

Original Research Article

Dengue infection in central India: a 5 years study at a tertiary care hospital

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Received: 17 March 2017

Accepted: 19 April 2017

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ABSTRACT

Background: Dengue is one of the most important mosquito borne viral disease with wide spectrum of clinical presentation and often with unpredictable clinical evolution and outcome. Approximately 50 million infections occur annually world-wide, but what's the real size of the problem in India? Nobody truly knows...!! Present study was carried out to determine seropositivity, clinical profile and seasonal variation of dengue infection in central India.

Methods: Study was carried out from January 2012 to December 2016. Blood samples were collected from 15,606 patients with dengue like clinical illness and serum was separated. All the samples were subjected to IgM antibody detection by dengue MAC ELISA.

Results: Prevalence of dengue in dengue suspected cases was found to be 24.49% (3,822/15,606). Maximum number of positive cases, 1,548 (40.50%) were in the age group of 0-10 years. Males (60.83%) were affected more than females (39.17%). Peak was observed in the months of August, September, October and November. Common presenting features were fever followed by myalgia, arthralgia, headache and bleeding manifestations. Significant drop in platelet count was observed in patients with dengue shock syndrome and dengue haemorrhagic fever.

Conclusions: Number of dengue cases in central India are on increase and continued surveillance is essential to determine epidemiological and seasonal trend.

Keywords: Central India, Dengue, Seropositivity, Thrombocytopenia

INTRODUCTION

Dengue is a most important mosquito borne viral disease in the world.¹ According to WHO approximately 2.5 billion people are at risk of acquiring infection. Upto 50 million infections occur annually and 5,00,000 cases of dengue hemorrhagic fever (DHF).¹ Shepard DS et al reported in their study that India had nearly six million annual clinically diagnosed dengue cases between 2006 and 2012 - almost 300 times greater than the number of cases that had been officially reported.²

Dengue fever is caused by dengue virus, a positive stranded RNA virus in the genus *Flavivirus*, family

Flaviviridae.³ There are four distinct dengue virus (DENV) serotypes that share antigenic relationships (DENV-1, DENV-2, DENV-3 and DENV-4).¹ Principle transmission vectors are arthropods of the *Aedes* (*Ae.*) genre, especially *Aedes aegypti*.⁴ Short term changes in temperature, precipitation and humidity are often correlated with dengue incidence. Other important factors include population growth, urbanization, lack of sanitation, increased long distance travel and ineffective mosquito control.⁵ All age groups and both sexes are affected.⁶

Primary infection typically results in a self-limiting disease characterized by sudden onset of fever lasting

from three to seven days and constitutional symptoms including severe headache with retro orbital pain, body aches, joint pain and rash.³ Secondary infection is caused by a second exposure by a different serotype and can result in dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Major clinical features include high fever, hemorrhagic events, circulatory failures and the fatality rate can be as high as 30%.⁷

At present, information about dengue infection is quite limited and officially reported cases are inadequate. Morbidity, mortality rates and economic burden are overwhelming. This problem is also exacerbated by the continuing dispersal of these viruses to new geographic regions. Understanding of dengue infection is essential to assist policy makers and public health managers to prepare for and control outbreaks with limited resources for both diagnosis and treatment. Thus, present study was carried out to study the seropositivity, clinical profile and the seasonal variations of dengue infection in our region.

METHODS

The present observational cross-sectional study was carried out in department of Microbiology at a tertiary care hospital, in central India which is a sentinel surveillance site under National Vector Borne Disease Control Programme (NVBDCP) from January 2012 to December 2016. Approval was obtained from the institutional ethical committee. Study group consisted of clinically suspected cases of dengue infection admitted in the hospital as well as those who attended outpatient department. Detailed history was obtained from each patient.

Blood samples were collected and serum was separated as per the standard guidelines.^{8,9} Blood collection tubes were kept upright after the blood was drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form. Blood sample was centrifuged at the end of the clotting time (30-60 minutes) in a manual top discharge centrifuge for 20 minutes at 1100-1300 rpm at room temperature. Serum was transferred with pipette. Specimens were stored at 2-8°C till processing. Repeated freezing and thawing was avoided.

The serum samples were subjected to dengue IgM antibody detection with ELISA (NIV DEN IgM Capture ELISA kit, NIV, Pune, India). The test was performed as per manufacturer's instructions. It is based upon the principle that IgM antibodies in the patient's serum are captured by anti-human IgM coated on to the solid surface (wells).

