



## SEROPREVALENCE OF DENGUE INFECTION IN A TERTIARY CARE HOSPITAL OF SOUTH GUJARAT, INDIA

|                                |                                                                                                                                                          |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dr. Rajeev Kumar</b>        | M.D, Associate Professor, Department of Microbiology, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat.                       |
| <b>Dr. Ashish Surana*</b>      | M.D, Associate Professor, Department of Microbiology, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat. *Corresponding Author |
| <b>Dr. Tanavi H. Chaudhari</b> | M.D, Ex Senior Resident, Department of Microbiology, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat.                        |
| <b>Dr. Mannu R Jain</b>        | M.D, Professor & Head, Department of Microbiology, Surat Municipal Institute of Medical Education and Research (SMIMER), Surat.                          |

**ABSTRACT** **Introduction:** The epidemiology of dengue fever is complex as all the four serotypes are circulating in the Indian subcontinent and serology remains the mainstay in the diagnosis of dengue infection. **Aim:** This study was conducted to observe the seroprevalence, demographic profile and seasonal variation of dengue infection in South Gujarat, India. **Materials And Methods:** A cross sectional study was conducted in south Gujarat using data of clinically suspected dengue patients attending a tertiary care hospital over a four-year period. A total of consecutive 14,492 blood samples obtained from these patients were tested for anti-dengue virus (DV) IgM antibodies and DV-Non Structural Protein 1 Antigen (NS1Ag) by ELISA. Seroprevalence was estimated by analyzing data for the results of both NS1 antigen and IgM antibody along with its relationship with the demographic profile and seasonal variation. **Results:** Out of total 14,492 samples, laboratory evidence of dengue infection was seen in 4,782 (32.99 %) ~1823 were positive for NS1 antigen, 2212 were positive for IgM antibody while 515 were positive for both NS1Ag and IgM antibody. Predominantly affected age group was 21-30 years (39.46%) followed by 11-20 years (36.16%) and the seroprevalence was found to be more among males (53.94 %) as compared to females (46.06 %) across all age groups. There was a significant yearly increase in the number of clinically suspected dengue cases, but a decreasing trend in the overall seropositivity rate was observed with highest prevalence seen during monsoon and the post monsoon season. **Conclusion:** Dengue still remains a major public healthcare concern although a slightly decreasing trends in its seroprevalence was observed in our study. A shift of seropositivity from paediatric to the adult population with slightly more involvement of males along with seasonal variation were also observed. Further, combination of detecting both anti-dengue virus IgM antibodies and DV NS1Ag remains the cornerstone in dengue diagnosis.

**KEYWORDS :** NS1 antigen, Dengue IgM antibody, Dengue Seroprevalence, South Gujarat

### Introduction

Dengue is the most rapidly spreading mosquito borne viral infection in the world<sup>1</sup>. The word "dengue" is derived from the Swahili phrase "*Ka-dinga pepo*", meaning "cramp-like seizure" caused by evil spirit<sup>2</sup>.

Dengue, now endemic in more than 100 countries, affecting around 2.5 billion people has emerged as a major global public health challenge in urban & semi-urban areas of tropical and sub-tropical regions<sup>3</sup>.

Since the record of India's first dengue like illness in Madras in 1780<sup>4</sup>, the disease has been changing incessantly in terms of strains, geographic locations, severity, prevalence etc. and has now become endemic in India affecting all the states and union territories. Every year around one lakh cases are reported with the highest prevalence being seen in the states of Karnataka, Kerala & Maharashtra<sup>5</sup>.

