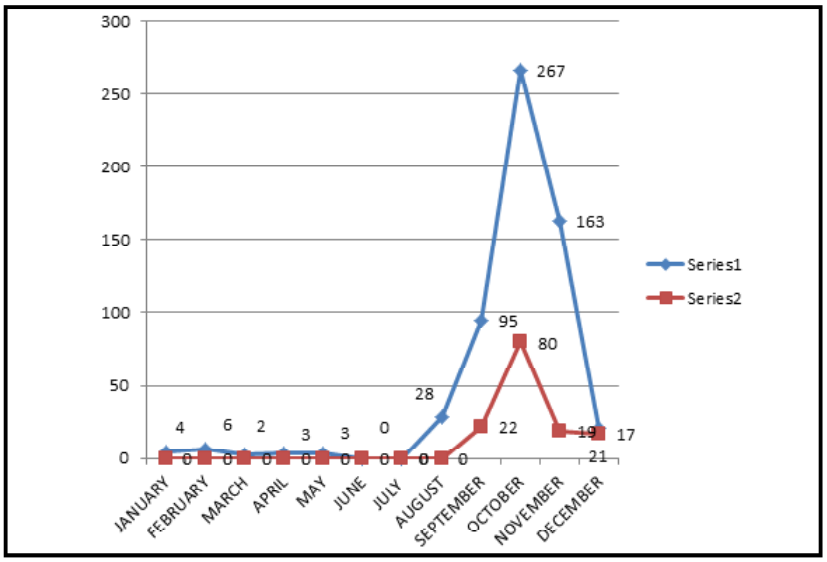
Table I shows the gender wise distribution of positive cases of dengue which indicates that 77(60.15%) of the positive samples were males and 51(39.84%) were from females

Tables II shows the age distribution of positive cases indicating the proportion of cases was almost equal in both< 15 years and 16-30 years age group whereas the rate of seropositivity was highest i.e 39.% in <15 age group followed by 16-30 years (39.06%) and the study found statistically significant association between rate of seropositivity and age group. Also, out of 128 positive cases, 102 (79.68%) seropositives were detected during the months of September and October collectively. (*Fig 2*)

Discussion

Dengue is an important emerging disease of the tropical and sub-tropical regions today. It is clear that since last decade, dengue have been occurring regularly with

Fig 2. Monthly distribution of dengue positive cases (both NS1 and IgM)



periodic surges in a number of cases.[13] Dengue infection is endemic in many parts of India.[14] WHO has declared dengue to be hyper endemic in India and cyclical epidemics of dengue are becoming more frequent.[1] Understanding the economic and disease burden of dengue in India is essential to assist policy makers and public health managers to prepare for and control outbreaks, and encourage international collaboration to develop and evaluate prevention, control and management measures and technologies to control further epidemics.[15] As effective control and preventive programmes for dengue infection are based upon improved surveillance data, the purpose of the study was to report the seroprevalence of dengue virus infection in Kathua district, where increased travel and migration is being observed as situated near Lakhanpur border and also geographical location of lower Himalayan region being an important factor.

In the present study, 21.6% patients were serologically positive for dengue infection either by NS1 or IgM ELISA. An unsteady surge in the number of dengue patients over years was noted. While this may be attributed to rapid unplanned urbanization with unchecked construction activities and poor sanitation facilities contributing fertile breeding grounds for mosquitoes, it is also true that an increase in alertness among medical fraternity following the start of epidemic and the availability of diagnostic tools in the hospital have contributed to the increased detection of cases.[16] The results were congruent with other studies like Garg A *et al* [13] reported 19.7% positivity and Sathish J.V *et al* [1] reporting 21.46% seropositivity, whereas few other studies have reported lower prevalence rate and some have reported higher prevalence ie 40%.[17, 18] The difference in sero prevalence could be due to differences in processing of samples. It was also observed that males (60.15%) were more commonly affected than females (39.84%). The higher sero positivity in males might be because of outdoor activities or increased exposure at work places.[4, 19, 20] another factor can be that there is less health care seeking attitude in females and also there is more trend in females to seek care from traditional practitioners which go unnoticed from public surveillance system. As far as age group is considered, the present study found maximum positive cases in the age group of less than 30 years which is similar to the findings of other studies.[1, 21] However rate of positivity was highest in age age group of less than 15 years ie 31.48% followed by 16-30 years ie 20 % similar to many studies done in India as well as international.[22, 23, 24] comparatively lower immunity and intrinsically more permeable vascular endothelium in children render them more susceptible to dengue infections.

A monthly distribution of the cases of dengue infections in this study shows an increase from the month of September onwards which concurs with other outbreaks in India.[25] Seasonal variation in the dengue infection corroborated by gradual increase in cases from August and it peaked in October. Climatic factors responsible for epidemics either alone or in combination are rainfall, variation in temperature sometimes humidity.[22] During the rainy season, the survival of the mosquito is longer

and chance of transmission of the virus is also greater. The post monsoon stagnant water pool also acts as a breeding ground which favours the increase in disease prevalence.[4] Furthermore, the correlation between occurrence of arboviral infections and monsoon season is clearly shown in this study and also supported by various previous studies.[26]

Conclusions

The present study found seroprevalence of dengue infection to be critical in the study setting and identifies paediatric group being most vulnerable for infection and also revealing monsoon season as the most favourable one for transmission of disease. To conclude, improved surveillance and reporting of dengue cases is important to understand the true impact of the problem which will help guide prioritization for research and health policy efforts and help understand the projected economic impact of preventing and controlling this disease and result in reduced hospitalizations, illnesses and mortalities.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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