A STUDY OF SEROPREVALENCE AND CHANGING TREND OF DENGUE IN A TERTIARY CARE HOSPITAL IN MANIPUR

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ABSTRACT

BACKGROUND

Dengue fever is a seasonal and emerging acute mosquito borne arbo-viral illness affecting tropical and sub-tropical countries. This illness ranges from mild asymptomatic form to severe dengue haemorrhagic fever with or without dengue shock syndrome. In India, the epidemiology of dengue virus infection is very complex and ever changing. Infection has expanded over the last two decades to all regions of the country including hilly states of the north-eastern region (Assam, Arunachal Pradesh, Nagaland and Manipur).

Aim- To identify dengue seropositive patients by NS1 antigen and anti-dengue IgM antibody detection by ELISA and correlate the changes in epidemiology.

MATERIALS AND METHODS

A cross-sectional study done from January 2016 to December 2017 with blood samples tested from clinically suspected cases of dengue virus infection.

RESULTS

During the study period from January 2016 to December 2017, a total of 1569 blood samples were tested, of which 251 were in 2016 and 1318 cases were in 2017. In the year 2016, 35 (13.94%) were NS1 positive, 18 (7.17%) were IgM positive and total dengue positive in 2016 was 53 (21.11%). In 2017, 208 (15.70%) were IgM positive and there were no NS1 positive cases in this year. Maximum number of positive cases, 85 (41.46%) belonged to age group 15-30 years in 2017 and 18 (33.96%) in the age group of 15-30 years in 2016 also.

CONCLUSION

Prevalence of dengue seropositive cases was 53 (21.11%) in 2016 and 208 (15.70%) in 2017 indicating a rapid decline in dengue infection, which may be attributable to the collective efforts taken by the healthcare workers and by the people during the timely delivery of preventive and control measures besides having increased awareness among the people.

KEY WORDS

Dengue Fever, Dengue Haemorrhagic Fever, DSS (Dengue Shock Syndrome), NS1Ag, IgM, ELISA.

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BACKGROUND

Dengue fever has developed into one of the world's major emerging infectious diseases. The infection is by now seen as global epidemic with recorded prevalence in more than 120 countries.^[1] It appears that dengue originated from Africa and was introduced to Asia some 6000 years ago. The first recognised dengue epidemics occurred almost simultaneously in Asia, Africa and North America in 1780s. Dengue is transmitted by *Aedes* mosquitoes, particularly *A. aegypti* and less important *A. albopictus*. These mosquitoes travel well particularly in cargo ships and four serotypes of dengue virus have spread to most tropical and sub-tropical countries in their wake. During the last 2000 years spread of dengue virus disease has increased, reaching epidemic

proportion during the last three decades. Since the last 1900s, dengue is the most important mosquito-borne disease affecting humans after malaria with around 40 million cases of dengue fever and several hundred thousand cases of Dengue Haemorrhagic Fever (DHF) each year. The main endemic areas are Latin America, the Caribbean, Africa, South and South East-Asia and parts of pacific region. For Europe dengue remains an important disease, even though *A. albopictus* has become established in some parts of the continent. Each year 50-100 million cases occur, hospitalisation for the infection have reached 500,000 and global death toll is > 20,000 persons.^[2] Globally, an estimated 2.5 billion cases are at risk of dengue and approximately 925 million of these live in urban areas of tropical and subtropical countries of East-Asia, the pacific and the America.^[3] Dengue virus belong to the genus flavivirus and family flaviviridae. There is 4-serotypes of virus referred to as DU-1, DU-2, DU-3 and DU-4, the virus is transmitted from humans to humans mainly through principle vector, the *A. aegypti* mosquitoes and sporadically by *Aedes albopictus*.^[1,4] Each serotypes of the virus produces a specific life-long immunity, but it produces only a short-term cross-immunity.^[5]

Transmission of Dengue virus infection is strongly influenced by rainfall and temperature and other weather