Dengue virus is a small positive-stranded encapsulated RNA virus, belonging to the genus *Flavivirus*, family *Flaviviridae*. There are four serotypes (DEN-1, DEN-2, DEN-3 & DEN-4) of dengue virus, each having multiple subtypes/genotypes. These serotypes are primarily transmitted from person to person through the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito. Each serotype is capable of causing full spectrum of dengue infection ranging from a subclinical infection to Dengue Fever (DF) to life-threatening Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS)<sup>6,7</sup>. In view of these broad range of clinical symptoms elicited by dengue virus, early and accurate laboratory diagnosis becomes imperative. Biomarkers playing pivotal role in laboratory diagnosis of dengue infection includes culture & isolation of dengue virus, detection of its genomic RNA, testing for non-structural (NS1) protein or measurement of dengue-specific immunoglobulin M [IgM] and immunoglobulin G [IgG]. Thus it can be concluded that dengue is associated with complex pathophysiological, economic and ecologic tribulations<sup>8</sup>.

Among the various laboratory modalities, isolation of dengue virus has remained as the standard traditional method for its diagnosis. However, in view of the limitations associated with availability of viral culture facilities & expertise<sup>9</sup> and simultaneous development of more

sensitive and easy to use laboratory methods, the culture has been superseded by newer diagnostics tests. Among these are reverse-transcription polymerase chain reaction (RT-PCR) and serological tests based upon detection of anti-dengue virus IgM/IgG and dengue NS1Ag. Accordingly, World Health Organization (WHO) has also advocated for preferred use of these serological tests over dengue virological tests in dengue-endemic areas<sup>9</sup>.

The NS1Ag, is a protein secreted in high levels from dengue infected cells and is present in measurable levels in the circulating blood during the first 9 days of infection<sup>10,11</sup>. Also as the NS1Ag levels correlates well with the viral titer, it can be viewed as a surrogate marker for viremia<sup>10</sup>. Further, as it is detectable at the same time as viral RNA and before any antibody response is elicited, it can be considered as a valuable early diagnostic tool.

Since the merit of each of these seromarker varies, standalone use of any one of these serological assays cannot be advocated for definitive diagnosis of dengue infection. In view of this, studies have found that combination of NS1 Ag with IgM detection has dramatically improved both the sensitivity and specificity in dengue diagnosis<sup>10,11</sup>.

Besides laboratory diagnosis, detection of acute dengue infection by active surveillance activities, prompt outbreak control measures, continuous academic (e.g. vaccine development) research and clinical trials also contributes significantly as both short and long case measures in prevention of dengue infection<sup>1</sup>.

In Gujarat State, the seroprevalence of dengue infection is reportedly high (33.4%)<sup>12</sup>; consequently, there has been an increase in the number of dengue diagnostic & reporting units during the past few years secondary to improved awareness towards surveillance system, active involvement of private health care hospitals and easy availability of serodiagnostic facilities at all levels of health centers<sup>13</sup>.

### Aim

The present study was conducted with the aim to determine seroprevalence of dengue virus infection among clinically suspected cases in south Gujarat. It also intended to study the associated

demographic patterns along with the seasonal variation of dengue infection.

## MATERIAL AND METHODS

The present study was conducted by the Department of Microbiology, of a tertiary care hospital in South Gujarat after obtaining due permission from the Institutional Ethics Committee (IEC). Our study was conducted over a period of four years i.e. from January 2021 to December 2024 and includes data of consecutive 14,492 patients with clinically suspected dengue patients as reported from various outpatient and inpatient departments of our hospital. The data pertaining to the demographic and clinical details of each of these patient was collected on a pre-structured datasheet.

Blood samples from all of these consecutive 14,492 patients were collected in plain vacutainer using standard procedure. These were then sent to the serology laboratory where the serum was separated from each blood sample and stored at 4°C till the time of testing. All of these samples were tested for anti-dengue virus (DV) IgM antibodies and dengue virus non-structural protein-1 antigen (NS1Ag) by Enzyme Linked Immunosorbent Assay (ELISA) tests.

The dengue specific IgM antibody was tested using Dengue IgM Microlisa kits supplied by ICMR-National Institute of Virology (NIV), Pune whereas NS1Ag testing was done using Ozolisa Dengue NS1 Antigen Test, supplied by Government of Gujarat. While performing the ELISA, manufacturer's instructions were followed strictly. Accordingly, values were calculated and results were interpreted as per manufacturer guidelines.