In the next step, dengue antigen was added which binds to captured human IgM in the sample. Unbound antigen was removed during the washing step. In the subsequent step, biotinylated flavivirus anti dengue monoclonal

antibodies were added followed by Avidin-HRP. Subsequently, chromogenic substrate (YMB/H₂O₂) was added, the reaction was stopped by 1N H₂SO₄. The intensity of color/ optical density was measured at 450nm within 10 minutes after termination of reaction.

If OD value of sample tested was less than OD value of negative control by factor 2.0, sample was considered as negative. If OD value of sample tested exceeded OD of negative control by factor 3.0, sample was considered as positive. If OD value for sample tested exceeded OD of negative control by a factor 2.0 but was less than OD of negative control by factor of 3.0, the sample was considered as equivocal and repeat sample was asked for.

Data was analysed using statistical package for social sciences version 16 (SPSS V16). Observations were presented as frequency and percentage distribution. Relationship between nonparametric discrete observations was analysed by non-parametric Chi square test. Significance value of less than 0.05 (p<0.05) was considered for statistical tests.

RESULTS

Total 15,606 serum samples from suspected dengue cases were collected and subjected to dengue IgM antibody detection by MAC ELISA. 3,822 (24.49%) samples were positive. Prevalence of dengue in dengue suspected cases was found to be 24.49% (Table 1).

Table 1: Positivity of dengue by MAC ELISA.

MAC ELISA (n= 15,606) (%)	
Positive	3,822 (24.49)
Negative	11,784 (75.51)
Total	15,606

Maximum number of MAC ELISA positive cases, 1,548 (40.50%) were in the age group of 0-10 years followed by 1,021 (26.71%) cases in the age group of 11-20 years (Table 2).

Table 2: Age wise distribution of suspected dengue cases and MAC ELISA positive cases.

Age (Yrs.)	No. of suspected dengue cases (n=15,606) (%)	No. of MAC ELISA positive cases (n=3,822) (%)
0-10	4,868 (31.19)	1,548 (40.50)
11-20	4,004 (25.66)	1,021 (26.71)
21-30	3,273 (20.97)	648 (16.95)
31-40	1,562 (10.01)	295 (07.72)
41-50	940 (06.02)	167 (04.37)
51-60	540 (03.46)	84 (02.20)
61-70	393 (02.52)	57 (01.50)
71-80	26 (00.17)	02 (00.05)
Total	15,606	3,822

Table 3: Gender wise distribution of suspected dengue cases and MAC ELISA positive cases.

Gender	No. of suspected dengue cases (n=15,606) (%)	No. of MAC ELISA positive cases (n=3,822) (%)
Male	8,447 (54.13)	2,325 (60.83)
Female	7,159 (45.87)	1,497 (39.17)
Total	15,606	3,822

Males (60.83%) were affected more than females (39.17%) (Table 3).

Out of the 3,822 MAC ELISA positive dengue cases, 1,726 (45.16%) were from urban area and 2,096 (54.84%) were from rural area (Table 4).

Table 4: Area wise distribution of suspected dengue cases and MAC ELISA positive cases.

Area	No. of suspected dengue cases (n= 15,606) (%)	No. of MAC ELISA positive cases (n = 3,822) (%)
Urban	7,431(47.62)	1,726 (45.16)
Rural	8,175 (52.38)	2,096 (54.84)
Total	15,606	3,822

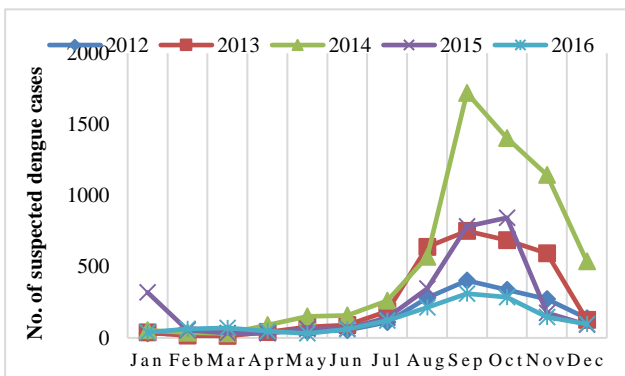


Figure 1: Month wise distribution of suspected dengue cases (n=15,606).

