

**Serological Evaluation of Dengue Infection Using IgM ELISA in Febrile Patients Attending in Patna Medical College & Hospital, Patna****Khushboo Kumari<sup>1</sup>, Sushma Kumari<sup>2</sup>, Vijay Kumar<sup>3</sup>, Pratulya Nandan<sup>4</sup>**<sup>1</sup>Tutor, Department of Microbiology, Patna medical College and Hospital, Patna, Bihar, India<sup>2</sup>Tutor, Department of Microbiology, Patna medical College and Hospital, Patna, Bihar, India<sup>3</sup>Professor and HOD, Department of Microbiology, Patna medical College and Hospital, Patna, Bihar, India<sup>4</sup>Professor, Department of Microbiology, Patna medical College and Hospital, Patna, Bihar, India

Received: 05-11-2025 / Revised: 24-11-2025 / Accepted: 28-12-2025

Corresponding Author: Dr. Sushma Kumari

Conflict of interest: Nil

**Abstract:**

**Background:** Dengue, a viral disease that is transmitted by mosquitoes, is caused by the dengue virus (DENV) and is considered a major public health problem in the tropical and subtropical areas. Its spread has been accelerated by rapid urbanization, growing populations, global warming, and, in addition, inadequate vector control; hence, the need for early detection and sero epidemiological surveillance as a part of the management process has become vital.

**Objective:** The objective of the research was to ascertain the seroprevalence of dengue infections in febrile patients visiting a higher-level medical facility in Patna, Bihar, and to elaborate on the related clinical and epidemiological traits.

**Methods:** A prospective observational study has been performed on 320 patients who were suspected of having dengue clinically. Blood samples were drawn from veins and tested for Dengue NS1 antigen, IgM, and IgG antibodies through ELISA method. Demographic, clinical, and laboratory data were collected using a previously designed proforma.

**Results:** Most patients belonged to the young adult group of 21–30 years (28.1%) with a mild male predominance (56.3%). People living in the cities made up 65.6% of the total cases. The symptoms that occurred most often were fever (100%), headache (78.1%), and muscle pain (68.8%). The results of serological testing showed that out of the total samples, 37.5% showed NS1 antigen, 34.4% showed IgM antibodies, and 25% showed IgG antibodies, suggesting mainly acute primary dengue infection.

**Conclusion:** Infection with dengue mainly concerns young adults living in the city, and the typical symptoms of fever are observed in them. Early diagnosis by means of NS1 and IgM ELISA assures prompt management. Continuous monitoring, controlling the vectors, and health interventions are major factors in cutting down the number of cases and deaths due to dengue in regions where it is commonly found.

**Keywords:** Dengue, ELISA, Febrile illness, IgM antibodies, NS1 antigen, Seroprevalence, Tertiary care hospital.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

**Introduction**

Particularly in tropical and subtropical areas of the world, dengue is regarded as the most dangerous virus spread by mosquitoes to people [1]. It is a disease brought on by the dengue virus, a single-stranded RNA virus that is a member of the Flavivirus and mainly spread through *Aedes aegypti* mosquitoes [2]. Over the last few decades, dengue has been recognized as a huge public health problem mainly because of the aforementioned factors such as rapid urban growth, population increase, changes in the environment, difficulties in vector control, and greater human mobility [3]. According to the WHO estimates, there are millions of cases of dengue infection that occur every year, and a large part of

the infected people has symptoms that need medical attention [4].

The clinical aspects of dengue infection show a large variety of signs and symptoms which would be in the simplest case, febrile illness that cannot be differentiated to in the most severe cases such as dengue shock syndrome and dengue hemorrhagic fever [4]. The primary signs of dengue are high-grade fever, headache, pain behind the eyes, muscle and joint pain, skin rash, and occasionally bleeding. Because these signs and symptoms can be confused with those of other diseases causing acute febrile illness like malaria, chikungunya, typhoid, and viral fevers, it is very important to confirm the diagnosis

by laboratory tests to treat and manage the patient properly [6].

Serological testing is a very important factor for the diagnosis of dengue, especially in areas where resources are limited. Among the different methods for diagnosis, the detection of dengue-specific (IgM) antibodies through (ELISA) is most popular because of their sensitivity, specificity, price, and simplicity of execution [7]. Dengue-specific IgM antibodies are usually detectable in the blood of patients within the first 3-5 days after onset and their levels gradually decrease over the next few weeks which makes the IgM ELISA a reliable diagnostic tool for the identification of recent infections and for blood serum studies [8].