The dengue seroprevalence was estimated by analyzing data for the results of both NS1Ag and DV-IgM antibody along with their relation with the patient's demographic profile, clinical profile and seasonal variation.

## RESULTS

In our study, 14,492 samples from clinically suspected dengue patients were evaluated over a period of four years, out of which 4,782 (32.99 %) were seropositive for active infection either by NS1 Ag, IgM ELISA or both. As evident from the table 1A, there was a significant yearly increase in the number of clinically suspected dengue cases, but a decreasing trends in the overall seropositivity rate was observed from the year 2021 to 2024.

**Table 1A: Year Wise Dengue Seropositivity**

| Year         | Total Sample Tested | Total Seropositive |                |
|--------------|---------------------|--------------------|----------------|
|              |                     | Numbers            | Percentage (%) |
| 2021         | 676                 | 243                | 35.95          |
| 2022         | 2861                | 1062               | 37.12          |
| 2023         | 4471                | 1350               | 30.19          |
| 2024         | 6484                | 2127               | 32.80          |
| <b>14492</b> | <b>4782</b>         | <b>32.99</b>       |                |

Tests for IgM antibody were found to be seropositive in 2,212 (15.26%) patients while seropositivity of NS1 antigen alone was observed in 1,823 (12.58%) patients. In 747 (5.15 %) patients, both NS1 antigen & IgM antibody were found to be seropositive (Table 1B).

**Table 1B: IgM/NS1 Ag Dengue Seropositivity**

| Samples Tested (2021-24) | Only IgM Positive |       | Only NS1 Ag Positive |       | Both IgM & NS1 Ag Positive |      | Total Seropositive Total Seropositive |       |
|--------------------------|-------------------|-------|----------------------|-------|----------------------------|------|---------------------------------------|-------|
|                          | No.               | %     | No.                  | %     | No.                        | %    | No.                                   | %     |
| 14492                    | 2212              | 15.26 | 1823                 | 12.58 | 747                        | 5.15 | 4782                                  | 32.99 |

Among the clinically suspected dengue patients 1193 (8.23 %) were from OPD out of which 258 (21.62%) were dengue seropositive while 13,299 were from indoor wards of which 4,524 (34.02%) showed evidence of active infection (Table 2).

**Table 2: Outpatient & Inpatient Hospital Distribution Of Dengue Cases**

|        | Samples Tested* | Dengue Seropositive |             |              | Total Seropositive (%) |
|--------|-----------------|---------------------|-------------|--------------|------------------------|
|        |                 | IgM Only            | NS1 Ag Only | IgM & NS1 Ag |                        |
| OPD    | 1193            | 121                 | 103         | 34           | 258 (21.62%)           |
| Indoor | 13299           | 2091                | 1720        | 713          | 4524 (34.02%)          |

|              |              |             |             |            |                      |
|--------------|--------------|-------------|-------------|------------|----------------------|
| <b>Total</b> | <b>14492</b> | <b>2212</b> | <b>1823</b> | <b>747</b> | <b>4782 (32.99%)</b> |
|--------------|--------------|-------------|-------------|------------|----------------------|

\*From Year 2021 to 2024

**Table 3: Incubation Period & Dengue Seropositivity**

| Incubation Period | Sample Tested | Only IgM Positive |              | Only NS1 Ag Positive |              | Both IgM & NS1 Ag Positive |             | Total Seropositive |              |
|-------------------|---------------|-------------------|--------------|----------------------|--------------|----------------------------|-------------|--------------------|--------------|
|                   |               | No.               | %            | No.                  | %            | No.                        | %           | No.                | %            |
| < 5 days of fever | 10083         | 1422              | 14.10        | 1669                 | 16.55        | 294                        | 2.92        | 3385               | 33.57        |
| ≥ 5 days of fever | 4409          | 790               | 17.92        | 154                  | 3.49         | 453                        | 10.27       | 1397               | 31.69        |
| <b>Total</b>      | <b>14492</b>  | <b>2212</b>       | <b>15.26</b> | <b>1823</b>          | <b>12.58</b> | <b>747</b>                 | <b>5.15</b> | <b>4782</b>        | <b>32.99</b> |

