



# Dengue Status in a Tertiary Care Rural Hospital

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## ABSTRACT

**Introduction:** Dengue is one the most common arthropod borne virus transmitted disease of the tropics. Though many remains asymptomatic, a huge population gets infected with some persons leading to the serious form of the disease. As rapid urbanization allows to thrive the *Aedes albopictus* mosquito which is mainly involved for the transmission of the disease, the seasonal outbreaks are becoming a common fact to deal the Acute Febrile Illnesses year after year.

**Aims/Objectives:** To study the epidemiology of dengue through the serological, demographic profile, regional distribution, vectors and circulating serotypes.

**Materials and Methods:** Demographic data and presenting symptoms of fever 10476 cases reported to the clinic were recorded. Suspected patients were tested for dengue, chikungunya, scrub typhus and malaria. Dengue-specific NS1 and IgM ELISAs were performed, followed by RNA extraction, PCR and sequencing to detect circulating DENV serotypes.

**Results:** Of the 10476 fever patients tested from July to September 2021, 2767 cases were Dengue NS1 positive, while 2624 were Dengue IgM positive. Of the 200 samples analysed, 19.1% had concurrent infection with multiple dengue serotypes DEN 1, 2 & 3; 63.8% was DEN 1, and 18.2% was DEN 2.

**Conclusion:** Wardha is endemic to Dengue, and concurrent infections with multiple DENV serotypes have become a frequent finding in this region. The most prevalent febrile illness nowadays is Scrub typhus or Dengue Fever in the rural part of central India, which has predominantly replaced typhoid fever and malaria. They may be asymptomatic or give rise to undifferentiated fever, Dengue Fever (DF), Dengue Haemorrhagic Fever (DHF), or Dengue Shock Syndrome (DSS). Annually, 100 million cases of dengue fever and half a million cases of DHF occur worldwide. It is essential to know the prevalent serotype and disease pattern for better recovery of the patients. Though much research is ongoing for a new vaccine against Dengue, reaching rural India will take time and effort. Early recognition and prompt initiation of appropriate treatment are vital if disease-related morbidity and mortality are to be limited.

**Key Words:** Acute Febrile Illness, Central India, Dengue serotyping, Epidemiology, Seasonality, WGS

## INTRODUCTION

The COVID-19 pandemic has placed immense pressure on healthcare and management systems worldwide. Many non-covid infections were neglected during the lockdown, especially vector borne diseases. However, the symptoms are similar to covid infections, and though the etiological agents are different, one RNA virus is trying to dominate another. Based on epidemiological data and previous understandings, we expect a surge in Dengue cases in India. In March 2020, after the Havoc of Covid, no other viral infection physician could think apart from Covid. But with the decrease in covid cases, patients with Acute Febrile Illnesses like Dengue/Scrub typhus/Chikungunya surged in, replacing the Covid infection just as the phenomenon of “dominance of viruses”.

Dengue is a self-limiting acute mosquito-transmitted disease characterized by fever, headache, myalgia, arthralgia, rash, nausea and vomiting. These infections may be asymptomatic or may lead to (a) “classical” Dengue fever, or (b) Dengue Hemorrhagic fever without shock, or (c) Dengue hemorrhagic fever with shock. Dengue fever (DF) and its severe forms - Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS) have emerged as significant public health problems in recent decades in terms of the mortality and morbidity associated with it.<sup>1</sup> Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature, relative humidity and unplanned rapid urbanization. Several states in the country are grappling with an outbreak of dengue fever. The disease is no longer

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confined to the tropics.<sup>2</sup> Dengue has four distinct but closely related serotypes of the virus that cause dengue: DENV-1, DENV-2, DENV-3 and DENV-4. World Health Organisation (WHO) has emphasized the importance of sustaining efforts to prevent, detect and treat vector-borne diseases such as dengue and other arboviral diseases during this crucial period, as the case numbers have increased in several countries, exposing urban populations at the highest risk for both diseases.<sup>3</sup> The combined impact of COVID-19 and dengue epidemics can potentially result in devastating consequences for at-risk populations.

This study reports a rapid surge in Dengue cases in the tertiary care rural hospital. Previously, we had never observed so many positive sera for NS1 antibody, which we have recorded only in three months, July-September 2021. Hence, we have compared the previous year's 2019 and 2020 dengue data in this study. Compared with our previous year's data with 2021 data, there is a marked increase in the number of Dengue NS1 cases in our hospital. During 2021, from June end, cases started coming for testing for dengue. This was the early monsoon period cases which were still on the rise. Here we highlight the data for Dengue NS1, and IgM Positive noted during 2021 with a comparison of 2019 and 2020.

