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Original Research Article

# Dengue virus: Investigate serological and hematological indicators for predictions

**Priya Laljibhai Bhojani[1]*, Preeti Jahveri[1], Krupali Patel[1], Suchita Mishara[1]**

[1] *Smt. NHL Municipal Medical College, Ahmedabad, Gujarat, India*

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ABSTRACT

**Background:** Timely and accurate diagnosis of dengue fever is essential for both efficient treatment and prevention of severe symptoms. Investigating hematological and serological markers for dengue virus infection is the goal of the current investigation.

**Materials and Methods**: A laboratory-based cross-sectional study was carried out among patients who visited SVP Hospital in Ahmedabad, Gujarat, between January 2022 and December 2023. Hematological parameters were assessed and blood samples from suspected dengue cases were analyzed using the rapid diagnostic immunochromatography (ICT) approach. Continuous variables were compared between groups infected with dengue and those that were not using the Mann-Whitney U test. A binary logistic regression analysis was performed to evaluate the correlation between dengue positive and other factors.

**Result:** Dengue-positive participants showed low mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), thrombocytopenia, leucopenia, erythrocytosis, and high hemoconcentration in comparison to dengue-negative people. On the other hand, dengue positivity was significantly predicted by platelet count, total leucocyte count, MCH, MCHC, neutrophil count, and lymphocyte count, according to binary logistic regression.

**Conclusion**: The features and demographics of dengue-positive cases were determined in this investigation, along with their associations with hematological markers. Moreover, by helping medical professionals diagnose and treat dengue patients faster, the discovered predictive indicators can lessen the disease's effects.



## 1. Introduction

In tropical and subtropical regions, dengue fever—a virus spread by mosquitoes—poses a serious threat to public health, with an estimated 3 million cases recorded annually. Biting female Aedes mosquitoes carrying the infection—more especially, Aedes aegypti and Aedes albopictus—can infect humans.[1] Due to the disease's rapid geographic expansion brought on by urbanization, international travel, trade, and climate change, as well as an increase in the frequency of large-scale outbreaks, dengue fever has become a serious global health concern.[2,3] Dengue is more common in regions with tropical and subtropical climates, such as Africa, the Eastern Mediterranean, South-East Asia, and the Western Pacific. The most affected regions include the Americas, South-East Asia, and the Western Pacific, with Asia accounting for almost 70% of the world's disease burden.[4] According to previous estimates from the World Health Organization (WHO), over 40% of the world's population is susceptible to dengue, and throughout the past 50 years, the number of dengue cases has increased by 30% worldwide.[5]

There are many degrees of dengue fever. Patients with dengue suffer from an acute febrile disease that lacks localizing signs and may be mistaken for another

* *Corresponding author*.
*E-mail address*: priyabhojani12345@gmail.com (P. L. Bhojani).

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infection.[6] The blood circulation has high concentrations of the NS1 antigen from the first day of the fever until day nine. While IgG appears on Day 14 and persists forever, IgM is detectable from Days 3 to 5 of the disease and lasts for 2 to 3 months.[7–9] The approved approach for identifying acute dengue infection is to use an in vitro immunochromatographic technology to identify Nonstructural 1 (NS1) protein and IgM and IgG anti-dengue viral antibodies. It has been discovered that dengue infection modifies a number of hematological indicators.[10] Thrombocytopenia, leukopenia, high hematocrit (Hct), and the presence of aberrant lymphocytes are common observations in dengue patients.[11,12] Nucleic acid amplification assays are the gold standard for dengue detection, however they are not commonly accessible in resource-poor nations. Therefore, lateral flow assays (LFA) or immunochromatography (ICT)- based detection techniques are usually used for dengue diagnosis in most developing nations.[13,14] Despite being user-friendly, easy to use, and having short turnaround times, active dengue detection with ICT has low sensitivity and specificity as well as increased cross-reactivity, which results in more false positives.[15] Hematological parameters can be helpful as a supplementary test for dengue diagnosis, in addition to quick dengue testing using ICT methods. ICT detects dengue-specific antigens or antibodies in a patient's blood, and hematological parameters assist in identifying dengue-related hematological changes such as thrombocytopenia and hemoconcentration.[16] Hematological indicators and the ICT technique help medical practitioners identify and treat dengue fever patients early, which improves patient outcomes and lowers the risk of severe consequences.