The measurement of dengue seroprevalence in febrile patients gives an informative view of the disease burden, seasonal patterns, and transmission dynamics in a specific area. It is the tertiary care hospitals that attend large and varied patients, and hence they are the key centers for monitoring dengue activity. The information produced from these types of investigations can be used for early detection, preventive strategies that are formulated, surveillance systems that are strengthened, and healthcare resources that are allocated [9].

The current study is designed to measure the seroprevalence of dengue infection through IgM ELISA among febrile patients visiting a tertiary care hospital. Knowing the extent of dengue infection in this environment will assist in providing improved clinical awareness, quicker diagnosis, and public health interventions that are more effective in preventing dengue-related sickness and death.

## Materials and Methods

**Study Design:** A future observational study was conducted to evaluate the seroprevalence of dengue infection amongst the febrile using ELISA.

**Study Area:** The research was conducted at the Department of Microbiology, Patna Medical College and Hospital (PMCH), Patna, Bihar, India for six months from May 2025 to October 2025

**Study Population:** The population of the study consisted of dengue fever and dengue hemorrhagic fever cases that were clinically suspected and visited different outpatient departments (OPDs) as well as inpatient departments (IPDs) of PMCH during the study period. The patients were included without consideration of their age, sex, occupation, religion, or economic status.

**Sample Size:** The research took into account all consecutive and non-repetitive serum samples coming from patients with a clinically suspected diagnosis of dengue during the research period of six months. In total, 320 serum samples were collected for analysis.

## Selection Criteria

### Inclusion Criteria

- Patients presenting with acute febrile illness clinically suspected of dengue infection
- Patients referred to for dengue serological testing
- Patients willing to give informed consent

### Exclusion Criteria

- Patients with confirmed alternative diagnosis
- Repeated samples from the same patient
- Inadequate, hemolyzed, or contaminated samples

**Data Collection:** During the research period, patients with clinically suspected dengue attending the outpatient departments (OPDs) and inpatient departments (IPDs) of Patna Medical College and Hospital were all prospectively included in the study. Carefully designed and previously tested forms were used to gather and document patient-related data such as demographic information (age and gender), clinical signs (duration of fever and associated symptoms like headaches, muscle pain, pain behind the eyes, rashes, and bleeding), and hospital admission status (as in OPD or IPD).

Laboratory details that were relevant were also recorded in an orderly manner; these included the date when the sample was taken, the type of test that was performed (NS1 antigen, IgM, and IgG antibodies), and the test results. To prevent duplication and to ensure that only non-repetitive samples were included, a unique identification number was assigned to each patient. Throughout the study, it was ensured that patient information was kept confidential.

**Study Procedure:** Blood (3-5 mL) from veins was collected under sterile conditions and universal safety guidelines from 320 patients with dengue symptoms. Blood samples were put on a rack at room temperature for about 30 minutes to allow clotting to take place. Next, the clot was broken and at the same time serum was separated by centrifugation at 3000 rpm for 5 minutes. The serum was then carefully moved into sterilized and correctly labeled microtubes, where it was either tested right away or kept under good conditions until analysis. The study only included serum samples that were non-repetitive.

**Laboratory Testing:** Dengue NS1 antigen, IgM antibody, and IgG antibody were detected in the serum samples with the help of enzyme-linked immunosorbent assay (ELISA) kits. Dengue NS1 antigen was detected by the Dengue NS1 Ag Microlisa method, and Dengue IgM and IgG antibodies were detected with the help of Dengue IgM and IgG Microlisa "J. Mitra & Co. Pvt. Ltd., India". Each assay was conducted in strict adherence

to the manufacturer's guidelines, and the tests included proper positive and negative controls for monitoring quality throughout the testing process.

**Interpretation of Test Results:** The assay results were interpreted in accordance with the kit instructions. The cut-off point was determined by taking the average optical density (OD) of the calibrator and multiplying it by the calibration factor that came with the kit.

