



## HEMATOLOGICAL ALTERATIONS IN DENGUE VIRUS INFECTION: INSIGHTS FROM NS1 ANTIGEN-POSITIVE CASES IN KASSALA STATE, SUDAN

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### ABSTRACT

**Background:** Dengue fever remains a significant public health concern in endemic regions, including Kassala State, Sudan. This study aimed to investigate hematological changes in dengue virus (DENV) NS1 Ag-positive cases and their diagnostic implications. **Methods:** A cross-sectional study was conducted in 2023 among 273 febrile patients at Kassala state, Eastern Sudan. A total of 273 febrile participants from Kassala State were included in study. All participants underwent NS1 Ag rapid testing during the acute phase of their illness to confirm early dengue virus infection. Hematological parameters, including hemoglobin (Hb), red blood cell count (RBC), total and differential white blood cell counts, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were analyzed and statistically correlated with NS1 Ag positivity. **Results:** Among the participants, 100 (36.63%) tested positive for NS1 Ag. The most common hematological abnormality observed was thrombocytopenia (73%), followed by lymphopenia (66%), leucopenia (53%), and low MCHC (56%), which were significantly associated with NS1 Ag positivity ( $p < 0.05$ ). Other findings included low Hb (45%), low RBC (20%), neutrophilia (11%), low MCV (22%), and low MCH (57%), though these were not statistically significant. **Discussion:** The findings highlight the diagnostic value of thrombocytopenia, leucopenia, and lymphopenia in dengue fever, reflecting immune dysregulation and bone marrow suppression caused by the virus. Additionally, abnormalities in MCHC suggest potential disruptions in red cell indices, warranting further investigation. These results align with global observations and reinforce the role of hematological parameters in diagnosing and managing dengue fever. **Conclusion:** This study underscores the importance of hematological profiling in early detection and management of dengue virus infection. Recognizing these patterns can guide clinical decision-making, particularly in resource-limited settings, and improve patient outcomes through timely interventions. Further research should explore the prognostic implications of these findings and their integration into predictive models for disease severity.

**KEYWORDS:** Dengue, Seroprevalence, Kassala, Sudan, Fever, Hematological changes.

### INTRODUCTION

Dengue is a highly dangerous disease, primarily spread by mosquitoes, posing significant public health risks and socioeconomic issues in tropical and semi-tropical areas. The World Health Organization (WHO) identified it as one of the most dangerous diseases in 2019, with over 3.9 billion people in 128 nations at risk.<sup>[1,2]</sup> Dengue fever has an estimated 390 million cases annually, with

severity and prevalence varying by year and location.<sup>[3]</sup> Factors contributing to the increase include insufficient mosquito control, global warming, and inadequate medical facilities. The virus infects around 100 million individuals annually, resulting in around 22,000 deaths.<sup>[4]</sup>

Dengue epidemics have become widespread across Africa, with 24 out of 54 nations having documented

cases. Since 1960, every subregion has experienced epidemics triggered via each of the four-dengue virus serotypes. Dengue fever is frequently detected in travellers returning from over 30 African countries, with Central Africa having high rates of infection.<sup>[5]</sup>

Dengue virus infection is influenced by environmental conditions, climate change, and geographic location in urban endemic areas. Susceptibility is higher in children and young adults, and previous infections can increase severity.<sup>[6]</sup> Travel to endemic regions also increases infection rates. Dengue fever is associated with haematological changes like thrombocytopenia, leukopenia, and haemoconcentration due to increased vascular permeability. These changes are crucial for early diagnosis and severity assessment, as they can lead to hypovolemic shock. These changes are vital for early diagnosis and assessment of the disease.<sup>[7]</sup>

Sudan's dengue prevalence is estimated at 27%, with cross-sectional studies showing 26% and 30% prevalence. Regional estimates vary by 23%, 24%, 36%, and 43% in different regions.<sup>[8]</sup> Dengue epidemics have been more frequent in eastern Sudan, particularly in Kassala state, with severe illness manifestations and fatalities. Dengue IgG and IgM antibodies prevalence in Kassala state is 9.4% and 0.6%, respectively.<sup>[9]</sup>

Four distinct serotypes of the dengue virus, DENNV-1, DENV-2, DENV-3, and DENV-4, which share a 65% genome similarity, have been identified based on their antigenic differences. A fifth serotype, DENV-5, has recently been discovered in Malaysia and is frequently identified in non-human primates. Based on the sequence variation in the envelope (E) gene, these four genotypes within each serotype [DENNV-1 (I-V); DENV-2 (Asian I, Asian II, Asian/American, American, Cosmopolitan, and sylvatic); DENV-3 (I-IV); and DENV-4 (I, II, III and Sylvatic)] can be phylogenetically defined.<sup>[10]</sup> It is recognized that all serotypes coexist and flourish in the same biological and geographical regions.<sup>[11]</sup>

## MATERIALS AND METHODS

This cross-sectional study investigated the prevalence of dengue virus (DENV) infection during a 2023 outbreak in Kassala State, Eastern Sudan. The study population included all febrile patients seeking treatment at healthcare facilities in the region during the outbreak. Approval was obtained from the Health Research Ethics Committee of the Ministry of Health and Social Development, Kassala State. Informed consent was secured from adult participants or from parents/legal guardians for children.

