International Journal of Community Medicine and Public Health
Pooja PS et al. Int J Community Med Public Health. 2025 Jun;12(6):2674-2679
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20251711

Original Research Article

Seroprevalence of dengue virus infection among the patients admitted with acute febrile illness in a tertiary care hospital in Central India

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Received: 18 March 2025
Revised: 13 May 2025
Accepted: 21 May 2025

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ABSTRACT

Background: Dengue is the most rapidly spreading mosquito-borne viral disease in tropical and subtropical regions, posing a major public health challenge. Seroprevalence studies are vital for understanding its burden and guiding control strategies.
Methods: This cross-sectional study was conducted in the Department of Microbiology at Government Medical College, Nagpur, from July 2022 to June 2024. A total of 7814 patients admitted with acute febrile illness (AFI) were screened, and 429 clinically suspected dengue cases were selected. Serum samples were tested for Dengue NS1 antigen, IgM, and IgG antibodies using enzyme-linked immunosorbent assay (ELISA).
Results: Out of 429 suspected cases, 109 were confirmed positive for dengue, yielding a seroprevalence of 25.41%. The highest incidence was observed in the 21–30 years age group (23.86%), with females (54.13%) being more affected than males. A majority of cases (74.31%) were from urban areas. Peak incidence occurred during the monsoon months, particularly in September.
Conclusions: The study highlights a significant seroprevalence of dengue in Central India, emphasizing the need for vigilant surveillance, timely diagnosis, and effective vector control measures, especially during the monsoon season.

Keywords: Dengue, IgM dengue antibody, IgG dengue antibody, NS1 antigen, Seroprevalence

INTRODUCTION

We stand on the brink of an era in which millions of people are likely to be suffer from some of the most terrifying and maiming diseases. Some new and previously unknown diseases continue to emerge, which are often labelled as 'emerging' and 're-emerging diseases, respectively. This crisis is a challenge for the public health system in many parts of the world.[1]

Viral haemorrhagic fevers are becoming increasingly common in the tropics and subtropics. Dengue fever is currently the most important arthropod-borne viral disease because of its widespread distribution in more than 100 countries and its potential for extensive outbreaks, which are life-threatening. Two-fifths of the world's population, or 2500 million people, are now at risk for dengue, and every year approximately 50 million new cases occur worldwide.[2]

Dengue is the most rapidly spreading mosquito-borne viral disease in tropical and subtropical regions of the world. The principal vector, *Aedes aegypti*, is a day-biting mosquito of public importance that breeds in natural or artificial waters. Dengue virus belongs to the broad group Arboviruses, family Flaviviridae, subfamily Flavivirinae and genus Flaviviruses. Dengue illnesses are caused by any one of the four serologically related viruses designated as DENV-1, DENV-2, DENV-3 and DENV-4. A full spectrum of disease ranging from a subclinical infection to a mild self-limiting disease, Dengue fever (DF) and a severe disease that may be fatal, the Dengue haemorrhagic fever/Dengue shock syndrome (DHF/DSS) is caused by all four serotypes.[3,4]

Early diagnosis is useful in triaging patients and has a central role in dengue case management, and plays a vital role in forecasting an early warning of an epidemic and in undertaking effective vector control measures.

The current study was carried out with the objective of estimating the seroprevalence and proportion of dengue cases among patients admitted with acute febrile illness (AFI) at a tertiary care hospital in Nagpur. The sociodemographic and seasonal patterns of the dengue cases were also studied.

METHODS

A cross-sectional study was conducted in the Department of Microbiology at Government Medical College, Nagpur, Maharashtra, India, from July 2022 to June 2024. The permission from the Institutional Ethical Committee was obtained. Ethics approval number 1713 dated 02 November 2022. The sample size was calculated by the following.

$$n = \frac{Z^2_{1-\frac{\propto}{2}} \times p(1-p)}{d^2}$$

Where, $1-\frac{\propto}{2}$ = 95% (desired confidence level), p=38.3% (expected prevalence), d=5% (absolute precision), and n=363 (estimated sample size).