## METHODS

**Diagnosis of Dengue Fever:** Serum samples from clinically suspected patients having a history of fever for 1-5 days were tested for NS1 antigen (Panbio Dengue Early ELISA, Standard Diagnostics, Korea). Those having fever for more than five days were tested for dengue-specific IgM antibodies by using an IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) kit (ICMR-National Institute of Virology, Pune). All the Fever cases suspected of having Acute Febrile illness other than dengue were screened for Malaria, Chikungunya and Scrub typhus. For malaria, microscopy of blood smear and for Chikungunya, ChikV IgM ELISA (ICMR-NIV, Pune). Dengue and Chikungunya IgM capture ELISA tests are intended for qualitatively detecting IgM antibodies in serum. For scrub typhus, IgM ELISA from Inbios with OD value > 0.5 is considered positive. The manufacturer's instructions were strictly followed for performing the test and interpreting the results. Optical Density (O.D) was measured at 450 nm using an ELISA reader (Bioscreen ELISA Reader and Washer Model: Tecan Infinite F 50 Family).

**Serotyping for Prevalent Serotype:** We have analysed 200 representative NS1 Dengue Sera positives to determine the serotype for which we have performed a PCR test. RNA extraction was done using Qiagen Viral RNA mini kit. A multiplex TaqMan real-time one-step PCR assay for detecting all four DEN serotypes was performed using ABI 7500 PCR thermal cycler (Applied Biosystem, USA).

**Recording clinical and demographic details of patients:** Clinical and demographic details of the patients were obtained from the patient's requisition forms entered in the hospital information system. The details were recorded in Microsoft-excel sheets, and those of dengue NS1 ELISA-positive patients were analysed to discern the pattern.

**Data comparison:** We have also compared our data with January to December 2019, 2020 NS1 and IgM positives with 2021 data.

## RESULTS

From July to September 2021, 10476 samples were received from patients with Acute Febrile Illnesses. These are the months when we get maximum cases of Acute Febrile illness. These 10476 samples were tested for Dengue NS1, IgM, Scrub typhus and Malaria. After analyzing the demographic data, we observed that all these patients were from in and around the villages to this tertiary care rural hospital. Maximum samples were from the central city and small villages around. A total of 2767 (26.41%) patients were NS1 positive out of 10476; out of 10109 IgM tested samples, 2624 (25.96%) were IgM positive. 34.72% of patients were from the age group 11 to 20 years, followed by 28.41% in the age group of 0 to 10 years of age group. A male preponderance was noted in (60.71%). Fever was the most common presenting feature, with a history of fever of fewer than 5 days. 94% of patients had Myalgia and Headache along with Fever. Vomiting was noted in 74% of cases. Haematological investigations revealed Thrombocytopenia in 72% of cases with raised liver enzymes.

If we compare our 2021 Dengue NS1 and IgM data with the previous two years' data - 2019 and 2020, there is an exponential surge in the number of cases and positivity for NS1 and IgM (July to September 2021) in these three months. In the year 2019 (January to December) total of 7,867 serum samples were tested, out of which 315 (4%) were NS1 positive and 6,661 tested for IgM, out of which 528 (8%) were positive. In 2020, 3526 samples were tested for NS1, out of which 360 (10.2%) were positive, and 3334 were tested for IgM, out of which 712 (21.35%) were positive.

During both years, male preponderance was typical. Age-wise distribution of cases was predominantly seen between the age group of 11 to 20 yrs (60.71%) of male and 41 to 50 yrs (51.31%) of female patients. The distribution of dengue cases across the monsoon and post-monsoon seasons of the study years showed remarkable differences in the prevalence of infection, which is statistically significant (chi-squared value 15.54;  $p < 0.05$ ). During the pre-monsoon season, from January to May for 2019 and 2020, there were hardly any Dengue-positive cases. In our hospital increase in the number of cases started from June onwards.

200 representative samples were tested for confirming serotypes by RT-PCR. About 63.8% of Dengue positive serum was DEN 1, 19.1% were collectively DEN 1, DEN2 and DEN 3, while 18.2 % of serum samples were DEN 2 alone.