- The sample OD ratio was calculated as:

**Sample OD Ratio = Sample OD / Cut-off Value**

- Interpretation criteria:
  - **OD ratio < 9:** Negative for Dengue NS1 / IgM / IgG
  - **OD ratio 9–11: Equivocal**
  - **OD ratio ≥ 11: Positive** for Dengue NS1 / IgM / IgG

**Statistical Analysis:** The data collected from 320 patients suspected of having dengue fever were recorded into Microsoft Excel and then assessed through SPSS software (version [insert version]). The analysis process started with the calculation of descriptive statistics, such as frequency, percentage, and proportion, for the different demographic characteristics, clinical symptoms, and the serological test results (i.e., NS1, IgM, IgG). The Chi-square test was used to compare the groups (e.g., age groups, sex, and residence), and the cut-off value for statistical significance was set at  $p < 0.05$ . To visually represent the distributions and trends in

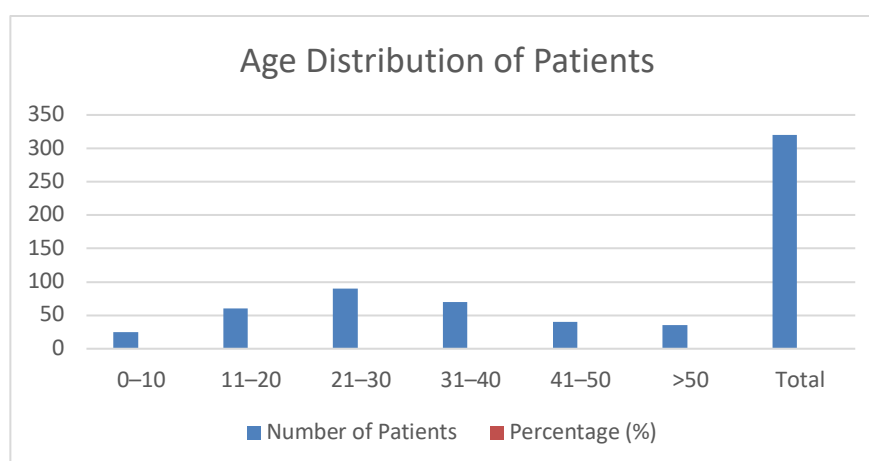
positivity, bar graphs, pie graphs, and stacked column graphs were used.

## Result

The present study included a total of 320 clinically suspected dengue cases which were analyzed for NS1 antigen, IgM, and IgG antibodies through the methods of ELISA. The study of population's demographic characteristics, clinical manifestations, and serological profiles were systematically evaluated to get an insight into the pattern of dengue infection. The results were analyzed concerning age, sex, and area of residence, including the association of common clinical symptoms with serological positivity. The findings are provided in tables and graphical representations to accurately illustrate the distribution and trends of dengue positivity among various patient subgroups.

A comprehensive evaluation of the study sample indicated that dengue seropositivity varied according to demographic and clinical categories. The pattern of NS1 antigen and dengue-specific antibodies distribution pointed to a major role of acute infections, especially in young adults and city dwellers. Clinical symptom-wise analysis showed that fever, headache, and myalgia were strongly associated with dengue positivity, while complications like rash and bleeding were seen only in a small percentage of cases. These results uncover the epidemiological and clinical characteristics of dengue infection among febrile patients visiting a tertiary care hospital.

Age Group (years)	Number of Patients	Percentage (%)
0–10	25	7.80%
11–20	60	18.80%
21–30	90	28.10%
31–40	70	21.90%
41–50	40	12.50%
>50	35	10.90%
Total	320	100%