Furthermore, as there are no licensed dengue vaccinations or targeted antiviral drugs, patient management is reliant on excellent supportive care. Accurate detection of dengue infection can help with both enhancing patient care and reducing further transmission through community-wide vector control activities.[17] Consequently, evaluating the hematological and serological traits of dengue virus-infected patients was the aim of the current investigation.

## 2. Materials and Methods

A laboratory-based cross-sectional study was carried out on patients who visited SVP Hospital, Ahemdabad between January 2022 and December 2023. Each research subject provided their informed consent.

### *2.1. Including and excluding criteria*

Criteria for inclusion and removal Participants with symptoms of dengue illness and positive dengue in ICT serology were enrolled after obtaining informed consent.

Individuals without dengue symptoms and those with negative ICT serology tests were eliminated.

On the other hand, patients who did not exhibit any symptoms of dengue or who tested negative for the virus were included in the control group.

### *2.2. Specimen collection and processing*

Collection and processing of specimens Venous blood samples were taken in accordance with standard operating procedures, placed in a K3 EDTA vacuum tube, and brought to the lab where the blood was gently mixed. An automated hematology analyzer (Sysmex XN-350) was used to perform a full blood profile, which included hemoglobin, RBC and RBC indices, hematocrit, total leukocyte count, differential leukocyte count, and platelets. Similarly, in order to identify dengue infection, serum samples were gathered in gel clot activator tubes. The quick immunochromatographic test (ICT) (BiolineTM DENGUE DUO, Dengue NS1 + IgM/IgG Combo quick Test, Abbott) served as the foundation for the qualitative dengue diagnostic process. Individuals who tested positive for dengue were evaluated for both IgM and NS1 positivity or for NS1 and IgM positivity.

Any result that was negative on any of these profiles was considered to be devoid of dengue. Patients were divided into groups: those with positive dengue and those with negative dengue.

### *2.3. Analytical statistics*

To evaluate the data, IBM SPSS version 25 was employed. The Shapiro-Wilk normalcy test was performed to see if the data had a normal distribution. For continuous variables, the median was shown (Q3- Q1). In the univariate analysis of the dengue positive and negative groups, which was appropriately conducted using the Mann- Whitney U test, a p-value of less than 0.05 was considered significant. Binary logistic regression was performed in accordance with the specifications, and the results were presented as crude and adjusted odds ratios with a 95% confidence interval (95% CI). An indicator of statistical significance for a variable was a p-value of less than 0.05.

## 3. Results

### *3.1. Characteristics and demographics of dengue-positive cases*

There were 689 cases of dengue positivity overall. Table 1 shows that of them, 71.4% (n = 492) were single positive, 13.20% (n = 91) were dual positive, and 4.93% (n = 34) were triple positive. Participants who tested positive for dengue had a median age of 30 years (Q3-Q1 = 40 years – 20 years). Of the 689 people who tested positive for dengue, 56.2% (n = 387) were men and 43.8% (n

= 302) were women. Additionally, it was discovered that the 20–29 age group had the highest number of positive cases, followed by the 30-39 age group. The age difference between the dengue-positive group (median = 30 years) and the dengue-negative group (median = 28 years) was statistically significant (p=0.005), according to the Mann-Whitney test (Table 2).

**Table 1:** Serological classification of dengue positive case

| Dengue Positive cases | | | Total |
|---|---|---|---|
| Single positive | NS1 only | 450 | 492 |
| | IgM only | 32 | |
| | IgG only | 10 | |
| Dual Positive | NS1+IgM | 62 | 91 |
| | NS1+IgG | 20 | |
| | IgM+ IgG | O9 | |
| Triple positive | NS1+IgM+IgG | 34 | 34 |
| Total (Overall Positive) | | | 689 |

### *3.2. Relationship between a hematological profile and dengue infection*

The Mann- Whitney association between the hematological profile of the dengue positive and negative groups is shown in Table 2. Simply said, compared to the dengue negative group, the dengue positive group had lower levels of platelet count, TLC, low MCH, low MCHC, low hematocrit, high neutrophil, low lymphocyte count, low monocyte count, and low Eosinophil.