In our present study, 10,083 (69.57 %) clinically suspected patients were having less than 5 days of fever while 4,409 (30.43 %) were having fever of more than or equal to 5 days. The seropositivity rates were found to be higher by all the methods among those tested during early febrile period i.e. 3385 (33.57%) as against 1397 (31.69%) seropositive patients with ≥ 5 days of fever (Table 3).

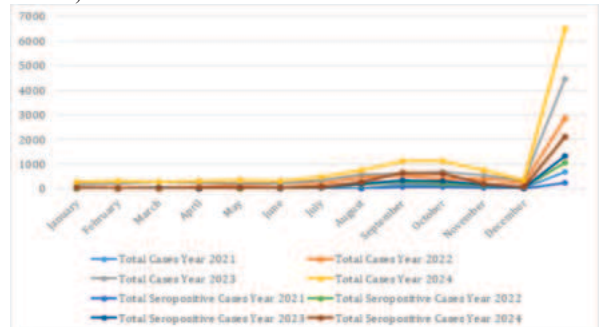
**Table 4: Distribution Of Dengue Cases Among Various Age-groups**

| Age Group   | Clinically Suspected Cases (2021-2024) |            | Total Seropositive (2021-2024) |            | Positivity for the age group (%) |
|-------------|----------------------------------------|------------|--------------------------------|------------|----------------------------------|
|             | Number                                 | Percentage | Number                         | Percentage |                                  |
| 0-10 Years  | 2975                                   | 20.53      | 751                            | 15.71      | 25.26                            |
| 11-20 Years | 3536                                   | 24.40      | 1279                           | 26.74      | 36.16                            |
| 21-30 Years | 3798                                   | 26.21      | 1499                           | 31.34      | 39.46                            |
| 31-40 Years | 1958                                   | 13.51      | 703                            | 14.71      | 35.92                            |
| 41-50 Years | 1176                                   | 8.11       | 300                            | 6.28       | 25.54                            |
| 51-60 Years | 447                                    | 3.08       | 121                            | 2.53       | 27.09                            |
| > 60 Years  | 602                                    | 4.16       | 128                            | 2.68       | 21.28                            |
|             | <b>14492</b>                           |            | <b>4782</b>                    |            |                                  |

Data analyzed for various age groups (Table 4) revealed that the maximum number of cases i.e. 3,798 & 3,536 were reported among those belonging to 21-30 years of age-group followed by 11-20 years respectively while the least incidence (447) was seen in the elderly age group of 51-60 years.

Similarly, highest seropositivity of 39.46% was observed among the patients belonging to the age group of 21-30 years closely followed by 36.16% among 11-20 age group while the least seropositivity (21.28%) was seen in those having more than 60 years of age.

Further, among the total samples tested, even though the majority 54.28% of the clinically suspected patients were female (7,866), as compared to 45.72% males (6,626), both the proportion of seropositive cases and the rate of seropositivity was observed to be higher among males (n=2,579, 53.94%) as compared to the females (n=2,203, 46.06%).



**Figure 1: Year Wise Monthly Distribution of Dengue Cases**

The number of clinically suspected patients tested for dengue and their seropositivity for various calendar months during the study period i.e. from 2021 to 2024 was also observed (Figure 1) and it was found that although maximum cases were reported in the month of September (2021~180, 2022~564, 2023~688 & 2024~1145), highest seropositivity rates were seen in the month of October (2021~46.0%, 2022~51.54%, 2023~58.34% & 2024~54.98%).

## DISCUSSION

In our study, seropositivity for dengue infection was found to be 32.99 % which is similar to the studies conducted in Saurashtra, Gujarat by Mistry et al<sup>12</sup> (33.4%) and by Ukey et al<sup>14</sup> (31.3%) in Central India suggesting continuous presence of dengue infection.