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variables. Dengue virus infection produces a spectrum of clinical illness, which ranges from asymptomatic and mild febrile illness to classic Dengue Fever (DF) to the most severe form of illness, the dengue haemorrhagic fever.^[6] Classical dengue fever [DF] is manifested by rapid onset of high-grade fever, headache, retro-orbital pain, diffuse body pain (both muscles and bones), weakness, vomiting, sore throat, an altered taste sensation and a centrifugal maculopapular rash.^[7] DHF is characterised by a high-grade fever, haemorrhagic manifestation, hepatomegaly in severe cases by Dengue Shock Syndrome (DSS). DHF leads to circulatory failure due to hypovolemic shock, which is called Dengue Shock Syndrome (DSS).^[3,8,9] Primary infection in non-immune person usually causes DF.^[4,10]

Epidemic of clinically dengue-like illness in India was recorded in Chennai in 1780, whereas the virologically confirmed outbreak of dengue fever occurred in Calcutta and eastern coast of India in 1963-1964,^[11] thereafter dengue virus infection spread throughout the entire country. The first nationwide outbreak occurred in 1996. Epidemiology of dengue fever in India has been very complex and has significantly changed over the last 6-8 decades in terms of geographical area, prevalent strain and severity of disease. Serotypes of disease keep on changing from year after year and in between 1996 – 2003. All the four serotypes (Dengue 1 to 4) were reported.^[4]

According to the estimate of national vector borne disease control programme, 47,209 dengue cases were reported in India in the year 2012.^[12] Serological survey conducted during 1963 in the North-Eastern Region (NER) of India recorded dengue outbreak in Lohit district of Arunachal Pradesh (AP) and Darrang district of Assam.^[13,14] Another report of dengue (Den V-2) in Assam and Nagaland appeared during the nineties.^[14,15] During 2009-2011, study carried out by Dutt *et al* (2012) reported 143 laboratory confirmed cases belong to Assam (82), Meghalaya (35), Nagaland (15), Manipur (8) and AP (3).^[14]

Dengue has been reported from valley as well as hilly region of India including some parts of North-East India. In July-August 2012, the outbreak of dengue was reported in Pashighat, a hilly station located at an altitude of 155 metres, MSL (Latitude: 28.07° N, Longitude: 95.33°E), situated in East Siang district of hilly state of Arunachal Pradesh, North-East India.^[16]

For laboratory diagnosis of dengue virus infection, virus isolation (Culture) is the gold standard and greatest disadvantage of this method is time consuming procedure required to obtain culture reports and it also needs expensive laboratory facilities for cell line preparation.^[17]

Relatively inexpensive, easy and reliable methods used in clinical laboratory are detection of antigen and antibody of dengue virus by serology. In primary infection, anti-dengue IgM antibody are produced from 5th day of infection and remain in circulation for 60-90 days. Anti-dengue IgG antibody are produced after one week and attain maximum after 2-3 weeks and thereafter remained in circulation life-long; in secondary infection IgG antibody are distributed at high level in acute stage of infection, while IgM at low level.^[18] For any virus infection the standard serological test, hemagglutination inhibition, neutralisation test, indirect

immunofluorescence antibody test, Enzyme-Linked Immunosorbent Assay (ELISA), complement fixation test or rapid immunochromatography test can be used. Out of these tests, ELISA is the most widely used method for routine diagnosis of dengue infection for its high sensitivity, specificity and also for its simplicity and cost effectiveness.^[19] Detection of NS1 (Non-Structural highly conserved glycoprotein-1) antigen is a new approach for the diagnosis of acute dengue, as it was found circulating in the blood during acute phase of disease in the patients from first day to 9th day of fever.^[18,19] Soluble form of NS1 antigen can be detected in the blood stream, test such as antigen capture-Enzyme-Linked Immunosorbent Assay (ELISA), lateral flow antigen detection by rapid method and measurement of NS1 specific immunoglobulin IgM and IgG responses have been developed.^[20] The sensitivity of NS1 antigen capture ELISA was significantly higher in acute primary dengue than in acute secondary dengue.^[21,22]

Two patterns of immune response are distinguished: primary and secondary. A person never previously infected with a flavivirus nor immunised with a flavivirus vaccine, mount a primary antibody response when infected with dengue virus. The dominant immunoglobulin isotype is IgM. Anti-dengue IgM detectable by IgM capture ELISA appears in half of the patients with a primary infection, while they are still febrile; in the other half it appears within 2-3 days of defervescence and thereafter anti-dengue IgG appears shortly afterwards (WHO).