Sample size is estimated on the basis of the percentage of seropositivity of suspected Dengue cases before the date in the research article "serological diagnosis of dengue in laboratory practice in Kolkata".[16]

Inclusion criteria

Among 7814 cases admitted with acute febrile illness in various wards and ICUs during the study period, blood samples were collected from 429 patients whose clinical features were suggestive of dengue infection. The clinical basis for suspecting dengue fever was based on standard criteria like presentation of febrile illness of 2–7 days duration with features like headache, myalgia, arthralgia, rash, hemorrhagic manifestations, and leucopenia.[4]

Exclusion criteria

The patients who were not willing to participate in the study were excluded.

Around 5 ml of blood sample was collected aseptically from suspected cases of dengue by venepuncture. The serum was separated by centrifugation and collected in a serum vial. The specimen was properly labelled with the serial number, name of the patient and date of collection.

All 429 samples were tested for the detection of dengue NS1 antigen by DENGUE NS1 Ag MICROLISA and dengue-specific IgM and IgG by capture ELISA. Tests were performed, and calculations were done according to the manufacturer's instructions (Figure 1).

Results obtained were analysed statistically by IBM statistical package for the social sciences (SPSS) statistics 20.0. The Chi-square test and Fisher's exact test were used in calculating the p value. The p values of less than 0.05 were considered statistically significant.

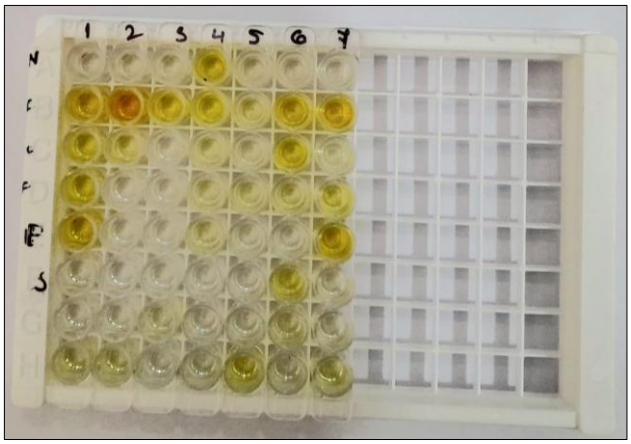

Figure 1: Microtiter plate showing the results of ELISA.

RESULTS

Among 7814 cases admitted with acute febrile illness (AFI) in various wards and ICUs, blood samples were collected from 429 suspected cases of Dengue. Out of 429 samples, 109 were positive for dengue. Thus, the proportion of dengue positive cases among AFI is 1.39%.

The seroprevalence of dengue infection was determined by the number of suspected cases of dengue that tested positive for dengue by one or more of the parameters (NS1 antigen, IgM antibodies and/or IgG antibodies) by ELISA. Seroprevalence in the current study was 25.41%.

The majority of dengue-positive cases are in the age group of 21-30 years. Out of 109 Dengue-positive cases shown by ELISA, 26 (23.86%) were in the age group of 21-30 years. This is followed by a higher incidence in the age group of 31-40 years (22.94%). This means that the age group of 21-40 years has the highest number of positive dengue cases. The least number of cases were reported in the extremes of age (Figure 2).

Gender-wise distribution of Dengue positive cases shows that females were more affected than males. Out of 109 positive cases detected by ELISA, 59 (54.13%) were females, and 50 (45.87%) were males. The ratio of male to female was found to be 1:1.18 (Figure 3).

Table 1 shows that dengue positive cases are found to be more prevalent in urban areas accounting for 74.31%.

While in rural areas, only 25.69% positive cases were detected.

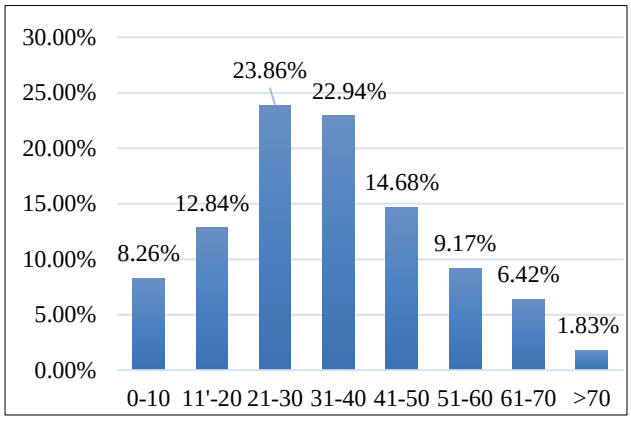

Figure 2: Age wise distribution of dengue positive cases.