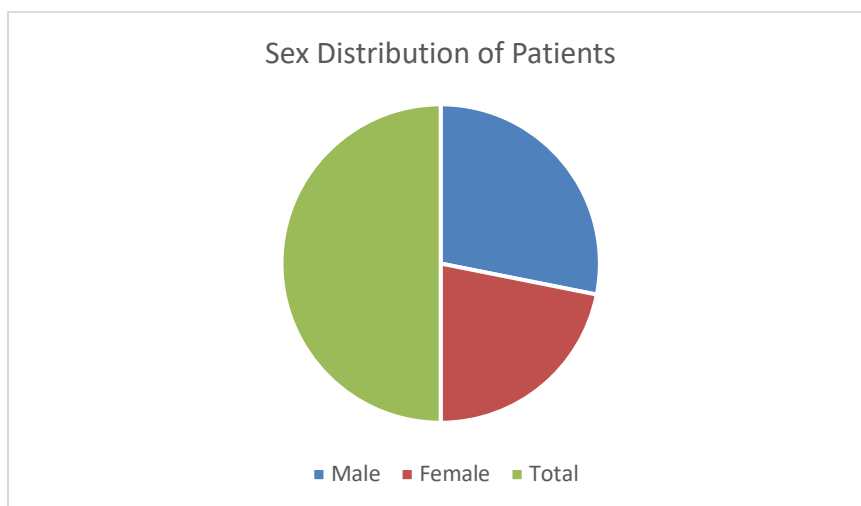


**Figure 1: Showed that Age Distribution of Patients**

As per Table 1, most patients belonged to the age group of 21–30 years (28.1%), followed by the age group of 31–40 years (21.9%). The age group of 11–20 years was responsible for 18.8% of cases, while the age group of 0–10 years had only 7.8% of the total population involved in this study. The age

group over 50 years made up 10.9%, while the age group 41–50 years constituted 12.5%. Thus, it can be concluded that the hospital-based population was largely composed of young and middle-aged adults mainly affected by dengue infection.

Sex	Number of Patients	Percentage (%)
Male	180	56.30%
Female	140	43.70%
Total	320	100%

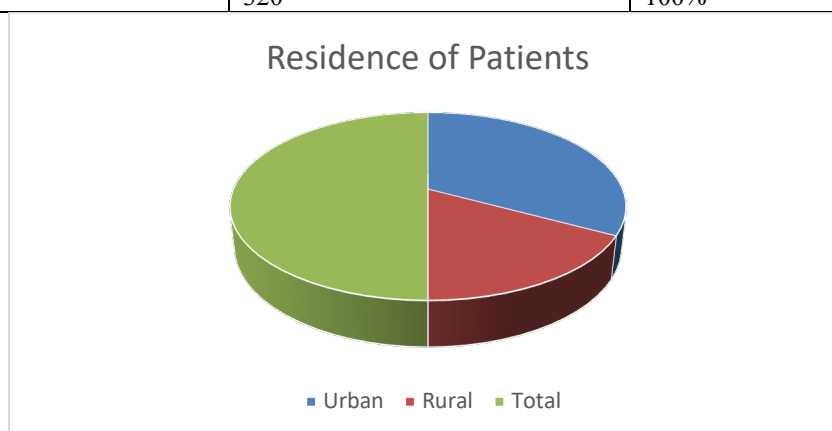


**Figure 2: Showed that Sex Distribution of Patients (n = 320)**

If we look at Table 2, the majority of the patients were men (56.3%) and only a minority were women (43.7%). This indicates that there was a small male dominance in the dengue cases of this research. The

difference might mirror the exposure patterns, the occupational activities, or the healthcare-seeking behavior among males versus females.

Residence	Number of Patients	Percentage (%)
Urban	210	65.60%
Rural	110	34.40%
Total	320	100%



**Figure 3: Showed that Residence of Patients (n = 320)**

Table 3 discloses that the majority of patients (65.6%) were from urban areas and only 34.4% from rural areas. This suggests that urban areas were the

main sites of dengue infections which could be related to the factors like dense population,

inadequate sanitation, and ample Aedes mosquitoes' breeding sites in cities.

**Table 4: Dengue ELISA Positivity by Age Group (n = 320)**

Age Group (years)	NS1 Positive	IgM Positive	IgG Positive	Total Patients
0–10	10	8	5	25
11–20	25	20	15	60
21–30	45	40	25	90
31–40	25	25	20	70
41–50	10	10	10	40
>50	5	7	5	35
Total	120	110	80	320

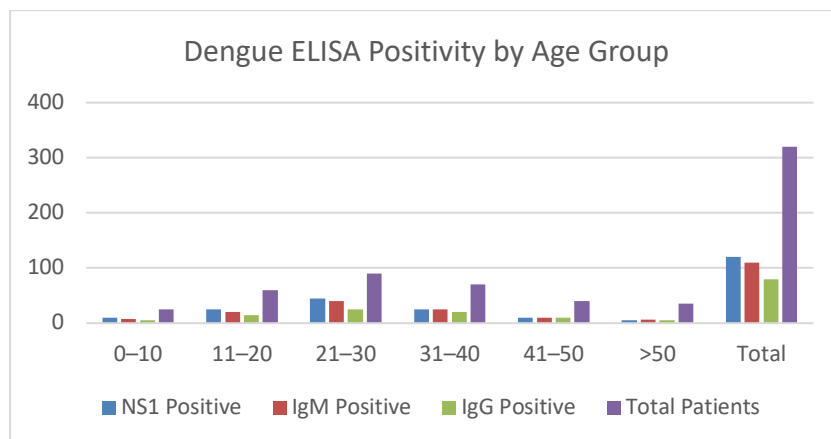


Figure 4: Showed that Dengue ELISA Positivity by Age Group (n = 320)

