*Original Research Article*

NS1 antigen and IgM antibody-based prevalence of dengue infection in the Patna Region: A five years study

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ABSTRACT

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| **Introduction:** Dengue virus is considered an important mosquito-borne virus that currently poses a major health risk and belongs to the Flaviviridae family. The study was conducted to estimate the NS1 antigen and anti- dengue IgM antibody-based prevalence of dengue infection in Patna, Bihar and also to investigate the association of seasonal variation along with the gender-specific prevalence also.  **Methods:** The study involved collection, classification, and analysis of laboratory-based data of blood sample a period of 5 years with reference to dengue infection.  **Results:** During this period a total 7415 samples were referred to the laboratory for screening from different hospitals. Then data were analysed to determine the NS1 and IgM based prevalence against dengue virus. A total of 29.77% were found positive for NS1 and 21.71% were found positive for IgM. Further 64.17% males and 35.82% females were found NS1 positive. Similarly, 66.52 males and 33.48 females were found positive for IgM. Most of the dengue infection was reported in the post monsoon season.  **Conclusions:** So, from study we can conclude that serological testing for presumptive identification of dengue infections is useful for prevention and monitoring the prevalence. Furthermore, a concerted effort is needed to create regional awareness among people in this area. |

*Keywords: Dengue virus, Dengue fever, NS1antigen and IgM antibody*

1. INTRODUCTION

Dengue virus (DENV) is considered an important mosquito-borne (female Aedes aegypti mosquitoes) virus that currently poses a major health risk and belongs to the Flaviviridae family (Racherla et al., 2018). The high mortality rate and lack of specific treatment have made dengue infection a major worldwide health concern. The World Health Organization reported that DENV occurs in more than 125 countries and also more than 50% of the population is at risk. Over 2.5 billion individuals in over 110 countries are at risk of developing these viruses each year because there are no effective drugs or treatments for them (Soe et al. 2018). It is estimated that there are between 50 and 100 million cases throughout more than 100 countries. India was one of them, with over 1.4 million documented cases, making it one of the worst affected countries (Ganeshkumar P et al., 2018, del Valle-Mendoza J et al., 2021).

Dengue fever is characterised by symptoms such as fever, headache, and rash, as well as a number of other nonspecific symptoms and indicators. Dengue hemorrhagic fever (DHF) may develop in the patient, which causes bleeding, pain in the abdomen, and possibly cardiac collapse. Dengue fever manifests itself in three stages: febrile, critical, and recovery phase. Thrombocytopaenia (a condition in which platelet count decrease below 100,000 per mm3 from baseline) and haemoconcentration (a 20% or greater increase in haematocrit) are detectable during the critical phase before fever and shock (Chuansumrit A et al., 2020). Dengue virus serotypes are classified as DENV-1, DENV-2, DENV-3, and DENV-4. Infection with any of these serotypes can result in a range of symptoms, including mild to severe dengue (SD) fever, as well as bleeding and plasma leakage (Soe et al. 2018, Dinesh DS et al., 2020, Trivedi and Chakravarty., 2022, Singh K et al., 2025).

The primary carrier of the dengue virus is Aedes aegypti which first and foremost thrives in specific environments, such as storage or water logging regions, unfurnished drainage networks in municipal regions that are semi-urban and rural, and everywhere there are insufficient waste disposal services (Anker, and Arima., 2011, Getachew D et al., 2015, Kumar S et al., 2012). There is a dearth of gender-specific data because these studies are not frequently carried out and most surveillance systems do not report or analyse data related to gender or rural/semi-urban places, which can related to the increased prevalence of dengue fever in such settings (Anker, and Arima., 2011, Kumar S et al., 2012, Kumar M et al., 2020). Several diagnostic methods are currently available for dengue virus detection, including serological assays, virus isolation, and molecular techniques such as Nucleic Acid Amplification Tests (NAATs). While NAATs and viral culture offer high specificity, their requirement for specialized equipment and higher costs limits their widespread use in routine diagnostics. Advanced molecular methods including RT-PCR, multiplex RT-PCR, real-time RT-PCR, and LAMP assays provide sensitive detection during the acute phase of infection. However, in resource-limited settings, cost-effective serological tests like ELISA remain the preferred choice for early diagnosis and timely clinical intervention due to their accessibility and reliability (Jain, C et al., 2023, Datta, S et al., 2010). Therefore, this study aims to assess the prevalence of dengue infection in Patna, Bihar, among suspected patients using NS1 antigen and IgM antibody serological testing. Additionally, it seeks to evaluate the association between seasonal variation, gender-specific distribution, and dengue virus infection rates.