### Sample size

Based on a prior report indicating a 23% prevalence of DENV among febrile patients in Kassala State, the sample size was determined accordingly.<sup>[12]</sup>

### Sample collection

Acute-phase blood samples were obtained from participants suspected of having dengue fever. A total of 5 mL of venous blood was collected under aseptic conditions and transferred to sterile ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Plasma was separated by centrifugation at 3000 rpm for 5 minutes at room temperature and aliquoted into labeled Eppendorf tubes for testing.

### NS1 antigen detection

The presence of DENV NS1 antigen was determined using the OnSite Dengue Ag rapid test (SD BIOSENSOR). Each test device was labeled with the corresponding specimen ID number.

**Procedure:** A volume of 60  $\mu$ L of plasma (or two drops) was added to the specimen well. Immediately, 40  $\mu$ L (or one drop) of sample diluent was added vertically to the center of the sample well. The test result was read after 15–20 minutes, ensuring adherence to the recommended waiting period for accuracy.

### Interpretation of assay result

**Negative result:** Only the control (C) line appeared, indicating no detectable dengue NS1 antigen.

**Positive:** Both the control (C) and test (T) lines appeared, indicating the presence of dengue NS1 antigen.

**Invalid:** If no control (C) line developed, the test was considered invalid regardless of the appearance of the test (T) line.

### Statistical analysis

The data collected in this study was entered into a computer and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 28 (SPSS Inc., Chicago, IL, USA). Binary and logistic regression analyses were performed to interpret the results and assess relationships between variables. Odds ratios (OR) with 95% confidence intervals (CI) were calculated, and statistical significance was set at  $P < 0.05$ . Numerical data was presented as frequencies and proportions, while quantitative data was analyzed using repeated responses and expressed as mean  $\pm$  standard deviation.

## RESULT

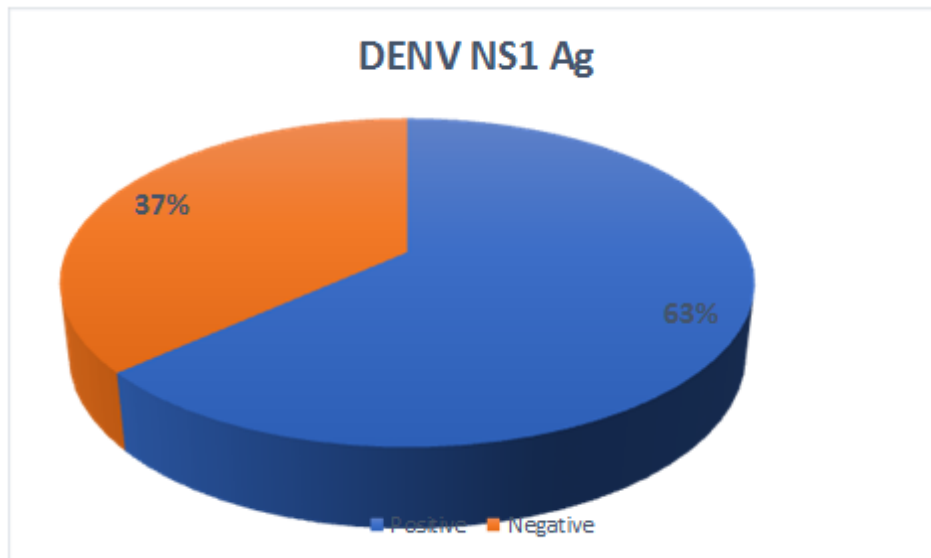
Two hundred seventy-three participants from Kassala state were included in this study, and all of whom had experienced fever. The mean age with standard error of the participants was  $30.4 \pm 0.84$  years, ranging from 2 to 74 years.

All participants were included in the study and underwent NS1 Ag rapid testing to detect early infection during the acute phase of the disease. Among these participants, 100 (36.63%) tested positive for NS1Ag (**Figure 1**).