### *3.3. Predictive markers and logistic regression*

Binary logistic regression was used to assess the correlation between laboratory parameters and the outcomes (dengue positive and negative). The inclusion of the following independent variables improved the model significantly: MCH (p<0.001, OR: 1.163, U5% CI: 1.070-1.263), MCHC (p<0.001, OR: 2.085, U5% CI: 1.751-2.483), platelets (p<0.001, OR: 1.000, U5% CI: 1.000-1.000), TLC (p<0.001, OR: 1.000, U5% CI: 1.000-1.000), and lymphocytes (p=0.031, OR: 0.861, U5% CI: 0.751-0.U86) (Table 3).

## 4. Discussion

To lower the risk of dengue-related morbidity and mortality, it is crucial to quickly identify the clinical and laboratory characteristics linked to severe dengue.[18] After an initial infection, dengue-specific antibodies start to show up on day five. By day three of most secondary infections, IgM and IgG-type antibodies are no longer visible.[19,20] However, the NS1 antigen is present in both primary and secondary infections from the very first day of dengue fever.[21] Consequently, the NS1 antigen is recognized as a specific viral characteristic and a reliable marker for dengue diagnosis.In this study, 647 (93.9%) of the 689 dengue positive cases exhibited positive NS1 findings, either on their own, in combination with IgM/IgG, or both.

Compared to the findings of Joshi A et al. and Kulkarni RD et al., this result is noticeably greater,[15,22] and less than research conducted in Nepal.[23] The endemicity may be the cause of these variations in dengue infection. According to the current study, the main reasons for the rise in dengue cases include the mosquito species Aedes spp.'s improved ability to adapt to relatively cold climates, growing urbanization, and cyclic dengue outbreaks with exponentially rising case counts.

This study provides important insights into the characteristics and makeup of dengue-positive people, as well as how they relate to hematological parameters. The results showed that single positive cases accounted for the bulk of dengue-positive cases, with dual and triple positive cases making up the minority. The greatest number of positive cases occurred in the age group of 20–2U years, followed by 30–3U years. Thirty was found to be the median age of dengue-positive people. These findings are consistent with past studies that found greater incidence rates of dengue in young people, possibly due to more outdoor activities, mosquito bite exposure, and social behaviors that promote the illness's transmission.[3,24]

The study found that the percentage of dengue-positive cases was somewhat greater in males than in females. There appears to be a gender difference in dengue infection rates, which could be explained by differences in male and female behavior, occupations, and exposure to mosquito bites.[25,26]

This study found that the sample population's hematological parameters differed. TLC was significantly lower in dengue-positive patients than in dengue-negative ones. This result is consistent with other research that found significant decreases in TLC in dengue patients.[27,28] As with Potts JA et al.,[27] and Rauniyar R et al.,[29] dengue patients had a markedly lower platelet count, according to our investigation.

Prior research has demonstrated that the primary causes of thrombocytopenia in dengue infection are elevated platelet degradation and reduced platelet production during dengue fever.[28] Our study did, however, show that dengue patients had increased RBC counts, hematocrits, and MCVs; these findings are consistent with those of previous pertinent studies conducted in Pakistan,[30] Ethiopia,[31] and Egypt.[32]

Regular hematological indicators that were potentially associated with dengue cases were investigated. The WHO states that the two most crucial tests evaluated during a dengue sickness are hematocrit and thrombocytopenia. The signs that demonstrated a substantial connection with dengue infection were leucopenia and thrombocytopenia. Several studies have shown a robust association between