However, when compared to other studies conducted by Kumar et al<sup>15</sup> (27.29%) and Patel et al<sup>7</sup> (25%), our seropositivity rates are slightly higher. This disparity in the seroprevalence could be attributed to various factors as differences in the season during which study was being conducted along with their study design (i.e. study settings, sampling techniques, data collection tools & sample processing procedure/ techniques).

The seroprevalence of dengue infection was observed slightly more among males (53.94%) as compared to females (46.06%) which though lower correlates well with the higher seropositivity seen among male population in the studies of Mistry et al.<sup>12</sup> (males, 68.7%), Patankar et al.<sup>16</sup> (males, 65%) and Patel et al<sup>7</sup> (males, 56.89%). These male–female differences in the seroprevalence of dengue infection can be attributed to local trend that men are more likely to seek health care than women. Besides, males have been associated with greater outdoor workplace presence, have long daytime workhours and frequent outdoor travelling all of which leads to comparatively more exposure of males to mosquitos carrying dengue viruses. Thus, our finding reinforces the importance of inclusion of demographic profile while studying seroprevalence of dengue infection.

The age group most affected by dengue infection was that below 30 years with highest seropositivity seen among age group 21-30 years (39.46%) followed by 11-20 years (36.16%) age group comparable to the findings of Ukey et al.<sup>14</sup> (15-30 years; 31.71%) and Patankar et al.<sup>16</sup> (18-35 years; 51%). These findings further strengthen the view that dengue though traditionally considered in SE-Asia primarily as a disease of paediatric age<sup>17</sup> have in recent years, been increasingly affecting adult population. Studies conducted in other SE-Asian countries where dengue has been epidemic since long, also reported similar observations regarding shift in epidemiology to older age groups<sup>18</sup>.

The number of samples tested increased significantly over the study period with around 9.5-fold increase i.e. from 676 sample in 2021 to 6484 in 2024. During all the years, highest number of suspected cases were reported during the month of September while the highest seropositivity was observed in October. Thus our study reinforces the findings of previous studies<sup>7,12,16,19</sup> that the dengue seropositivity shows seasonal variation with increased rates observed during monsoon & post-monsoon season i.e. both the number of cases and seropositivity increases from the months of June to October.

This signifies that environmental factors including weather variables play a pivotal and favorable role in promoting breeding of the vector and hence increased transmission of dengue<sup>20</sup>. This knowledge about local trends in seasonal variation of dengue infection provides important information for planning and preparation of various control measures to be employed in anticipation so as to reduce dengue infection.

Diagnosis of dengue infection is principally done on clinical basis but laboratory tests are essential in confirming it<sup>9</sup>. Though culture along with nucleic acid detection techniques are definitive and more specific detection methods as compared to serology, these techniques are expensive, require sophisticated laboratory setup and are labour intensive thereby restricting their usage as routine and common diagnostic tool for acute dengue cases. In contrast combination of NS1Ag and IgM detection yields more accurate results in contrast to the tests being done individually<sup>11</sup>. Further, the tests to detect NS1Ag and IgM are technically simple to perform, rapid, cost-effective and sensitive enabling them in emerging as key players in serodiagnosis of acute dengue infection<sup>1</sup>.

## CONCLUSION

Slightly decreasing trends in its seroprevalence, shift of seropositivity from paediatric to the adult population with slightly more involvement of males along with its seasonal variation were classically observed in our study.

Also acknowledged is the fact that its diagnosis based on a single biomarker has limitations hence combination of detecting both anti-

dengue virus IgM antibodies and dengue NS1Ag is recommended for early and confirmatory laboratory diagnosis of dengue infection.

Since dengue still remains a major public healthcare concern, it is imperative that local laboratory services be organized in the context of dengue prevalence rates, patient's needs and dengue control strategies.

