Microbiology Section

Emergence of Dengue as a Febrile Illness in Rewa and Nearby Districts of Madhya Pradesh during the Year, 2021: A Cross-sectional Study

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ABSTRACT

Introduction: Dengue is a mosquito borne viral disease, found in tropical and subtropical countries. Dengue virus (DENV) infected mosquitoes of Aedes species are crucial for the transmission of disease. It has emerged as a threat to the public health systems. Dengue is endemic in many parts of India but still the status of dengue cases in Rewa Madhya Pradesh is not reported convincingly.

Aim: To investigate the presence of dengue in Rewa district of Madhya Pradesh.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology at Shyam Shah Medical college Rewa under National Vector Borne Disease Control Programme (NVBDCP), Rewa, Madhya Pradesh, India, including 1113 Outpatient/ Inpatient (OPD/IPD) Department samples received during March 2021 to October 2021. Blood samples were collected from patients having febrile illness and after serum separation, serum were subjected to NS1 Enzyme Linked Immunosorbent Assay (ELISA) test. Descriptive statistics and Chi-square tests were applied for data analysis.

Results: A total of 1113 sample were received and tested for dengue NS1 out of that 108 sample were found NS1 positive by ELISA. The cases of dengue started from the month of July 2021. But in the month of October dengue positivity was highest in number. Dengue cases reported were 6.73 in the rainy season (July-August), but the dengue positivity increased 12.3 in the post rainy season (September-October). Overall prevalence of dengue was higher in the 21-30 years (34.3%) age group followed by 11-20 years (24.1%), 31-40 years (18.5%), 41-50 years (18.5%), 51-60 years (7.4%) and >60 years (3.70%) age groups with respect to total positive cases. The prevalence of dengue was higher in male (12.94%) in comparison to females (5.54%).

Conclusion: This study warrants the dengue virus infection as one of the important causes of fever during rainy and post rainy season in this region. Early diagnosis and reporting of cases are important for the better management of disease.

Keywords: Dengue virus, Enzyme-linked immunosorbent assay, Fever, Serology

INTRODUCTION

Dengue fever is a viral disease transmitted by infected Aedes aegypti mosquitoes in tropical and subtropical regions. It is a major public health problem especially in tropical region [1]. The incidence of dengue cases have increased over the years. The morbidity and mortality associated with dengue virus were reported significantly in many parts of the world, including India [2]. According to an estimate 3.9 billion people are at risk of dengue viruses' infection. 70% of the actual burden is bearded in Asia itself [3]. In India, the number of dengue cases and severity of illness have been increasing year by year [4]. Geographical distribution of DENV includes not only urban but also semi urban and rural areas [5].

Dengue viruses (DENV) belong to the genus Flavivirus of family *Flaviviridae*. It is a single stranded Ribonucleic Acid (RNA) virus and has four distinct serotypes (DENV 1-4) [6]. Usually, the condition of dengue disease occurs suddenly after 2 to 7 days of incubation with the onset of a high fever. Fever may be accompanied by headache, myalgia, thrombocytopenia, haemorrhagic manifestation, arthralgia and rigors [7,8]. Dengue infection can also cause severe disease lead to haemorrhagic manifestation, bleeding, plasma leakage, and organ impairment [9]. In some cases, conditions of Dengue Haemorrhagic Fever (DHF) and/or Dengue Shock Syndrome (DSS) appeared that are fatal [7,8,10]. The DHF and DSS case fatality rate can be as high as >40% [11].

Increased aspartate and alanine aminotransferase liver enzyme levels are other prominent clinical characteristics of dengue patients [9]. Circulation of all DENV 1-4 serotypes has already been reported in Central India, Madhya Pradesh [12,13]. Several reports of febrile illness like typhoid, malaria in Rewa district of Madhya Pradesh are available but exclusively dengue prevalence has not been studied for this region [14,15]. This study aimed to determine the emergence and presence of DENV in Rewa district of Madhya Pradesh in year 2021.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at Shyam Shah Medical college Rewa under National Vector Borne Disease Control Programme (NVBDCP), Rewa, Madhya Pradesh, India, including 1113 Outpatient/Inpatient Department (OPD/ IPD) samples received during March 2021 to October 2021. Shyam Shah Medical College Rewa was authorised for serology based dengue/chikungunya testing as Sentinel Surveillance Hospitals (SSH) by National Vector Borne Disease Control Programme (NVBDCP), Government of India in year 2021 [16].