2. material and methods

This prospective laboratory-based study was conducted at an Indian Council of Medical Research (ICMR) at Patna, Bihar in northeastern India. The study incorporated a retrospective analysis of dengue data spanning a five-year period (2017-2021) obtained through routine diagnostic procedures at the Viral Research and Diagnostic Laboratory (VRDL). The study cohort comprised 7,415 serum samples referred from both public and private healthcare facility throughout Bihar for dengue virus screening. Blood samples were obtained from clinically suspected dengue cases during the acute phase of the disease (1 to 6 days post-onset), presenting with symptoms such as fever, headache, myalgia, retroorbital discomfort, skin rashes, and hemorrhagic manifestations. Samples were collected in plain vacutainers, processed immediately, and centrifuged to separate serum from blood. The serum was aliquoted and stored at -20°C for subsequent analysis. Only samples with complete demographic data and results for both NS1 antigen and IgM antibody tests were included in the analysis. The NS1 antigen was detected using the QUALISA Dengue NS1 ELISA kit (Qualpro Diagnostics Pvt. Ltd., Goa, India), while IgM antibodies were tested with the MICROLISA IgM ELISA kit (J. Mitra and Co., New Delhi), following the manufacturers’ instructions and both tests were performed on the same day. Data were entered and cleaned in Microsoft Excel (2007 version) to ensure accuracy before generating graphs and tables. Statistical analyses, including descriptive statistics, were performed using IBM SPSS Statistics for Windows, version 22.0.