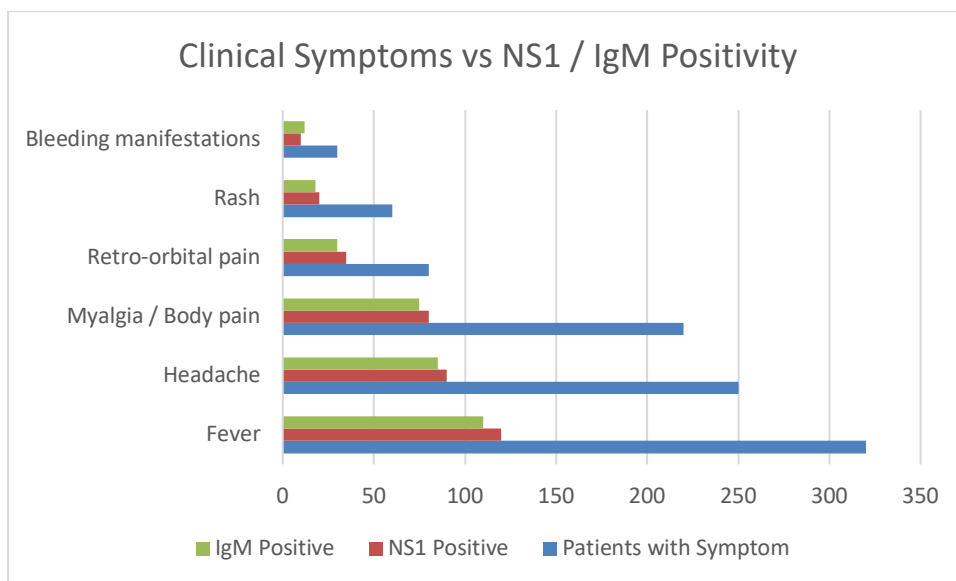
As outlined in the table, the 21–30 years age group had the highest NS1 positivity (45 cases) and IgM positivity (40 cases), which means that young adults were the most affected age group for acute dengue infection. Also, IgG positivity, reflecting past exposure or secondary infection, was the highest in this age group (25 cases). The 31–40 years age group had a moderate level of positivity for NS1 (25 cases), IgM (25 cases), and IgG (20 cases). In the 0–10 years age group, NS1, IgM, and IgG positivity were concerning low (10, 8, and 5 cases

respectively), indicating that there was a lower incidence of dengue in this age group.

The 41–50 age group and those over 50 years of age showed the lowest positivity rates across all three trials. In the end, NS1 antigen positivity (120/320, 37.5%) was marginally higher than IgM positivity (110/320, 34.4%), while IgG positivity (80/320, 25%) was the lowest, showing that a major part of the patients was facing the primary or acute stage of dengue infection.

**Table 5: Clinical Symptoms vs NS1 / IgM Positivity**

Symptom	Patients with Symptom	NS1 Positive	IgM Positive
Fever	320	120	110
Headache	250	90	85
Myalgia / Body pain	220	80	75
Retro-orbital pain	80	35	30
Rash	60	20	18
Bleeding manifestations	30	10	12



**Table 5: Clinical Symptoms vs NS1 / IgM Positivity**

As indicated by the table, every patient (100%) had fever, and among these, NS1 positivity was noted in 120 cases and IgM positivity in 110 cases, thus making fever the most prominent symptom in dengue infection. Next in line were headaches (78.1%) and myalgia/body pain (68.8%), which were still quite strong NS1 and IgM positive symptoms. Symptoms like retro-orbital pain (25%), rash (18.8%), and bleeding manifestations (9.4%) which were seen less often, also had lower NS1 and IgM positive cases respectively. This means that although fever, headache, and myalgia are the typical signs of dengue at an early stage, more particular ones like rash and bleeding are seen less often, probably because of differences in the severity and progression of the disease.