In 2019, out of 7867 NS1 tested samples, 315 (4%) were positive and 6661 serum samples were tested for IgM, and 528 (7.9%) were positive. In 2020, 3526 tested for NS1 Positive 360 (10%), whereas 3334 tested for IgM 712 (21%) were positive. The number of samples tested and the number of positivity for the year 2021, only in 3 months, was remarkably higher than the 2019 and 2020 whole-year data. An increase in Dengue samples was seen from July to October in all those three years. These are the monsoon and post-monsoon seasons here. A decline in the number of tested samples was seen from October onwards.

## DISCUSSION

Infection with more than one serotype was also observed, as reported earlier by Afreen, et al. 2014, Bharaj, et al. 2008, and Tazeen, et al. 2016.<sup>4,5,6</sup> Dengue patients infected with multiple serotypes are more prone to severe infections.<sup>7</sup> Further, severe clinical manifestations are commonly seen with DENV -2, hemorrhagic manifestations with DENV-4, and liver involvement with DENV- 3; while DENV-1 usually has mild clinical presentation.<sup>8,9</sup> The year 2021 will be a memorable year for Introducing covid vaccination globally and with a surge in Dengue cases in many states, especially Maharashtra and Uttar Pradesh. It may be due to heavy rainfalls in these areas, and post covid lockdown travel has increased the surge in Dengue cases. An Increase in cases was not just doubled in our area, but a four-fold rise in the number of cases. This increase in cases year after year is a matter of concern. Dengue was first isolated in India in 1945. After a long gap in 1963-1964, the first epidemic of Dengue Fever reported on the Eastern Coast of India spread northwards and reached North India. Simultaneously, it also reached the southern part of the country and gradually, the whole country was involved with widespread epidemics followed by epidemics of all four serotypes of DEN.<sup>10</sup>

Dengue fever is also described as break bone or crippling fever hence arthralgia and sudden rise in fever are predominant features of Dengue Fever. These symptoms are associated with headache; vomiting, which has been noted by many studies.<sup>11</sup> In our study, haematological investigations showed about 72% of cases were associated with thrombocytopenia. The majority of the cases were from the age of 11 to 20 yrs, with male preponderance. Similar findings were noted by Chandralekha, et al.<sup>12</sup> While a study conducted by Oza, et al. from Rajkot has shown the prevalent age group as 15-44 yrs of age, Ashwini Kumar, et al. in Karnataka and Saini, et al. from Western Maharashtra, India reported 70% cases in the

age group of 11 to 40 yrs.<sup>2,13,14</sup>

In our study, hepatomegaly and encephalitis were the complications in 1% of cases. Abdominal pain was the common manifestation in our study, similar to a study done by Patil, et al., who noted it in 28% of cases.<sup>15</sup> The same study also noted that myalgia, vomiting, and a headache were typical clinical manifestations. Our study has shown that with the onset of monsoon, the number of NS1 positivity has increased. In July, it was 33% and IgM 35% . Though there was a decrease in the Positivity percentage in the following months, the number of samples tested also doubled in August and September. A study conducted in North Gujarat has mentioned patients were admitted during September-December. Similarly, 90% of cases were reported by Patil, et al. in Wardha, Maharashtra.<sup>15</sup>

Table Number 1 and Figure Number 1 depicts the NS1 positivity for the year 2021. Total positivity was 20.18%, with maximum positivity from July to September 2021. From October, the positivity has decreased. But IgM positivity remained high from June onwards till December. Though the total positivity was more than NS1, i.e. 25.56%. It has been noted that IgM may be positive even in Covid positive patients. Hence due to this, the positivity for IgM remains high (Table Number 2 and Figure Number 2). Because NS1 is a highly conserved glycoprotein that seems essential for the viability of the dengue virus but has no established biological activity.<sup>16</sup> It is produced both in membrane-associated and secretory forms by the virus.

Enzyme-linked immunosorbent assays (ELISA) directed against NS1 Antigen (NS1 Ag) have demonstrated its presence at high concentrations (1 to 10 mcg/ml) in the sera of dengue virus-infected patients during the early clinical phase of the disease.<sup>17</sup> Other diagnostic methods currently available include viral culture, viral RNA detection by "Reverse Transcriptase PCR" (RT-PCR) and serological tests such as "Immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay" (MAC-ELISA). The first two methods are expensive, and their routine use in clinical diagnostic facilities is complex in rural settings. MAC-ELISA kits are available for commercial use. However, they cannot be used for early diagnosis because IgM does not become detectable until 5 to 10 days after the onset of illness in the case of primary dengue infection and until 4 to 5 days after the start of illness in secondary infections. Thus it can be stated that along with the supportive clinical symptoms, the detection of NS1 antigen for early diagnosis of dengue cases in the first 5 days of fever. Beyond that, it may be diagnosed by IgM ELISA alone.