3. results and discussion

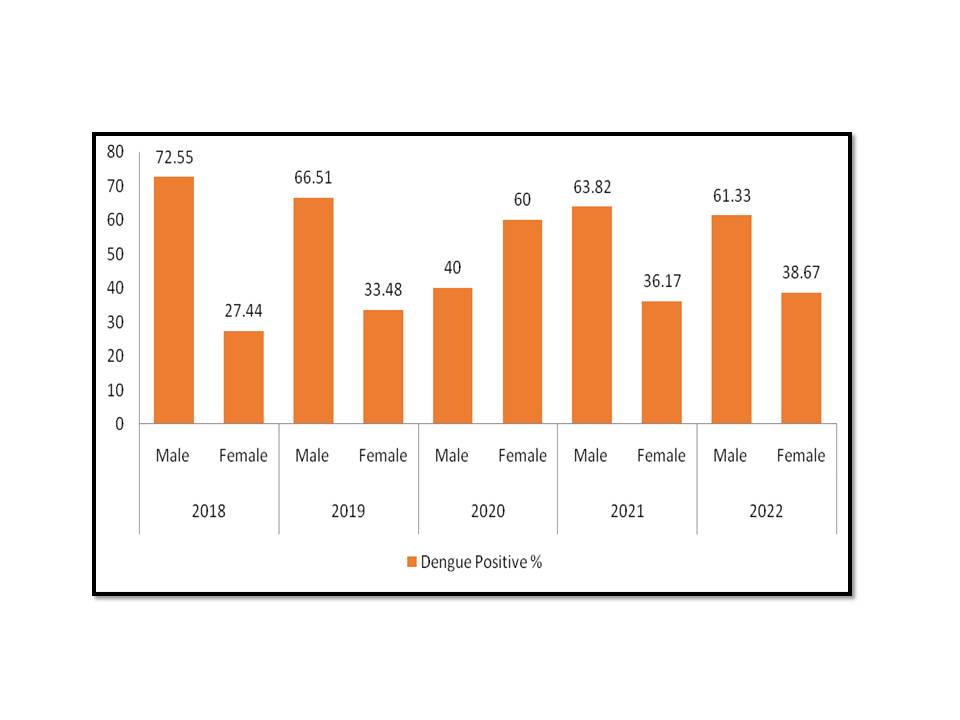
3.1. Result

Out of 7,415 blood samples tested, 2,208 (29.77%) were positive for the NS1 antigen, while 10 (0.13%) yielded equivocal results. For IgM antibodies, 1,610 (21.71%) tested positive, and 824 (11.11%) were equivocal. Additionally, 681 (9.18%) patients were confirmed dengue-positive through both NS1 and IgM markers, as detailed in Table 1. The overall percentage of dengue for the year January 2018 and December 2022 has been described as macro information, in which positive, negative, and equivocal results has been shown in quarterly manner like January to March (Q1), April to June (Q2), July to September (Q3) and October to December (Q3) (Table 1). Most of the positive cases of dengue NS1 were identified in the year 2022 (37.69%), followed by 2018 (28.15%), 2019 (27.21%), and 2021 (8.52%). But during the year 2020, very few samples were tested and all were found negative this due to SARS-CoV-2 pandemic. But in the case of IgM antibody most positive cases were identified on the year 2019 (28.91%), followed by 2018 (27.21%), 2022 (16.57%), 2020 (9.43%), and 2021 (6.59%).

**Table 1. Summarized tabulation of overall data for the year 2018-2022.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** |  | **Dengue NS1** | | |  | **Dengue IgM** | | | **Both NS1 & IgM** | |
|  |  | **Total**  **sample**  **tested** | **Positive** | **Negative** | **Equivocal** | **Positive** | **Negative** | **Equivocal** | **Positive** | **Equivocal** |
| **2018** | **Jan-Mar** | **72** | **0** | **72** | **0** | **2** | **69** | **1** | **0** | **0** |
| **April-Jun** | **36** | **0** | **36** | **0** | **0** | **36** | **0** | **0** | **0** |
| **July-Sep** | **288** | **34** | **250** | **4** | **47** | **206** | **35** | **12** | **3** |
| **Oct-Dec** | **677** | **268** | **408** | **1** | **243** | **412** | **22** | **106** | **1** |
| **Total** | **1073** | **302** | **766** | **5** | **292** | **723** | **58** | **118** | **4** |
| **2019** | **Jan-Mar** | **26** | **1** | **25** | **0** | **2** | **22** | **2** | **0** | **0** |
| **April-Jun** | **94** | **6** | **87** | **1** | **20** | **32** | **42** | **5** | **1** |
| **July-Sep** | **441** | **78** | **363** | **0** | **110** | **249** | **82** | **32** | **0** |
| **Oct-Dec** | **2137** | **649** | **1488** | **0** | **648** | **1103** | **386** | **253** | **0** |
| **Total** | **2698** | **734** | **1963** | **1** | **780** | **1406** | **512** | **290** | **1** |
| **2020** | **Jan-Mar** | **53** | **0** | **53** | **0** | **5** | **46** | **2** | **0** | **0** |
| **April-Jun** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **July-Sep** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **Oct-Dec** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **Total** | **53** | **0** | **53** | **0** | **5** | **46** | **2** | **0** | **0** |
| **2021** | **Jan-Mar** | **33** | **0** | **33** | **0** | **0** | **33** | **0** | **0** | **0** |
| **April-Jun** | **9** | **0** | **9** | **0** | **1** | **8** | **0** | **0** | **0** |
| **July-Sep** | **305** | **9** | **296** | **0** | **19** | **263** | **23** | **0** | **0** |
| **Oct-Dec** | **275** | **44** | **231** | **0** | **21** | **232** | **22** | **8** | **0** |
| **Total** | **622** | **53** | **569** | **0** | **41** | **536** | **45** | **8** | **0** |
| **2022** | **Jan-Mar** | **88** | **1** | **85** | **2** | **4** | **80** | **4** | **1** | **0** |
| **April-Jun** | **143** | **0** | **143** | **0** | **10** | **123** | **10** | **0** | **0** |
| **July-Sep** | **897** | **338** | **557** | **2** | **142** | **687** | **68** | **84** | **0** |
| **Oct-Dec** | **1841** | **780** | **1061** | **0** | **336** | **1380** | **125** | **181** | **0** |
| **Total** | **2969** | **1119** | **1846** | **4** | **492** | **2270** | **207** | **265** | **0** |
|  | **Grand Total** | **7415** | **2208** | **5197** | **10** | **1610** | **4981** | **824** | **681** | **5** |

A total of 7415 tested samples, 4690 were male and 2725 were female. Out of which 1417 (64.17%) males and 791 (35.82%) females were found NS1 antigen positive. Similarly in the case of IgM antibody 1071 (66.52) males and 539 (33.48) females were found positive. Further gender distribution percentage of male was more as compared to female in dengue suspected cases consecutive for years from 2018 to 2022 as shown in figure 1.