**Discussion:**

Dengue fever (DF) continues to be a major issue for public health, especially in the case of tropical and subtropical areas like India. The study conducted included the evaluation of 320 clinically suspected dengue cases through NS1 antigen, IgM and IgG antibodies production using ELISA to find out the seroprevalence and the clinical profile of dengue infection at the tertiary care hospital.

When we looked at age-wise distribution, we find that most cases are in the 21-30 years range, followed by 31-40 years. NS1 and IgM positivity were also in the highest ranks of the young adults, reflecting that the age group was facing the acute dengue infection the most. This observation is in line with various hospital-based studies done in India and Southeast Asia, where Islam et al. (2012) reported that the young adult population is the one most affected by the disease, probably because of their daily exposure outdoors, the nature of their jobs, and the fact that they are more mobile [10]. Glynn et al., (2020) stated that in the case of

children (0-10 years) and older adults (>50 years), the seropositivity was found to be lower, which points to these groups either having less exposure to the virus or being infected with milder strains [11].

The study findings on sex distribution showed a small predominance of males (56.3%). Previous research has reported similar trends that might indicate the same reasons for males being more affected than females such as gender-related issues in exposure, outdoor activities, and medical treatment-seeking behavior. However, the contrast was not significant, which means that dengue infection is equally prevalent among both genders [12].

According to their place of residence, urban areas account for the majority of patients (65.6%), which reinforces the idea of dengue transmission being an urban-centric phenomenon. The spread of dengue is facilitated more in urban areas that have high population density, poor sanitation, and lots of Aedes mosquito breeding sites [13]. There were fewer cases in the countryside, but they were still significant, indicating that the geographical distribution of dengue is expanding (WHO) [14].

The assessment of clinical symptoms indicated that the fever was the first symptom detected in all patients followed by headache, myalgia, and retro-orbital pain. Anand et al., (2012) indicated that rash and bleeding were comparatively minor issues. The NS1 and IgM seroconversions were most in the patients having fever and headache suggesting that these symptoms are the most dependable early markers of dengue virus infection [15], on the other hand, Chaudry et al., (1993) indicated that the bleeding might be a sign of the more severe or secondary infection [16]. Jones et al., (2019) reported that their observation was in line with the earlier reports which stated that classical dengue is

mainly characterized by fever, myalgia, and headache while bleeding and rashes are less obvious and variable [17].

The cumulative seroprevalence reported in this research was 37.5% for NS1, 34.4% for IgM, and 25% for IgG that indicated a large part of the patients were undergoing acute primary dengue infection. The detection of the NS1 antigen was just slightly above the IgM positivity which was a clear indication of the early phase of infection among hospitalized patients. The IgG positivity was low which pointed to the fact that secondary or past infections in this cohort were limited. A similar observation was made by Shukla et al., (2017) who indicated that NS1 and IgM ELISA are effective for early diagnosis and sero-surveillance in hospital settings through their studies [18].

Altogether, the research finds that dengue mainly impacts young cities' inhabitants, accompanying the typical febrile symptoms. NS1 antigen and IgM ELISA early detection are of paramount importance for quick treatment and, consequently, the prevention of heavy disease. Besides, public health measures such as vector control, awareness campaigns, and early diagnostic facilities should be prioritized to reduce the impact of dengue in the areas where it is endemic.

As a result, the ongoing research presents significant enlightenment concerning the dengue infection's epidemiological and serological profile at a tertiary care hospital in the Indian state of Bihar. The results highlight the importance of constant monitoring, rapid detection, and specific preventive actions, especially in the case of urban populations and the young adults who are most affected. Additionally, it is suggested that further extensive community-based research should be conducted to investigate risk factors, seasonal variations, and the magnitude of secondary dengue infections in the area.