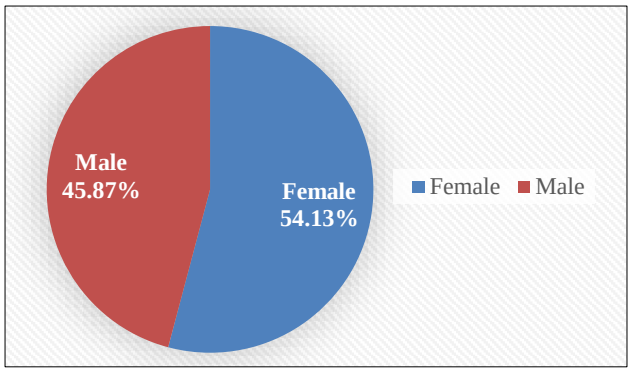

Figure 3: Gender wise distribution of dengue positive cases.

Table 1: Regional distribution of dengue-positive cases (n=109).

Region of residency	Number of dengue positive cases	Percentage (%)
Urban	81	74.31
Rural	28	25.69

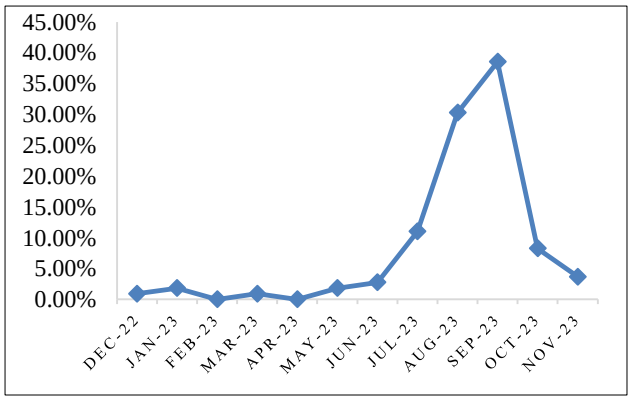

Figure 4: Month-wise distribution of Dengue positive cases.

The maximum number of dengue cases were diagnosed in September (42/109, 38.53%), followed by August (33/109, 30.28%). There is an increase in dengue positivity from July to September, which might be due to the rainy season. During this 3-month period, a total of 170 (39.63%) out of 429 samples were collected from suspected cases of dengue. A total of 87 (79.82%) out of 109 cases were diagnosed during this season. There is a significant correlation between the rainy season and dengue positivity (p value <0.0001) (Figure 4).

DISCUSSION

Dengue is an important vector-borne disease. Dengue infection has been endemic in many parts of India for over two centuries as a benign and self-limited disease. Dengue epidemics are increasing in frequency, and it is becoming hyperendemic in India.[5-7]

In the present study, among 7814 patients admitted with AFI in various wards and ICUs in a tertiary care hospital in Central India, 109 (1.39%) cases tested positive for dengue by ELISA. Only 10 (0.8%) of the 1281 AFI patients in the study by Kigozi et al tested positive for dengue, which is a slightly lower percentage than in the current study.[8] Compared to the present study, Prasad et al found that out of 54578 instances of AFI, 572 (1.05%) tested positive for dengue using virus isolation, NAAT, or serology.[9] The proportion of patients who tested positive for dengue by ELISA was 47 (10.47%) out of 449 hospitalised patients with AFI in a study by Shelke et al, which is significantly higher in comparison to the present study.[10]