## Conflict Of Interest

Nil

## REFERENCES

- Dengue: guidelines for diagnosis, treatment, prevention and control, New Edition 2009. A joint publication of the World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR).
- Emerging Infectious Diseases; Centers for Disease Control and Prevention (CDC); page 887, vol. 12, no. 6, June 2006.
- Guidelines for Prevention and Control of Dengue 2006. A joint publication of the Zoonosis Division, National Institute of Communicable Diseases and World Health Organization (WHO), Country Office for India, pp 1.
- Nivedita G, Srivastava S, Jain A, Chaturvedi UC; Dengue in India; Indian J Med Res 136, September 2012, pp 373-390.
- Dengue Situation in India; Dengue Cases and Deaths in the Country since 2018; National Center for Vector Borne Diseases Control (NCVBDC); Ministry of Health & Family Welfare-Government of India.
- Liliana Sánchez-González, Laura Adams, Gabriela Paz-Bailey; Dengue Centers for Disease Control and Prevention (CDC) Yellow Book 2024, pp 8.
- Sangeeta DP, Nisarg T, Gaurishankar S, Sima B., Seroprevalence of dengue virus in civil hospital, Gandhinagar, Gujarat; Saudi Journal of Pathology and Microbiology, March 2019; 4(3): pp 168-171
- Murray NE, Quam MI B, Wilder-Smith A; Epidemiology of dengue: past, present and future prospects; Clinical Epidemiology 2013;5 pp 299–309
- Singh M, Chakraborty A, Kumar S, Kumar A. The epidemiology of dengue viral infection in developing countries: A systematic review. J Health Res Rev 2017; 4:104-7.
- David AM, Alexandra CID, Paul RY; The Journal of Infectious Diseases, Volume 215, Issue suppl 2, 1 March 2017, pp S89–S95
- Stuart DB, Richard GJ, Robert VG et.al. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. Diagnostic Laboratory Immunology, Vol 19, Iss 5, May 2012, pp 804-10.
- Mistry M, Chudasama RK, Goswami Y, Dalwadi C, Mitra A, Mehta G; Epidemiological characteristics of dengue disease in Saurashtra region, India, during year 2015. J Family Med Prim Care 2017;6: 249-53.
- Shikha Jain S, Dixit GT, Kohli V.; Trend and Epidemiological Profile of Dengue Fever/Dengue Haemorrhagic Fever in Ahmedabad City, Gujarat, India; Healthline Journal Volume 10 Issue 1 (January-June 2019), pp 62-67.
- Ukey PM, Bondade SA, Paunipagar PV, Powar RM, Akulwar SL; Study of Seroprevalence of Dengue Fever in Central India; Indian Journal of Community Medicine/ Vol 35/Issue 4/October 2010, pp 517-519.
- Kumar V, Chaturvedi M, Agrawal A, Singh G, Maurya P, Goyal A, Kumar S; Dengue infection in Agra region; Asian Journal of Medical Sciences | Dec 2022 | Vol 13 | Issue 12, pp162-168.
- Patankar MC, Patel BV, Gandhi VP, Shah PD, Vegad MM. Seroprevalence of dengue in Gujarat, Western India: a study at a tertiary care hospital. International Journal of Medical Science and Public Health, 2014, vol 3, issue 1, pp 16-18.
- Ministry of Health and Family Welfare, Government of India; India fights dengue; strategy and plan of action for effective community participation for prevention and control of dengue.
- Bhatia R, Dash AP, Sunyoto T; Changing epidemiology of dengue in South-East Asia WHO South-East Asia J Public Health. 2013; 2, pp 23–7
- M. Murhekar et al.; Epidemiology of dengue fever in India, based on laboratory surveillance data, 2014–2017/ International Journal of Infectious Diseases 84S (2019), pp S10–S14.
- S. Wongkoon, M. Jaroensutasinee, and K. Jaroensutasinee; Distribution, seasonal variation & dengue transmission prediction in Sisaket, Thailand; Indian J Med Res. 2013 Sep; 138(3), pp 347–353