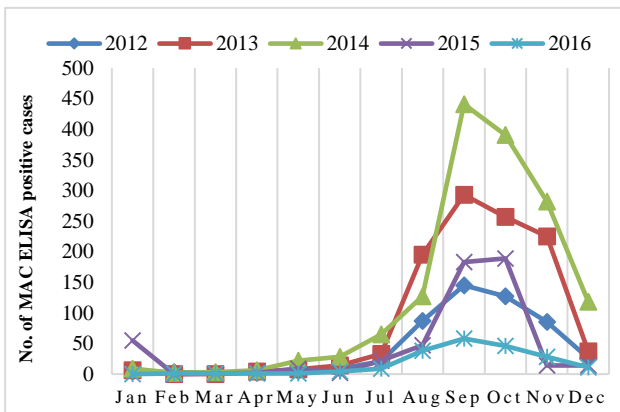


Figure 2: Month wise distribution of MAC ELISA positive cases (n=3,822).

Maximum number of suspected dengue cases and MAC ELISA positive cases were reported in the months of August, September, October and November as depicted graphically (Figure 1 and 2).

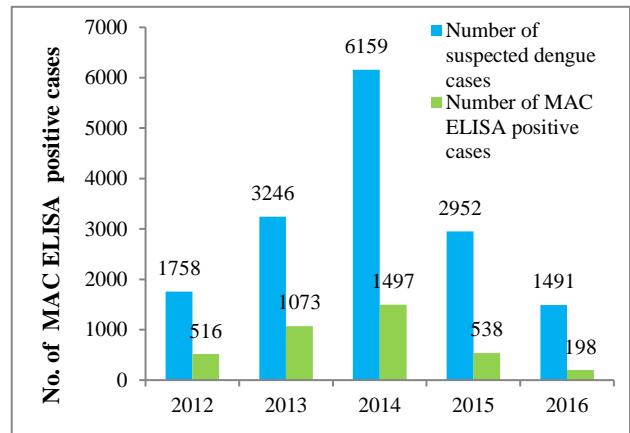


Figure 3: Year wise distribution of suspected dengue cases and MAC ELISA positive cases.

Table 5: Clinical presentation of suspected dengue cases and MAC ELISA positive cases.

Clinical presentation	No. of suspected dengue cases (n= 15,606) (%)	No. of MAC ELISA positive cases (n=3,822) (%)
Fever	15,606 (100)	3,822 (100)
Myalgia	11,648 (74.64)	2,408 (63.00)
Arthralgia	10,263 (65.76)	2,179 (57.01)
Headache	9,507 (60.92)	2,133 (55.81)
Rash	2,499 (16.01)	495 (12.95)
Bleeding Manifestations	1,457 (09.34)	389 (10.18)
Circulatory Failure	164 (01.10)	87 (02.28)

Common presenting features were fever followed by myalgia, arthralgia and headache. Bleeding manifestations like petechiae/purpura, haematemesis/melena, overt bleeding was also observed. Circulatory failure was seen in few cases (Table 5).

Table 6: Platelet count and distribution of MAC ELISA positive cases (n=3,822).

Platelet count(/mm ³)	No. of cases (%)	Total (%)
<25,000	287 (07.51)	2,378 (62.22)
25,000 - 50,000	409 (10.70)	
50,000 -75,000	673 (17.61)	
75,000 -1,00,000	1,009 (26.40)	
>1,00,000	1,444 (37.78)	1,444 (37.78)
Total	3,822	3,822

Death was reported in 40 (01.05%) cases. Relationship of platelet count was studied with category of dengue

infection (Table 7).

Table 7: Relationship between platelet count and category of dengue infection (n=3,822).

Platelet count (/mm ³)	Category of dengue			Total (%)
	DF (%)	DHF (%)	DSS (%)	
<25,000	14 (13.73)	27 (26.47)	61 (59.80)	102
25,000-50,000	32 (14.55)	163 (74.09)	25 (11.36)	220
50,000-75,000	467 (69.81)	201 (30.04)	01 (00.15)	669
75,000 -1,00,000	1,385 (99.86)	02 (00.14)	00	1,387
>1,00,000	1,443 (99.93)	01 (00.07)	00	1,444
Total	3,341 (87.41)	394 (10.31)	87 (02.28)	3,822

DISCUSSION

WHO has declared dengue to be hyper endemic in India and cyclical epidemics of dengue are becoming more frequent. Official reported cases of dengue fever are very inadequate. There has been a steady rise in number of dengue cases every year and mortality rate has been going down steadily as well.^{1,2}

Seroprevalence of dengue infection

In the present study, out of 15,606 dengue suspected cases, 3,822 cases were positive for dengue IgM antibody by ELISA. Prevalence of dengue in dengue suspected cases during our study period was thus found to be 24.49%. Higher seroprevalence of 31.3% was reported by Ukey PM et al in central India.⁶ Saini S et al reported seropositivity of 30.6% in western Maharashtra.¹⁰ Sood S reported 18.99% seroprevalence of dengue in Rajasthan, India.¹¹ Seropositivity of 17.7% was reported by Rao MS et al in Andra Pradesh, India.¹²