**Figure 1. Figure showing the gender wise distribution of the dengue cases.**

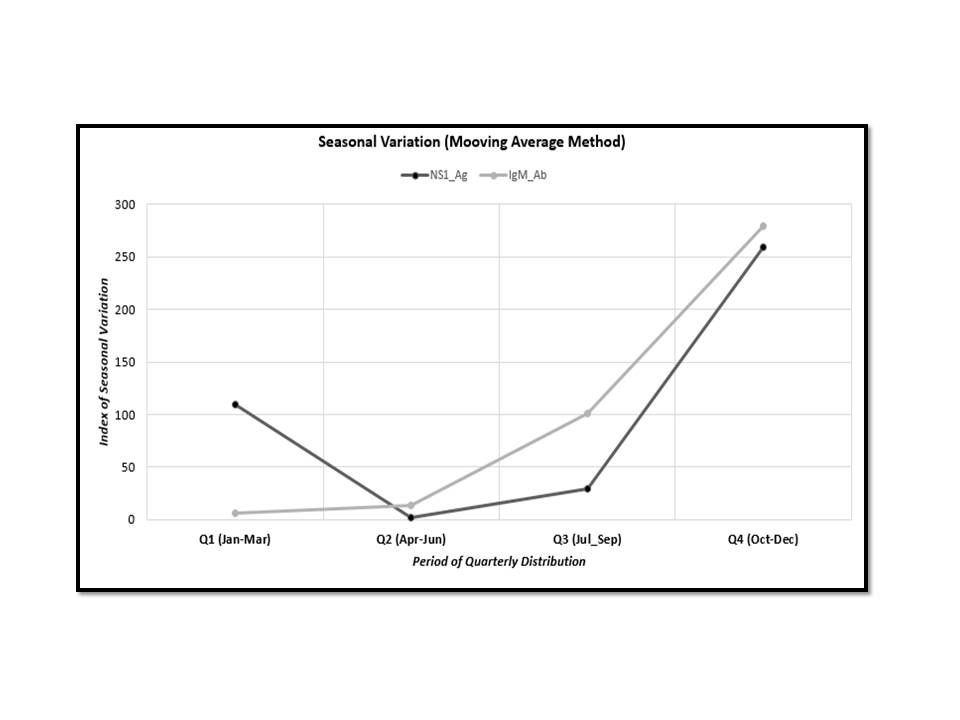
The demography of the patients showed that most of the patients with dengue positive and equivocal cases were found at the median age 23 years with CI at 95% (25.47 to 26.62) (Table 2 & 3). Most of the dengue infection was reported in the month of October to December (Q4). Index of seasonal variation was analyzed with the help of moving average method, positive cases of IgM was continuously increases as compared to NS1. Low seasonal variation of IgM positive cases was found between first and second quartile, slightly high in the fourth quartile. Whereas in case of NS1 positive cases seasonal variation was found irregular in different season, such as start deceasing from first quartile to second quartile, and constantly increasing from third to fourth quartile (Figure 2).

**Table 2: Table showing the distribution of age and statistical for the study.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Condition** | **Median** | **Range** | **Standard Deviation (SD)** | **CI for mean at 95%** |
| Age\_NS1-Ag\_Positive Cases | 24 | 79 | 13.75 | 25.47-26.62 |
| Age\_NS1-Ag\_Equivocal Cases | 14 | 33 | 12.19 | 6.58-24.02 |
| Age\_IgM-Ab\_Positive Cases | 23 | 82 | 13.58 | 24.48-25.81 |
| Age\_IgM-Ab\_Equivocal Cases | 21 | 94 | 15.31 | 23.57-25.66 |
| Age\_Overall Cases | 23 | 95 | 13.99 | 25.06-25.86 |