In the current study, the seroprevalence of dengue is 25.41%. In a study by Raji et al, it was observed that out of 1194 samples tested, 258 samples (21.6%) gave a positive serological test for acute dengue viral infection, which is comparable to the present study.[11] In a study by Ukey et al, it was found that out of 131 cases, 41 (31.3%) were found to be positive for dengue infection.[12] In the study conducted by Thai et al, in 2005 in Vietnam in primary school children, the seropositivity was 65.7%, which is high in comparison to the present study.[13] In a study by Prakash et al, out of the 4019 suspected patients of dengue, 886 (22%) showed laboratory evidence of dengue virus infection. Of these, 19%, 17% and 27% were dengue positive in 2011, 2012 and 2013, respectively.[14] A total of 917 blood samples were tested for dengue viral infection under a study by Saini et al, of which 281 (30.6%) blood samples showed positive result for one or more parameters (NS1, IgM and/or IgG).[15] In a study by Bhattacharya et al, out of 1892 suspected persons' dengue tested positive in 725 (38.3%) of total cases.[16]

In our study, the age group with maximum number of positive dengue cases were 21–30 years. Out of 109 dengue-positive cases, 26 (23.86%) were in the age group of 21–30 years. This is followed by the age group of 31–40 years (22.94%). In the study by Ukey et al, it was

observed that the most affected age group was 15 to 30 years with 13 (31.71%) cases, followed by the 5 to 9-year-old age group with 8 (19.51%) cases, and 2 to 4 years of age, more than 30 years with 6 (14.63%) cases each.[12] In a study by Nepal et al, it was found that the highest number of dengue cases was observed in the 21–30 age group, in accordance with this study. Among a total of 50 positive cases, 22 (44.0%) belonged to the 21–30 age group. In contrast, there was not a single dengue-positive case in the age group below 10 years.[17] In a study by Patankar et al, out of all those who tested positive for dengue, the adult age group of 18 to 35 years old had the greatest positivity rate (n=534, 58%), followed by the younger age group of 5 to 17 years old (n=219, 24%).[18]

In our study, the gender-wise distribution of dengue-positive cases shows that females were more affected than males. Out of 109 dengue-positive cases, 59 (54.13%) were females and 50 (45.87%) were males. The male-to-female ratio was found to be 1:1.18. In a study by Khan et al in Arunachal Pradesh, female patients comprised 67.2% of the dengue-positive cases.[19] In a study by Sarkar et al, females were more affected (46.5%) than males (45.1%).[20] In a study by Chakaravarti et al, it was found that dengue positivity was higher among females (52.5%) than males (47.5%). The ratio of male to female was found to be 1:1.1, and the findings are comparable to the present study.[21] Khan et al stated that the majority of the vector mosquitoes, *Aedes sp*., are domestic and peridomestic in nature, meaning that housewives and other females are more likely to be bitten by them.[19] In contrast to the above findings, a study by Raji et al observed that among the positive cases of dengue, 57.36% were males and 46.64% of them were females. The ratio of male to female was found to be 1.3:1.[11] Similarly, in a study by Ukey et al, it was observed that among males, about 28 (68.3%) study participants were positive cases of dengue, while among females, 13 (31.7%) cases were positive. The ratio of male to female was found to be 2.15:1.[12] In a study by Nepal et al, it was 149 found that 62% of positive cases were males (31/50) and 38% were females (19/50), with a male-to-female ratio of 1.6:1.[17]

In the current study, out of 109 dengue ELISA-positive cases, 81 (74.31%) belong to urban areas and 28 (25.69%) to rural areas. In the Nepal et al study, 39 of 277 suspected cases from an urban location were determined to be dengue-positive (14.1%). In contrast, only 11 of 313 suspected cases from a rural setting tested positive (3.5%).[17] In a study conducted by Mistry et al in the Saurashtra region, India, during the year 2015, it was found that almost two-thirds (65.7%) of dengue-positive cases were residing in urban areas of the Saurashtra region. This finding is comparable to that of the current study.[22] The urban population (75.05%) was more affected than the rural population (24.95%) in a study conducted by Dinkar et al from 2012 to 2017.[23] In a study conducted by Sami et al in Bangladesh in 2022, a total of 79.9% of patients were residents of metropolitan areas, while the remaining 20.1% were from either semi-urban or rural areas.[24] In a study by Chew et al, the rate of seroprevalence in urban locations ranged between 61% and 92% and in rural locations, it ranged between 28 and 91%.[25] According to Khan et al, solid garbage, air conditioners, air coolers, flower pots, and other similar objects are key factors in the growth of *A. aegypti* mosquitoes, which are the primary vectors of dengue.[19] According to Chakravarti et al, dengue was once thought to be an urban illness. Midway through the 20th century, developing nations saw rapid urban growth, which led to an increase in the number of unstable housing units, inadequate water supplies, and inadequate wastewater treatment facilities. The vector and the virus were able to proliferate and disseminate, attributable to these favourable circumstances. The spread of dengue from urban to rural regions is believed to be associated with socio-economic and human ecological changes like increased mobility and contact with transportation, as well as the growth of peri-urbanisation. *Aegypti* mosquitoes invaded rural regions as a result of these changes. Improved reporting may also play a role. In India, a condition that was formerly primarily urban is now a major health issue.[21]