**Table 2:** Hematological profile of dengue positive and negative cases

| Parameters | Dengue Negative (n= 788) Median (Q3- Q1) | Dengue Positive (n= 788) Median (Q3- Q1) | p- value † |
|---|---|---|---|
| Age (years) | 28 | 30 | 0.005 |
| Hemoglobin (gm/dl) | 14.3 | 14.5 | |
| RBC (X 1012/L) | 4.60 | 5.01 | <0.001 |
| HCT (%) | 40.3 | 44.7 | <0.001 |
| MCV (fl) | 89.7 | 90.97 | |
| MCH (pg) | 28.2 | 29.5 | <0.001 |
| MCHC (gm/dl) | 34.3 | 34.4 | <0.001 |
| TLC (cells /cumm) | 6640 | 3200 | <0.001 |
| Neutrophil (%) | 64.5 | 72 | <0.001 |
| Lymphocyte (%) | 30 | 22 | <0.001 |
| Monocyte (%) | 6.3 | 4 | <0.001 |
| Eosinophil (%) | 2 | 1 | <0.001 |
| Platelets (cells/cumm) | 30,5000 | 14,0000 | <0.00 |

**Table 3:** Binary logistic regression analysis for different parameters in overall dengue-positive patients

| Parameter | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | ORC (95% CI) | p-value | ORA (95% CI) | p-value |
| Age | | | | |
| HCT | 0.904 (0.883- 0.925) | <0.001 | | |
| MCH | 1.295 (1.222- 1.373) | <0.001 | 1.163 (1.070- 1.263) | <0.001 |
| MCHC | 2.674 (2.388- 2.995) | <0.001 | 2.085 (1.751- 2.483) | <0.001 |
| TLC | 1.001 (1.000- 1.001) | <0.001 | 1.000 (1.000- 1.000) | <0.001 |
| Neutrophil | 0.U66 (0.957- 0.975) | <0.001 | 0.816 (0.713- 0.U34) | 0.003 |
| Lymphocyte | 1.012 (1.002- 1.021) | 0.015 | 0.861 (0.751- 0.986) | 0.031 |
| Monocyte | 1.328 (1.276- 1.382) | <0.001 | | |
| Eosinophil | 1.688 (1.535- 1.857) | <0.001 | | |
| Platelets | 1.000 (1.000-1.000) | <0.001 | 1.000 (1.000-1.000) | <0.001 |

dengue illness and thrombocytopenia, which was confirmed in our study.[33,34] In a binary logistic regression research, the RBC indices (MCH, MCHC), neutrophil count, and lymphocyte count were independent predictors of dengue positivity. These findings are consistent with prior studies showing that dengue infection modifies the quantity of blood cells.[25,35]

The incapacity to use the polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA) for qualitative or quantitative detection places limitations on the current study. It has been found that ELISA and RT-PCR are more sensitive than ICT-based testing. Furthermore, there are significant disadvantages of ICT-based rapid tests, namely heightened cross-reactivity that may result in false positive results. Furthermore, neither the severity of the patient's sickness nor its clinical aspects were assessed. However, the findings of this study may offer practical routine laboratory markers for dengue identification in endemic areas, thereby improving the surveillance of the medical sciences technician diagnosing dengue.

## 5. Conclusion

Hematological outcomes, including erythrocytosis, high hematocrit, thrombocytopenia, neutrophilia, and lymphocytopenia, were revealed to be significant predictors of dengue positivity in the study. Antiviral drugs and dengue vaccinations have not yet been licensed for the treatment of dengue illnesses. For this reason, effective patient management depends on prompt diagnosis and excellent supportive care. Thus, the usefulness of comprehensive hematological markers in predicting dengue infection can be advantageous for the early diagnosis and treatment of dengue cases. However, more studies including a larger population and longitudinal investigations are required to confirm and build upon these findings.

## 6. List of Abbreviations

Interval of Confidence (CI) Dengue Virus (DENNV), Hemoglobin (Hb), Immunoglobulin G (IgG), Immunoglobulin M (IgM), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC),

Mean Cell Volume (MCV), Non-Structural Protein 1 (NS1), RBC (Red Blood Cell), and WBC (White Blood Cell) are among the terms used.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

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## Author's biography

**Priya Laljibhai Bhojani,** Resident

**Preeti Jahveri,** Associate Profesor https://orcid.org/0009-0002-7330-1915

**Krupali Patel,** Assistant Professor https://orcid.org/0009-0003-0994-557X

**Suchita Mishara,** Assistant Professor https://orcid.org/0000-0003-0636-5775

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