Briefly, clinically suspected dengue febrile Outpatient/Inpatient Department cases were sent to the Department of Microbiology for serological diagnosis under the programme of NVBDCP. All details including symptoms, case history and consent (from clinician and patient/guardian of patient) were taken for serological diagnosis as per case report, Case Record Form (CRF), of Department of Health Research (DHR)- Indian Council of Medical Research (ICMR) (CRF format was provided by ICMR-National Institute of Epidemiology under DHR-ICMR VRDL Network as Department of Microbiology SSMC Rewa is a part of the VRDL network). Based on the serological diagnosis results for dengue under NVBDCP programme in year 2021, data were analysed (before rainy season: March-June; rainy season: July-August; post rainy season: September-October).

Inclusion and Exclusion criteria: As per NVBDCP program inclusion and exclusion criteria were based on clinical diagnosis. Briefly, clinically dengue suspected OPD/IPD patients who presented with an acute febrile illness or following clinical features were included in the study: fever, headache, myalgia, thrombocytopenia, haemorrhagic manifestation, arthralgia, rigors. Confirmed or suspected cases for other infections like malaria, hepatitis B, hepatitis C, scrub typhus and leptospirosis were excluded for dengue diagnosis.

Diagnosis of Dengue by NS1 ELISA

The testing was done retrospectively as batched samples using Dengue NS1 Antigen ELISA, (J.Mitra, India) by serum/blood samples collected from OPD/IPD patients. As per the manufacturer's specifications, this is a qualitative ELISA for the detection of NS1 antigen in human serum. Briefly 50 μ L of serum samples with positive and negative control were separately added to the plate. Then 100 μ L of Horseradish Peroxidase (HRP) labelled diluted (1:50) conjugate was added to each well followed by primary incubation at 37°C for 90 min. After incubation, the plate was washed 6 times with a 1x wash buffer. Then 150 μ L of working substrate Tetramethyl Benzidine/Hydrogen Peroxide (TMB/H₂O₂) was added to each well and incubated at room temperature for 30 min in dark. Then 100 μ L of stop solution was added to each well and the plate was read at 450 nm with a microplate reader.

STATISTICAL ANALYSIS

Descriptive statistics were applied for data analysis. Statistical significance was evaluated using Chi-square tests. The level of statistical significance was kept at p-value <0.05. Analysis was performed by using statistical software GraphPad PRISM 8.0.2.

RESULTS

Over last decades dengue has emerged as a predominant febrile illness in India including Madhya Pradesh and other states. However status of dengue in Rewa has not been shown convincingly till date. As per our previous records of dengue testing in Department of Microbiology, SSMC, Rewa, only four positive cases out of 370 samples in year 2017 (unpublished data) were observed. During 2018-2020 no dengue positive cases in Rewa was recorded. In year 2021 dengue cases were increased in Rewa. Hereby, authors are showing the status of dengue cases in Rewa and nearby districts during the 2021 (tested and identified under NVBDCP programme).

Incidences of dengue cases in Rewa and other nearby districts of Madhya Pradesh: Serological diagnosis (based on NS1 Ag ELISA) was carried to know the incidence of dengue fever. The NS1 Ag ELISA was reactive with all four dengue virus serotypes. We identified positive cases regardless of serotypes. A total of 1113 serum samples were tested. Out of 1113 samples, 527 samples were collected from IPD cases and remaining 586 samples were collected from OPD. Over all positivity was 9.70 % (n=108). Out of 108 positive cases, 72 samples were collected from IPD cases and remaining 36 samples were collected from OPD. The prevalence of dengue fever was significantly higher (p-value <0.0001, Odd ratio: 1.630 to 3.962) in males (12.94%, n=81) in comparison to females (5.54%, n=27), [Table/Fig-1].

Out of 108 dengue positive cases, the number of positive cases were higher in Rewa (n=90) followed by Satna (n=9), Panna (n=4), Sidhi (n=4) and Shahdol (n=1), [Table/Fig-2].