Diagnosing rapidly with a sensitive laboratory test is essential to avoid mortality due to Dengue Fever. Within 5 days of infection, NS1 antigen is the earliest marker in the acute stage of infection. Antigen levels as high as 2 to 10 mcg/ml may be found in acute stage sera, while in the convalescent

stage, these may be 0.4 mcg /ml or less.<sup>18</sup> In our study, we had sent the representative for serotyping; hence we could not make a correlation between NS1 positive and DEN serotype. In our area, DEN 2 serotype was predominantly seen. Along with DEN2, DEN 1 & DEN3 were also present in about 200 Serum samples. Which has revealed that patients may get infected with two serotypes. One serotype does not confer immunity against other serotypes. Patients with other risk factors may develop severe diseases, including DHF and Dengue shock syndrome. A study from Mangalore by Damodhar et al. has shown three circulating serotypes during the epidemic.<sup>11</sup> In our study, only one serum sample showed DEN 4 serotype. In many outbreaks, DEN -2 has been reported as the causative serotype causing DHF and DSS, the incidence for which has risen. Infection with more than one serotype was also observed and reported earlier by Afreen et al., Bharaj et al., Tazeen et al. and those patients infected with multiple serotypes are more prone to severe infections.<sup>4,5,6</sup> Two DENV serotypes can be present in one mosquito and the bite of the same can spread co-infection, especially in the endemic areas.<sup>19</sup> Co-infection may also be possible if two mosquitos bite the patient quickly. The chances of dual dengue infections in humans are further enhanced because of the feeding behaviour of *Aedes aegypti*. Research shows that low income groups with no air-conditioning and poor street drainage are most likely to be infected.<sup>20</sup>

## CONCLUSIONS

Rapid growth in population and urbanization, along with climate change, have contributed significantly towards the cases of DF /dengue haemorrhagic fever in India. The main factor in dengue resurgence is unplanned urbanization with an overcrowded population characterized by a lack of basic infrastructures, substandard housing conditions, and water supply and sewage management deficiencies. Rapid urbanization is a major threat as the breeding ground of the vector is rampant in water accumulation methods in the present practices followed. Rapid global migration with modern transport systems creates an extensive urban network, increasing the potential for vector and virus dissemination to new territories. To overcome the resurgence of Dengue Fever, it is necessary to implement a policy regarding controlling the larval form of the vector and education in the community about cleanliness around houses before the beginning of the monsoon. With the onset of clinical symptoms, the laboratory testing for Acute Febrile Illnesses should be increased with a primary focus on Dengue.

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## Authors' Contribution:

Dr Vijayshri Khairkar –Conceiving the idea, gathering necessary approvals and drafting the manuscript.

Dr Deepashri Maraskolhe – Data analysis and drafting the manuscript.

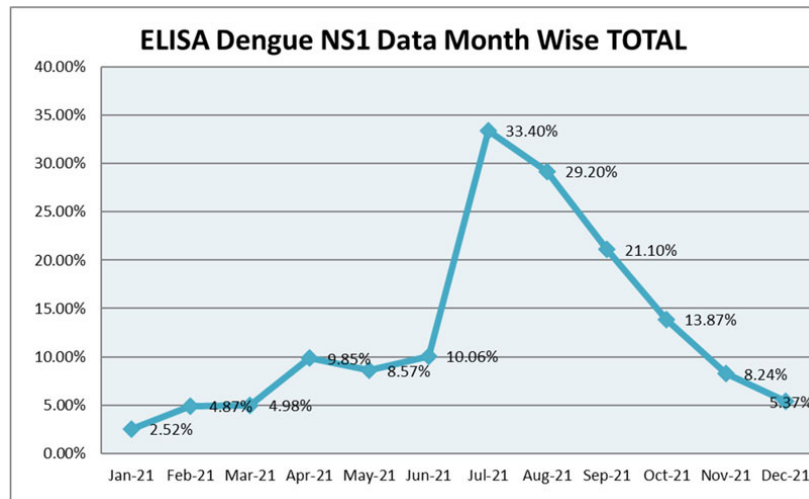
Dr Ruchita Attal – Data analysis and drafting the manuscript.