Hematological changes noticed in DENV NS1 Ag positive were as follows: Low Hb 45(45%), low RBCs

20(20%), leucopenia 53(53%), lymphopenia 66(66%), neutrophilia 11 (11%), thrombopenia 73(73%), low MCV 22(22%), low MCH 57(57%), and low MCHC 56(56%), The correlations were ( $X^2=0.594$ ,  $P=0.451$ ), ( $X^2=0.558$ ,  $P=0.520$ ), ( $X^2=13.6$ ,  $P=0.003$ ), ( $X^2=2.9$ ,

$P=0.001$ ), ( $X^2=1.21$ ,  $P=0.315$ ), ( $x_2=81.5$ ,  $P=1.4$ ), ( $X^2=0.44$ ,  $P=0.888$ ), ( $X^2=3.50$ ,  $P=0.078$ ), and ( $X^2=4.44$ ,  $P=0.044$ ), respectively. A significant association was seen in leucopenia, lymphopenia, and MCHC (**Table 1**).



**Figure 1: Incidence of Positive and Negative results for the DENVNS1 Ag test.**

**Table 1: Associations of Hematological and Biomarkers data with infected Dengue viruses by NS1 Ag among attended patients in Kassala State.**

Variable	Positive N(%)=100	Negative N(%)=173	Total 273 (100%)	X <sup>2</sup>	P
Low HB	45(45)	69(39.9)	114(41.8)	.594	0.451
Low RBCs	20(20)	29(16.8)	49(17.9)	0.558	0.520
Leucopenia	53(53)	41(23.7)	94(34.4)	13.6	0.003
Lymphopenia	66(66)	76(43.9)	142(52)	2.90	0.001
Thrombocytopenia	73(73)	26(15)	99(36.3)	81.5	1.44
Neutrophilia	11(11)	24(13.9)	35(12.8)	1.21	0.315
Low MCV	22(22)	40(23.1)	62(22.7)	0.44	0.888
Low MCH	57(57)	80(46.2)	137(50.1)	3.50	0.078
Low MCHC	56(56)	72(41.6)	128(46.2)	4.44	0.044

**DISCUSSION**

The findings of this study, conducted on 273 febrile participants in Kassala State, highlight significant hematological changes associated with dengue virus infection, as confirmed by NS1 Ag positivity in 37% of participants. This result aligns with local data indicating a high prevalence of dengue in endemic regions, particularly east Sudan.<sup>[8,13]</sup>

Thrombocytopenia was the most prominent hematological abnormality (73%) observed among NS1 Ag-positive cases. This finding is consistent with prior studies that identify thrombocytopenia as a hallmark of dengue infection, often resulting from increased peripheral destruction of platelets, reduced production, and immune-mediated mechanisms.<sup>[14]</sup>

Leucopenia (53%) and lymphopenia (66%) were significantly associated with NS1 Ag positivity ( $p =$

0.003 and  $p = 0.001$ , respectively). Leucopenia is a critical diagnostic marker, reflecting bone marrow suppression due to viral replication.<sup>[15,16]</sup> Lymphopenia, frequently noted in dengue, indicates the immunosuppressive effects of the virus, potentially exacerbating disease severity.<sup>[7]</sup>

Abnormalities in MCHC (56%) also showed a significant correlation ( $p = 0.044$ ) with NS1 Ag positivity. This finding is less commonly reported in the literature but suggests that dengue infection may influence red cell indices, potentially through plasma leakage and hemoconcentration.<sup>[17]</sup>

Other hematological parameters, such as low hemoglobin (45%), low RBCs (20%), and low MCV (22%), while not statistically significant in this study, are consistent with observations from other studies. These parameters

often reflect the systemic effects of dengue, including plasma leakage and hemodynamic changes.<sup>[18]</sup>

The lower prevalence of neutrophilia (11%) underscores the viral nature of the infection, as bacterial co-infections are rare during the acute phase of dengue.<sup>[19]</sup>

### Implications for Diagnosis and Management

These hematological findings emphasize the critical role of laboratory parameters in diagnosing and managing dengue fever. Recognizing patterns such as thrombocytopenia, leucopenia, and lymphopenia can guide clinicians in early detection, risk stratification, and targeted management of patients. Further studies should investigate the clinical implications of changes in red cell indices, such as MCHC, to understand their diagnostic and prognostic significance.

### CONCLUSION

This study highlights the significant hematological changes associated with dengue virus infection in Kassala State, Sudan. Among NS1 Ag-positive cases, thrombocytopenia, leucopenia, and lymphopenia emerged as the most prominent abnormalities, with statistically significant associations underscoring their diagnostic relevance. These findings corroborate existing literature and emphasize the critical role of hematological parameters in diagnosing and managing dengue, particularly in resource-limited settings where advanced diagnostic tools may be unavailable. Additionally, the observed alterations in red cell indices, such as MCHC, suggest potential areas for further research to elucidate their clinical significance in dengue pathophysiology. Early recognition of these hematological patterns can improve patient outcomes through timely interventions and risk stratification. Future studies should focus on longitudinal assessments and the integration of these parameters into predictive models for disease severity and outcomes.

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