The physiological definition of primary infection is the one characterised by a high molar fraction of anti-dengue IgM and a low molar fraction of anti-dengue IgG. In contrast to primary infection, secondary infection by dengue virus results in the appearance of high levels of anti-dengue IgG before or simultaneously with the IgM response. Once detected IgG levels rise quickly, peak about 2 weeks after the onset of symptoms and then decline slowly over 3-6 months. The physiological definition of a secondary infection is characterised by a low molar fraction of anti-dengue IgM and a high molar fraction of IgG (WHO).

The present study was undertaken to determine seroprevalence of dengue virus infection by detecting IgM antibody against the virus and NS1 in all clinically suspected cases of dengue infection depending on the duration of fever (more than/ less than) 5 days at the time of presentation in a tertiary care hospital (both outpatient and inpatient departments) in Manipur, India and to analyse the changing trends of this infection essential for planning the necessary control and preventive measures in the forthcoming years.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of two years from January 2016 to December 2017 at the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, a tertiary care teaching hospital in Manipur, India where the Microbiology Department operates viral diagnostic laboratory and it serves as sentinel surveillance unit of dengue, JE and chikungunya under National Vector Borne Disease Control Programme (NVBDCP) guidelines. The testing formality during the study was done just like other daily routine laboratory tests, i.e. requisition format duly signed by the respective attending physicians on duty and 3mL of blood sample taken from patients having clinical

suspicion of dengue viral infection with a short history and duration of fever on the day of presentation in the hospital was submitted to VDL unit of the Microbiology Dept., JNIMS. Non-repetitive blood samples of 1569 in number were collected during the study period. Haemolysed and lipaemic samples were excluded in the study.

Serum Collection and Processing

Blood samples received were stored at fridge and processed for serum separation within 24 hrs. The separated serum samples were subjected for serological test depending on the duration of fever at the time of presentation of the patient to the hospital (less than/ more than 5 days). Samples were respectively chosen to be processed for NS1 antigen detection and IgM antibody detection.

Serological ELISA Test

NS1 antigen was done by ELISA using dengue NS1 antigen micro ELISA kit (J. Mitra and Co. Pvt, New Delhi 110020 India. E-mail: Jmitra@jmitra.co.in internet www.jmitrthan for the patients having fever less than 5 days and IgM detection by MAC-ELISA using NIV DEN MAC ELISA kit (version no. 2.4 prepared by National Institute of Virology, 20-A Dr. Ambedkar Road, Pune 411001 (Maharashtra), India for the patients having fever more than 5 days. Use of version no 2:4 of NIV Dengue Mac ELISA kit was effective from 01/02/2013. Procedures for both the tests were followed as per manufacturer's instruction.

Inclusion Criteria

All the samples referred for suspicion of dengue shock syndrome by Public Health Department, Private Clinics and Hospitals across the state are included for the study.

Statistical Analysis

The data collected during the study were delinked from any identifier and Microsoft Excel was used for statistical analysis. Chi-square test has been used to detect the statistical significance of the data. P-value is < 0.05 , which is statistically significant.

RESULTS

During the study period from January 2016 to December 2017 a total of 1569 blood samples were tested, of which 251 were in 2016 and 1318 cases were in 2017. In the year 2016, 35 (13.94%) were NS1 positive, 18 (7.17%) were IgM positive and total dengue positive in 2016 was 53 (21.11%). In 2017, 208 (15.70%) were IgM positive and there were no NS1 positive cases in this year. Overall, there has been increase in incidence of dengue cases from the year 2016 to 2017 and this increase in positive cases of dengue is found to be significant at $p < 0.05$. (The Chi-square statistic is 4.3259. The p -value is 0.037536) (Table 1).