Hindol Maity – Final compilation, editing and submission of the manuscript.

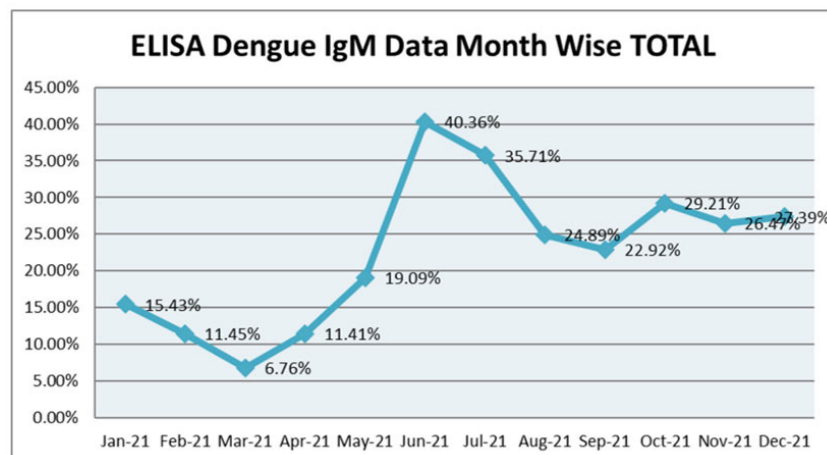
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**Figure 1:** ELISA Dengue NS1 Data month-wise Total.



**Figure 2:** ELISA Dengue IgM Data month-wise Total.

**Table 1: ELISA Dengue NS<sub>1</sub> Data Month Wise**

Month	Total Elisa Dengue NS <sub>1</sub> Sample Tested	Total Elisa Dengue NS <sub>1</sub> Positive Sample	Total Male	Total Female
Jan 2021	476	12(2.52%)	9(75%)	3(25%)
Feb 2021	390	19(4.87%)	8(42.10%)	11(57.89%)
March 2021	341	17(4.98%)	12(70.58%)	5(29.41%)
April 2021	203	20(9.85%)	13(65%)	7(35%)
May 2021	373	32(8.57%)	19(59.37%)	13(40.62%)
June 2021	330	53(10.06%)	31(58.49%)	22(41.50%)
July 2021	1991	665(33.40%)	400(60.15%)	265(39.84%)
Aug 2021	4017	1173(29.20%)	690(58.82%)	483(41.17%)
Sept 2021	4033	851(21.10%)	453(53.23%)	398(46.76%)
Oct 2021	2393	332(13.87%)	186(56.02%)	146(43.97%)
Nov 2021	1249	103(8.24%)	60(58.25%)	43(41.74%)
Dec 2021	595	32(5.37%)	19(59.37%)	13(40.62%)
<b>Total</b>	<b>16391</b>	<b>3309(20.18%)</b>	<b>1900(57.41%)</b>	<b>1409(42.58%)</b>

**Table 2: ELISA Dengue IgM Data Month Wise**

Month	Total Elisa Dengue IgM Sample Tested	Total Elisa Dengue IgM Positive Sample	Total Male	Total Female
Jan 2021	460	71(15.43%)	46(64.78%)	25(35.21%)
Feb 2021	384	44(11.45%)	21(47.72%)	23(52.27%)
March 2021	325	22(6.76%)	13(59.09%)	9(40.90%)
April 2021	184	21(11.41%)	14(66.66%)	7(33.33%)
May 2021	352	67(19.03%)	23(34.32%)	44(65.67%)
June 2021	332	134(40.36%)	58(43.28%)	76(56.71%)
July 2021	1848	660(35.71%)	371(56.21%)	289(43.78%)
Aug 2021	3897	970(24.89%)	581(59.89%)	389(40.10%)
Sept 2021	3970	910(22.92%)	470(51.64%)	440(48.35%)
Oct 2021	2273	664(29.21%)	324(48.79%)	340(51.20%)
Nov 2021	1205	319(26.47%)	153(47.96%)	166(52.03%)
Dec 2021	595	163(27.39%)	71(43.55%)	92(56.44%)
<b>Total</b>	<b>15825</b>	<b>4045(25.56%)</b>	<b>2145(53.02%)</b>	<b>1900(46.97%)</b>