Age wise distribution

In the present study, out of 3,822 MAC ELISA positive cases, 40.50% cases were in the age group of 0-10 years, followed by 26.71% cases in the age group of 11-20 years. Rao MS et al also observed maximum seropositivity of 35.84% in the age group of 0- 10 years, followed by 22.66% in the age group of 11-20 years.¹² Ukey PM et al reported highest seropositivity of 43.90% in children < 10 years followed by 31.71% in age group of 15-30 years.⁶

Kumar A et al observed that maximum number of cases, 57.3%, were in the age group of 15-44 years, while 7.5% cases were among the under-five children.¹³

In general, persons of all age groups sustain dengue infections but the greater relative prevalence of dengue infection is seen in children compared to adults. It is believed to be due to the intrinsically more permeable

vascular endothelium and comparatively lower immunity in children rendering them more susceptible to dengue infections.¹⁴

Gender wise distribution

We observed that males (2,325 (60.83%)) were affected more than females (1,497 (39.17%)). The ratio of male to female in MAC ELISA positive cases was found to be 1.55: 1 (Table 3). Karoli R et al reported 58% male patients and 42% females with M: F of 1.38: 1.¹⁵ Male to female ratio of 1.82:1 was reported in another study.¹³ However, approximately equal number of affected females 550 (50.70%) and males 535 (49.30%) were reported by Murugananthan K et al.¹⁶ Higher seropositivity in males might be because of increased exposure at work places or outdoor activities.

It had been suggested by Halstead SB et al that immune responses in females are more competent than in males, resulting in greater production of cytokines rendering them more immune to dengue infection than males.¹⁷

It is widely recognised that in many of the Indian communities, lower disease incidence in women may be a statistical artefact related to lower reporting and care-seeking for women from traditional practitioners who do not report to public surveillance systems. By the same token, women are less likely to be taken for care at a hospital when ill or are taken at late stages of disease, when no other options are available.¹⁸

Area wise distribution

It was observed that out of the 3,822 MAC ELISA positive dengue cases, 1,726 (45.16%) were from urban area and 2,096 (54.84%) were from rural area. Our findings are in accordance with Ukey PM et al who reported majority of the dengue-positive patients from the rural areas of Maharashtra.⁶

Historically, DF/DHF has been reported as occurring predominantly among urban populations where *Ae. aegypti* breeding is more common as well as density of dwellings and short flying distance of the vector create the right conditions for transmission. However, the trend is now changing due to socio economic and man-made ecological changes like increased transport contact, mobility and spread of peri-urbanisation etc. As well as there is scarcity of water in the rural settings and large containers are used for water storage which then acts as favoured breeding sites for mosquitoes resulting into invasion and increased transmission of dengue infection in rural regions.^{1,7}

Month wise distribution

We found that maximum number of dengue suspected cases were reported in the months of August, September, October and November. Amongst the MAC ELISA positive cases, peak was seen in the months of September, October and November (Figure 1 and 2). Similar findings were reported by Kumar A et al who observed a gradual increase in cases from June with a peak in September, during all the seven years of the study.¹³ Gunasekaran P et al also reported high percentage of IgM positivity during the months of September and October in all the three years.¹⁹

Ae. aegypti has an average adult survival of fifteen days. During the rainy season, survival is longer and therefore the risk of virus transmission is greater. During post monsoon period, stagnant water pool collected during rainy season acts as favourable breeding sites and along with lower temperature during this period, there is an increase in transmission of dengue infection.^{1,7,20}

We reported an epidemic of dengue infection with 1,497 positive cases in the year 2014, almost triple rise from 516 cases reported in the year 2012 (Figure 3). However, this seropositivity decreased to 538 and 198 cases of dengue in the year 2015 and 2016 respectively. This might be because of preventive and integrated precautionary measures for disease control that were taken in collaboration with municipal corporation and public health sector like using insecticides or biological control agents.

Clinical presentation

The most common presenting feature in suspected dengue cases was fever (100%), followed by myalgia in 74.64%, arthralgia in 65.76% and headache in 60.92% cases. Rash was present in 16.01% cases. Bleeding manifestations and circulatory failure were seen in 09.34% and 01.10% cases respectively (Table 5).