Maximum number of positive cases, 85 (41.46%) belonged to age group 15-30 years in 2017 and 18 (33.96%) in the age group 15-30 years in 2016 also. The raise in positivity among the age group from the year 2016 to 2017 is found to be significant at $p < 0.05$. The chi-square statistic is 15.3988. The p -value is .003942 (Table 2).

Infection was most common in males 54.7% (29/53) as compared to females, 45.28% (24/53) in the year 2016. In 2017 also, infection was most common in males 57.69%

(120/208) as compared to females, 42.30% (63/208). Maximum cases were seen in October and November in 2016 and July and August in 2017.

Year	Total Sample Tested	NS1 Positive	IgM Positive	Total
2016	251	35 (13.94%)	18 (7.17%)	53 (21.11%)
2017	1318	0	208 (15.70%)	208 (15.70%)
Total	1569	35	226	261 (16.6%)

Table 1. Year Wise distribution of Positive Dengue Cases

Figure 1. Histogram showing Positive Case distribution between 2016 and 2017

Age	2016	2017
<15	7 (9.43%)	62 (29.80%)
15-30	18 (33.96%)	85 (40.86%)
31-45	15 (28.30%)	29 (13.94%)
46-60	09 (16.98%)	23 (11.05%)
>60	04 (7.54%)	04 (4.32%)
Total	53 (96.29%)	208 (99.98%)

Table 2. Age Wise distribution of Dengue Positive Cases

Figure 2. Histogram showing the Age Groups Positivity comparison between 2016 and 2017

Age	2016	2017
Male	29 (54.7%)	120 (57.69%)
Female	24 (45.28%)	88 (42.33%)
Total	53 (99.99%)	208 (99.99%)

Table 3. Gender Wise distribution of Dengue Positive Cases

Figure 3. Histogram showing Gender Wise distribution of Dengue Positive Cases between 2016 and 2017

	Months	Total Suspected Cases	Serologically Positive Cases (%)
	January	NIL	NIL
	February	NIL	NIL
	March	NIL	NIL
	April	NIL	NIL
	May	NIL	NIL
	June	NIL	NIL
	July	NIL	NIL
Negative	August	NIL	NIL
4	September	6	2 (3.77%)
116	October	153	37 (69.81%)
66	November	78	12 (22.64%)
12	December	14	2 (3.77%)
	Total	251	53 (21.11%)

Table 4. Seasonal variation of Dengue/ Month Wise distribution of clinically diagnosed and Serologically Positive Cases during the DF Outbreak, 2016

Negative	Months	Total Suspected Cases	Serologically Positive Cases (%)
4	January	4	0
0	February	0	0
0	March	0	0
0	April	0	0
1	May	2	1 (0.480%)
22	June	33	11 (5.28%)
337	July	416	79 (37.98%)
460	August	537	77 (37.01%)
165	September	183	18 (8.65%)
70	October	86	16 (7.69%)
34	November	39	5 (2.40%)
17	December	18	1 (0.48%)
1110	Total	1318	208 (15.78%)

Table 5. Seasonal variation of Dengue/ Month Wise distribution of clinically diagnosed and Serologically Positive Cases during the DF Outbreak, 2017

Figure 4. Line Graph showing Month Wise Dengue Positivity distribution in the Year 2016 and 2017

DISCUSSION

Worldwide large scale reappearance of dengue in the past few decades has turned this into a serious public health problem, especially in tropical and subtropical countries.^[2,3,23] In India also, dengue infection with rapidly changing epidemiology has been seen in the last few decades.^[4] It is becoming an emerging viral diseases in India.^[5] The rapid increase in the global dengue burden and inability of accurate clinical diagnosis because of its wide clinical spectrum ranging from mild febrile illness to severe syndrome has promoted social interest in using laboratory diagnosis of dengue infection.^[24] Diagnosis of dengue by viral

culture and RT-PCR are time consuming, very costly and also it needs experienced laboratory personnel.^[17] Detecting antigen and antibody of dengue virus by serology, which is quick and relatively inexpensive and most feasible method used in most of clinical laboratories.^[18,19,25]