Distribution of dengue cases: Month-wise and season-wise incidence of dengue cases were analysed to know the trend of dengue in respect to month and season. To show the particular





month and season-wise trend of dengue, dengue positivity (%) of each month and season were calculated out of sample tested during that month and season only. The dengue cases were started from the month of July (161 cases) and initially the positivity was 6.83% (11/161). In month of August, the positivity was 6.62% (n=9/136). Then, the dramatic increase in dengue cases were observed in September (10.27%, n=34/331). In October, dengue cases were further increased (14.14%, n=54/382). Season-wise distribution of dengue cases revealed that, before the rainy season (March-June) dengue cases were not seen. However in the rainy season (July-August) dengue positivity was 6.73% (n=20/297). After the rainy season dengue (September-October) positivity increased and it was 12.3% (n=88/713). This seasonal variability was significant (p-value <0.001) in terms of dengue positive cases in the rainy and post rainy [Table/Fig-3].

Month	Sample tested (n)	Positive (n)	Positivity (%)
March	28	0	0.0
April	53	0	0.0
May	8	0	0.0
June	14	0	0.0
July	161	11	6.83
August	136	9	6.62
September	331	34	10.27
October	382	54	14.14
Season	Sample tested (n)	Positive cases	Positive cases
Before rainy season (March-June)	103	0	0
Rainy season (July-August)	297	20	6.73%
After rainy season (September-October)	713	88	12.3%
[Table/Fig-3]: Distribution of dengue cases month-wise distribution, season-wise distribution (N=1113) {p-value=0.003, Chi-square test=(11.38).			

Age group wise distribution and locality of Dengue positive cases: The age group of 21-30 years was highly affected with dengue (37, 34.3%) followed by 11-20 years (26, 24.1%), 31-40 years (20, 18.5%), 41-50 years (9, 8.3%), 51-60 years (8, 7.4%), 1-10 years (4, 3.7%) and >60 years (4, 3.7%). Overall 64, 79.01%

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of males and 22, 81.48% females in rural areas were found more affected with dengue. However, in urban areas dengue positivity was 17, 20.99% in males and 5, 18.52% in females [Table/Fig-4,5].

Positive cases (N=108) (n,%)
Nil
4 (3.7%)
26 (24.1%)
37 (34.3%)
20 (18.5%)
9 (8.3%)
8 (7.4%)
4 (3.7%)

[Table/Fig-4]: Age group-wise distribution of dengue positive cases (N=108).



Clinical manifestation of dengue positive cases: The most common clinical features were fever, headache, myalgia, thrombocytopenia, haemorrhagic manifestation, arthralgia, rigors. All positive samples (N=108) were having fever history (100%). Headache, myalgia and arthralgia were also evident in dengue positive cases which may include thrombocytopenia, haemorrhagic manifestation, rigors [Table/Fig-6].

Symptoms	n, %	
Fever	108 (100%)	
Headache	57 (52.78%)	
Myalgia	42 (38.89%)	
Thrombocytopenia	9 (8.33%)	
Haemorrhagic manifestation	1 (0.93%)	
Arthralgia	35 (32.41%)	
Rigors	1 (0.93%)	
[Table/Fig-6]: Clinical features and laboratory parameters of dengue positive cases (N=108).		

DISCUSSION

Dengue cases have emerged and grown dramatically around the world over past decades. Late or misdiagnosis of Dengue as other febrile illnesses is still a major problem in the management of disease burden [17]. According to an estimate 96 million dengue cases manifest clinically out of 390 million of dengue cases per year. 70% of the actual burden is in Asia [3]. In 2020,

during covid-19 pandemic dengue affected many countries including India and still continues to affect in 2021 [2]. In 2020, 44585 dengue cases along with 56 deaths were reported in India as per National Vector Borne Diseases Control Programme (NVBDCP). Total 806 cases were reported from Madhya Pradesh (MP) without any deaths. But in the year 2021 (till October) MP has a contribution of 11354 cases out of 123106 (including 90 deaths) cases in India [18]. At the end of 2015, Madhya Pradesh raked 14th countrywide in the number of dengue cases (2108 cases with 8 death) reported [18]. Now MP ranked 3rd in the number of dengue cases has been drastically increased from 2015 to the end of 2021 [Table/Fig-7] [18].