**Table 3: Table showing the age distribution of positive and equivocal cases.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Age (Years)** | **NS1\_Ag n (%)** | **IgM\_Ab n (%)** | **Both (NS1+IgM) n (%)** |
| <20 | 793 (35.75) | 947 (38.91) | 1740 (37.40) |
| 20-30 | 759 (34.22) | 808 (33.20) | 1567 (33.68) |
| 31-40 | 343 (15.46) | 337 (13.85) | 680 (14.62) |
| 41-50 | 187 (08.43) | 194 (07.97) | 381 (08.19) |
| >50 | 136 (06.14) | 148 (06.08) | 284 (06.10) |
| Total | 2218 | 2434 | 4652 |



**Figure 2: Figure showing the seasonal variation of dengue cases.**

**3.2. Discussion**

Dengue fever is a mosquito-borne virus that causes significant morbidity and economic losses in many tropical and subtropical locations across the world. Dengue fever is prevalent in most regions of India since it affects a large portion of the country. Most of those affected people were belongs to middle class (Das B et al., 2013). Further as a result of urbanisation, climatic changes, and increased human migration, dengue virus is spreading rapidly. Dengue virus has become the most common vector-borne viral infection of the twenty-first century (Eldigail MH et al., 2018). This is extensive investigation on the prevalence of dengue infection from Patna, Bihar, in India's north-east during 2018-2022. In the current investigation, 29.77% and 21.71% of the suspected cases tested positive in NS1 and IgM antibody test for dengue infection. The other dengue suspected patients who tested serologically negative may really have one of the numerous disorders that exhibit similar symptoms to dengue fever. A similar study prevalence of dengue fever in Uttar Pradesh reported 23% seropositive either by NS1 or IgM ELISA. Although the fact that the gender specific distribution of dengue infection is not well known, few studies have been conducted to describe the gender-based prevalence of dengue cases (Kumar M et al., 2020, Chakravarti A et al., 2012, Goswami L et al., 2018).

In the present study, 64.17% males and 35.82% females were found NS1 antigen positive. Similarly in the case of IgM antibody positive 66.52% males and 33.48% females were found positive. So according to our findings, males were more affected than females. Many additional independent Indian investigations yielded similar results (Kumar M et al., 2020, Agarwal R et al., 1999, Wali JP et al., 1999).. Similarly, comparable findings have been recorded around the world, with males being more affected than females (Brown MG et al., 2009, Ang LW et al., 2015, Rafique I et al., 2017, Abdullah QY et al., 2020).

In this study, dengue infection was found peak in the month of October to December (Q4) which slowly increase from month of July to September (Q3) and decrease from peak for each year from 2018 to 2022. This is owing to the high humidity that follows the rainy season, which provides ample breeding grounds for mosquitoes. Similar results were found in India and all around the world where dengue fever was most common in the post-monsoon season (October to December) period as compared to the monsoon period (Sharma RS et al., 2005, Ali A et al., 2013).

The study predicts the NS1 and IgM ELISA prevalence and included large number of samples from different districts of Bihar. Further the combined results of the NS1 and IgM ELISA demonstrate that the ability to identify dengue fever with general accuracy and capacity may lead to an increase in diagnostic sensitivity.

4. Conclusion

This study demonstrated the utility of serological testing (NS1 antigen and IgM antibody) in determining dengue prevalence among suspected patients in Patna, Bihar. Our findings revealed significant seasonal variation, with the highest infection rates occurring during the post-monsoon period, as well as a gender disparity, with males exhibiting higher susceptibility. The observed gender differences need further investigation into potential behavioral or biological risk factors.

To mitigate dengue transmission, we recommend that government authorities intensify efforts to control vectors through integrated measures, including biological, environmental, and chemical interventions. Concurrently, community education programs should be implemented to promote early medical seeking behavior and enhance healthcare professionals' capacity to recognize dengue symptoms promptly.

Furthermore, sustained regional awareness campaigns are essential to encourage residents to adopt preventive measures and curb viral spread. Therefore, we advise that every single case of dengue should be serologically tested for NS1 and IgM, independent of gender, regardless of the male preponderance.

Ethical approval AND CONSENT

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of ICMR- RMRIMS, (Ethics Committee Letter No: RMRI/EC/21/2023, dated 24.02.2023). The Ethics Committee waived the requirement for written consent, as all samples utilized in the study were previously collected during routine medical care based on clinicians’ requests.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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