In the year 2003 India had experienced one of the wettest monsoon in 25 years, which led to the rapid growth of mosquitoes creating an alarming situation of mosquito-borne disease in Delhi and many other states.^[25] Most of the vector borne disease exhibit a distinctive seasonal pattern and climatic factors such as rainfall, temperature, humidity and other weather variables which in many ways influence vector and pathogens they transmit it.^[23,26] For assessing the seasonal variations of the disease, analysis of data was done on a monthly basis. Worldwide studies have proposed that ecological and climatic factors influence the seasonal prevalence of both *A. aegypti* mosquito and dengue virus.^[24] In the present study during 2016 a fewer cases were detected in the month of September, peaking in October 37 (69.80%) of serologically positive dengue cases followed by successive decrease (declining) in the number of dengue cases by November. This increase and peaking in cases of dengue infection during post-monsoon period is also reported by various studies in India.^[27,28,29] In our neighbouring country, Bangladesh also post-monsoon period is the most affected part of dengue infection.^[30] Seropositive dengue cases during study period were 261 (16.6%). Year-wise distribution of the study population showed a steady decrease in the incidence of dengue; 21.1% were reported in 2016 and just 15.70% was noted in 2017. During 2017 of the present study gradual increase was observed from May and peaking in July, 79(37.98%) in serologically positive dengue cases and successively declining from September onwards till December, which is corresponding to monsoon season of this part of the country, clear relation between monsoon season and dengue occurrence (outbreak) is also supported by studies done in Karnataka and other parts of India.^[31,32,33,34] True endemicity will be reduced only when adult infection declines and only new entrants into the population, that is children are affected by this disease. Higher prevalence of infection in the present study in males, 29 (54.70%) in comparison to females 24 (45.23%) in 2016 and 120 (57.69%) males in comparison to females 88 (42.33%) in 2017. Similar findings of higher prevalence in males in dengue cases were also reported by some authors.^[32,35,36] Higher prevalence of dengue virus infection in males as compared to females is probably due to social, cultural and exposure differences.

The scope of the present study was to highlight the changing trend of dengue virus infection in Manipur, where no previous research in dengue virus infection has been performed. The present study has its shortcomings in terms of correlating the patient's clinical histories and symptomatology with occurrence of dengue seropositivity. Further studies of longer duration are needed to pursue on lines of serotyping of prevalent strains of virus and differentiating primary and secondary infection in this particular geographical areas to reduce the morbidity and mortality due to DHF/ DSS; however, no death from dengue virus infection has been reported during the study period in this region, but may happen anytime in future. Because of the scarcity of resources and facilities we could not perform

molecular level study to counter check the serological test result of the present study. If the WHO's goal of reducing morbidity by about 25% and mortality by 50% by 2020^[37] is to be achieved, we need continuous sero-epidemiological surveillance for timely formulation and implementation of effective dengue control programme with delivery of effective vaccine before onset of monsoon season.

CONCLUSION

The study concludes a rapidly declining seroprevalence of dengue viral infection in this particular geographical area of the country from 2016 to 2017, which may be attributable to the collective effort of health care workers and people during timely delivery of preventive and control measures besides having increased awareness among the people.

Strict vigilance by the health care workers of Manipur health services in taking all measures to increase awareness of public in preventive and control measures through Information, Education and Communication (IEC) networks with integrated vaccination program before onset of monsoon season are still required to further decrease dengue viral disease. Further health services of Manipur and NVBDC, Ministry of Health and Family Welfare, Government of India, New Delhi should make all arrangements to provide testing kits for early and quickest diagnosis and serotyping of prevalent strains of dengue virus along with differentiating primary and secondary dengue virus infection for the timely management of severe morbidity and mortality, which may come up anytime in future among the people having secondary dengue virus infection.

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