In our study, the maximum number of specimens was received in September (74/429, 17.25%), followed by August (57/429, 13.29%). The maximum cases diagnosed were also in September (42/109, 38.53%), followed by August (33/109, 30.28%). In a study by Raji et al, it was observed maximum number of specimens received was during September, but seropositive cases were more in July, (25.88% for NSI antigen and 15.85% for IgM antibody).[11] In a study by Ukey et al, it was found that in 2005, the highest number of suspected dengue patients admitted was in November, which was 41 patients with 15 (36.59%) positive followed by October which was 28 patients with 7 (25%) positive. However, in 2006, the highest number of dengue patients admitted was in September, i.e., 24 with 14 (58.33%) positive, followed by November, i.e., 5 with 2 (40%) positive.[12] In a study by Mistry et al, cases of dengue were reported throughout the year from Rajkot and other districts of the Saurashtra region. Reporting of dengue-positive cases remained low during the first 6 months of 2015, followed by a significant increase from July to September and again during December. An increasing number of cases was reported from July 2015 and reached a peak during September 2015, followed by a decrease in cases until December 2015.[22] In a laboratory based study on dengue fever surveillance conducted by Victor et al in 2007, the data on month-wise incidence of dengue in Tamil Nadu for the past nine years revealed that the number of cases increased from June to December, confirming that the active transmission period is during monsoon and post-monsoon periods every year.[26] The data on month-wise dengue cases in a metropolitan city of Maharashtra for the 10 years from January 2009 to December 2018 were reported in Pol et al. It showed an increasing trend of dengue cases from June to November and a drop in December. The highest number of dengue cases was in September, and the lowest was in March.[27] In the current study, the highest number of

dengue cases was reported in the monsoon and post-monsoon periods. Patil et al stated that during the monsoon period, heavy rains lead to the stagnation of large amounts of water. These are the favourite breeding places for vectors.[28] This indicates that to lower the occurrence of dengue cases during this period, suitable vector control measures must be put in place.[26]

Newer techniques like multiplex PCR are available for serotyping of dengue virus, which will shed light on the prevalent circulating serotype of the region. This was not carried out in the current study, which is a limitation. Moreover, cross-reactivity with other flaviviruses is also not ruled out.

CONCLUSION

Dengue infections occur yearly, and Central India is also experiencing an epidemic of the disease. Dengue seroprevalence studies play a crucial role in understanding the epidemiology of dengue virus infections in different populations. It helps to assess the true burden of the disease. By identifying high-transmission areas, seroprevalence data can help prioritise mosquito control programs and other interventions. It also helps to assess the effectiveness of the existing control measures. It is ideal to test all suspected cases for all three parameters, i.e. NS1 Ag, IgM and IgG antibodies, so that we will not miss any cases. Dengue morbidity and mortality can be decreased with appropriate investigation, vigilant monitoring, and quick supportive therapy.

ACKNOWLEDGEMENTS

Authors would like to thank the Department of Microbiology, GMC Nagpur, for providing the necessary resources and facilities to carry out this research. They would also like to thank colleagues and friends for their constant support and helpful discussions.

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: Pooja PS, Kokate SB, Shrikhande S. Seroprevalence of dengue virus infection among the patients admitted with acute febrile illness in a tertiary care hospital in Central India. Int J Community Med Public Health 2025;12:2674-9.