In 3,822 MAC ELISA positive cases, fever was present in all (100%) cases, followed by myalgia in 63%, arthralgia in 57.01% and headache in 55.81% cases. Rash was present in 12.95% cases. Bleeding manifestations

and circulatory failure were seen in 10.18% and 02.28% cases respectively (Table 5). Gum bleeding was the major haemorrhagic manifestation, followed by melena, petechiae/purpura, haematemesis and haemoptysis. Respiratory symptoms including cough, sore throat and rhinitis were also observed in children. Similar findings were reported by Turbadkar D et al who observed that fever was the major presenting complaint followed by icterus in 25.8%, myalgia in 25% and headache in 13.9% in the suspected cases of dengue.²¹ Mandal SK et al also reported fever in all cases followed by headache (62.16%), rash (37.84%) and bleeding (13.51%). They also observed ascites in 8.1%, pleural effusion in 18.91% and neurological features in 11.11% cases.²²

Thrombocytopenia

In the present study, out of the 15,606 suspected dengue cases, thrombocytopenia (Platelet count < 1,00,000/mm³) was observed in 8,936 patients. Amongst the 3,822 MAC ELISA positive cases, 2,378 (62.22%) patients had thrombocytopenia. Khan MU et al reported thrombocytopenia in 44.4% cases.²³ A higher percentage of 71% and 86.30% were observed by Khan DM et al and Kauser MM et al respectively.²⁴⁻⁵

Although, an exact underlying reason for thrombocytopenia in dengue fever is not known but it has been suggested that dengue virus inhibits in vitro megakaryopoiesis and induces apoptotic cell death in a subpopulation of early megakaryocytic progenitors which may contribute to thrombocytopenia in dengue cases.¹⁸ In another study, it was shown that dengue virus may directly interact with and activate platelets and thus may be responsible for thrombocytopenia.²⁶

Dengue infection is classified by WHO as dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).¹ Dengue serotype 2 is most commonly circulating in central India causing dengue fever but primary and secondary infections caused by other dengue serotypes are also reported causing DF and DSS, although less common.⁷

In the present study, DF was seen in 3,341 (87.41%) cases, DHF in 394 (10.31%) cases and DSS in 87 (02.28%) cases. Death was reported in 40 (01.05%) cases. Our findings are congruent with Kumar A et al who found 83.9% cases presented with DF, 8.8% DHF and 7.3% with DSS. Deaths were reported in 2.4% cases.¹³ Avarebeel S et al found that 81% cases had DF, 18.65% had DHF and 0.74% had DSS.²⁷ In present study, DSS and deaths are less, resulting in a lower case-fatality rate for dengue, likely representing hospitalization of less severe cases.

In the present study, we studied a relationship between platelet count and disease severity. Maximum number of DSS (86/87) and DHF (190/394) cases had platelet count <25,000/mm³ and between 25,000-50,000/mm³. Only 14

cases of DF had platelet count $<25,000/\text{mm}^3$ and 32 cases had count between $25,000\text{-}50,000/\text{mm}^3$. A significant drop ($p<0.001$) in platelet counts was noted as the patient presented with symptoms of DHF/DSS.

Our findings are similar to Khan DM et al and Jayashree K et al who reported a statistically significant association between thrombocytopenia and clinical presentation of dengue infection cases.^{24,28}

Laboratory findings such as thrombocytopenia and a rising hematocrit in DHF cases are usually observed by day 3 or 4 of the illness. Thrombocytopenia is observed in DF but is a constant feature and one of the diagnostic criteria of DHF and DSS. Though the dengue virus induced bone marrow suppression decreased platelet synthesis, an immune mechanism of thrombocytopenia caused by increased platelet destruction appears to be operative in patients with DHF.²⁴

Dengue disease continues to involve newer areas, newer populations and is increasing in magnitude, epidemic after epidemic. Every aspect of dengue viral infection continues to be a challenge; the pathogenesis of severe dengue disease is not known, no vaccine is yet available for protection and the vector control measures are inadequate. Scientific studies addressing various problems of dengue disease have been carried out at limited number of centers. Ongoing surveillance, vector surveys and epidemiologic studies to identify risk factors will provide key information for controlling dengue.

CONCLUSION

The present study provides a baseline data on seroprevalence of dengue in central India. We conclude that dengue cases are on increase in central India and with early diagnosis and treatment, morbidity and mortality can be decreased. Our study results call attention to the need for continuous surveillance and individual and community action for dengue control.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Deshkar ST, Raut SS, Khadse RK. Dengue infection in central India: A 5 years study at a tertiary care hospital. *Int J Res Med Sci* 2017;5:2483-9.