### Conclusion

The current research work was done to establish the dengue virus outbreaks pattern in Bihar, by evaluating 320 cases, with dengue considered as the possible cause, admitted to a tertiary care hospital. NS1 antigen, IgM, and IgG ELISA techniques were used to determine the presence of the virus. The results demonstrated that the majority of the infected people were young adults (21–30 years age group). There was an inclination towards males, and the disease was more often seen in cities. Fever, headache, and myalgia were the main clinical symptoms, while rash and bleeding occurred less frequently. The serological examination showed NS1 positivity (37.5%), IgM positivity (34.4%), and IgG positivity (25%), indicating that majority of the patients were suffering from acute primary dengue infection. The study points out that NS1 and IgM ELISA have a key role in the early diagnosis that

leads to early clinical management and also reduces the risk of complications.

In general, the entire piece of research insists on the need of constant monitoring, creating health awareness among the public and taking actions against vector control particularly in the cities and the major young adult risk groups. The earlier the detection and the quicker the intervention, the more control strategies remain to struggle against the prevalence of dengue infection in the places where they are endemic.

### References

1. Mairuhu AT, Wagenaar J, Brandjes DP, Van Gorp EC. Dengue: an arthropod-borne disease of global importance. *European journal of clinical microbiology and infectious diseases*. 2004 Jun;23(6):425-33.
2. Das B, Ghosal S, Mohanty S. Aedes: What Do We Know about Them and What Can They Transmit? In *Vectors and vector-borne zoonotic diseases 2018* Nov 5. IntechOpen.
3. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Tropical medicine and health*. 2011;39(4SUPPLEMENT):S3-11.
4. World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. *Epidemic, Pandemic Alert. Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization; 2009.
5. Ranjit S, Kissoon N. Dengue hemorrhagic fever and shock syndromes. *Pediatric critical care medicine*. 2011 Jan 1;12(1):90-100.
6. Bothra A, Maheswari A, Singh M, Pawar M, Jodhani K. Cutaneous manifestations of viral outbreaks. *Australasian Journal of Dermatology*. 2021 Feb;62(1):27-36.
7. Baje ZE, Lawal N, Bello MB, Imam MU. Dengue fever diagnosis in resource-limited settings. *Epidemiology & Infection*. 2025 Jan;153: e105.
8. Mamatha V. Usefulness of Dengue-NS1 Antigen as an Early Marker of Dengue Virus Infection in a Tertiary Care Hospital (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
9. Mohan K, Srinivasan S, Selvaraj S, Suresh A, Krishnamoorthy A. A study on Dengue infection, seroprevalence and its seasonal distribution among patients attending a Tertiary Care Hospital, Kanchipuram, India. *Journal of Applied Pharmaceutical Science*. 2025 Jan 5;15(2):200-4.
10. Islam MT. A Comparative Study On Epidemiological Mapping for Dengue Cases In

- Singapore And Bangladesh (Doctoral dissertation, © University of Dhaka).
11. Glynn JR, Moss PA. Systematic analysis of infectious disease outcomes by age shows lowest severity in school-age children. *Scientific data*. 2020 Oct 15;7(1):329.
  12. World Health Organization. Addressing sex and gender in epidemic-prone infectious diseases.
  13. Kolimenakis A, Heinz S, Wilson ML, Winkler V, Yakob L, Michaelakis A, Papachristos D, Richardson C, Horstick O. The role of urbanisation in the spread of *Aedes* mosquitoes and the diseases they transmit—A systematic review. *PLoS neglected tropical diseases*. 2021 Sep 9;15(9):e0009631.
  14. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*. 2013 Aug 20:299-309.
  15. Anand A. Study on parameters of severity in Dengue infection with both NS1 Antigen and Dengue IgM reactive on the same day, in a tertiary care hospital.
  16. Chaudry IH, Ayala A. Mechanism of increased susceptibility to infection following hemorrhage. *The American journal of surgery*. 1993 Feb 1;165(2):59S-67S.
  17. Jones ZA, Thomas SJ. Yellow Fever and Dengue: Fever, Hepatitis, and Jaundice in a Returning TravelerFever, Retro-Orbital Headache, Generalized Myalgias, Arthralgias and Bone Pain in a Returning Traveler. In *Introduction to Clinical Infectious Diseases: A Problem-Based Approach 2019* Feb 15 (pp. 375-383). Cham: Springer International Publishing.
  18. Shukla MK, Singh N, Sharma RK, Barde PV. Utility of dengue NS1 antigen rapid diagnostic test for use in difficult to reach areas and its comparison with dengue NS1 ELISA and qRT-PCR. *Journal of medical virology*. 2017 Jul;89(7):1146-50.