This dramatic increase in dengue cases in Madhya Pradesh raises the concern about the development of better disease management strategies to combat dengue and other vector borne diseases. Similar situation was observed in neighbour state of Madhya Pradesh i.e. Chhattisgarh, Gujarat, Maharashtra, Rajasthan and Uttar Pradesh. In these states also, drastic increase in dengue cases were seen from 2015 to the end of 2021 [Table/ Fig-7] [18]. As per study of Indian Council of Medical Research-National Institute of Malaria Research (ICMR-NIMR), New Delhi, 11 districts of Madhya Pradesh were identified as dengue infected areas (i.e. Ashoknagar, Bhopal, Chhindwada, Gwalior, Indore, Jabalpur, Mandla, Morena, Narsinghpur, Sagar and Shivpuri) [19]. In Rewa and nearby districts of Madhya Pradesh Dengue cases are still under reported [19]. Although, authors have observed four dengue positive cases in Rewa out of 370 during 2017, but during 2018-2020 authors have not found any positive dengue cases (unpublished data). In view of this, the present cross-sectional study was conducted under NVBDCP program in SSMC Rewa Madhya Pradesh to elucidate the emergence of dengue cases in this region during 2021.

Current study observed 9.70 % Dengue positivity (based on NS1 ELISA) in Rewa including nearby districts (Satna, Panna, Sidhi and Shahdol). The positivity of this region was slightly higher than the overall dengue positivity (9.47%) in Madhya Pradesh for the year 2021 [18]. The number of positive samples was higher in Rewa in comparison to other nearby districts. New dengue cases were reported in July during the rainy season and at the end of October (after the rainy season) the number of dengue cases dramatically increased. The ICMR-NIMR study in Madhya Pradesh has shown that dengue cases were recorded during the monsoon (June-August) and post monsoon seasons (September-November). Maximum cases (70%) of the cases were reported between September and November [19]. Disease prone environment exposure due to water logging and mosquito breeding could be one of the regions for the incidence of dengue cases in Rewa and nearby districts. As it is already well established that stored water

due to extended rainy season, with ambient relative humidity and temperature, favours the breeding of *Aedes aegypti* (a vector of dengue virus) [20,21].

The prevalence of dengue fever was significantly (p-value < 0.0001) higher in males in comparison to females. The present study showed higher dengue prevalence in males is concurrent with other studies [20]. Dengue positivity was highest (77.78%) in the adult age group (18-59 years) followed by 15-18 (13.89%), >60 (4.63%), 9-14 (4%) and 1-4 (4%) age group, out of all positive samples. Other studies have also shown higher dengue prevalence in persons ranging between the ages of 20-59 years (60.54%, 36384 cases out of 60096 positive cases) [22]. Another study of ICMR also supports these findings as they have shown 56.2 %dengue positivity in 18-45 year age group [23]. Authors observed that 75.61 % (62 out of 82 positive cases) of male dengue cases were belongs to 20-59 year age group. In case of female it was 84.62% (22 out of 26 positive cases) in 20-59 year age group. Thus male and females of this age group (20-59 year) have more risk of dengue.

Dengue cases were detected from both rural and urban areas. Usually urban areas are thought to be more prone for dengue because mosquito find more breeding grounds in urban area as there are more drains, deserted coolers, flower pots, unused tyres etc [24]. But authors observed higher dengue prevalence in rural areas in both male (79.01%) and female (81.48%) when compared to urban areas. The reason behind this could be the frequent travelling of rural people for their livelihood and water logging/staging in and after the rainy season [20,21,25].

The most common clinical feature in dengue positive cases was fever (100%), as dengue is one of the leading causes of febrile illness in Asia [26]. Other clinical features were also evident i.e. headache (52.78%), myalgia (38.89%), and arthralgia (32.41%). Haemorrhagic manifestation was observed in only one case and thrombocytopenia was observed in nine cases. Several clinical features in dengue positive cases have already been shown by other researchers such as fever (93%), headache (71%), retroorbital pain (35%), bodyaches (66%), joint pain (44%), rash (21%) , nausea (30%), diarrhoea (10%), sore throat (38%) and cough (38%). These clinical features were suggestive for selection of suspected dengue cases [27]. It is an alarming situation for dengue emergence even in rural area of Rewa and other nearby districts of Madhya Pradesh. Government authorities need to take vital steps to combated and control the dengue with special emphasis in rural area as they have limited access of health facilities.

Limitation(s)

There were several limitations in this study as some epidemiological factors like travelling, socio-cultural habits were not collected that may influenced the seropositivity. Differential diagnosis were not carried for dengue negative but symptomatic cases as samples were referred as clinically suspected dengue cases.

CONCLUSION(S)

This study elucidates the emergence of dengue in Rewa and other nearby districts of Madhya Pradesh. Males have more at risk of dengue infection in comparison to females. It has reached rural areas during the rainy and post rainy season. Now the time has come to initiate continuous surveillance and individual and community action for dengue control not only in urban area but also in rural areas.

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