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Leukopenia and thrombocytopenia in dengue patients: a cross-sectional study from a tertiary hospitals in Koshi Province, Nepal

Birendra Prasad Gupta[1*], Surendra Uranw[2], Vivek Prasad Gupta[1], Elisha Deuba[3], Ajay Kumar Sah[3], Santosh Chaudhary[4] and Chandramani Wagle[3]

Abstract

Introduction Dengue fever remains a public health challenge in Nepal, with hematological abnormalities like leukopenia and thrombocytopenia often observed. However, their prevalence and clinical significance outside the Kathmandu Valley remain poorly characterized. This study examines these markers in Koshi Province, a region prone to recurrent outbreaks but with limited data, to enhance regional understanding and early severity detection.

Methods A cross-sectional study was conducted across four tertiary hospitals in Sunsari district, Koshi province of Nepal during the 2022 dengue outbreak. A convenience sample of 325 laboratory-confirmed dengue patients (NS1 antigen/IgM/IgG positivity) was enrolled. Data on demographics, clinical features, leukocytes count, and platelet counts were collected using Case Report Form (CRF). Hematological parameters (leukocyte count < 4,000 cells/mm^3; and platelet count < 150,000 cells/mm^3) were analyzed alongside clinical severity (WHO 2009 classification: DWWS, DWS, SD). Chi-square tests, adjusted residuals and logistic regression (adjusted for age and diabetes) were used to assess associations between these hematological markers and dengue severity. Additional analyses were performed to examine associations of leukopenia and thrombocytopenia with demographic characteristics (e.g., age, sex) and clinical symptoms (e.g., joint pain, rash, retro-orbital pain, nausea).

Results Leukopenia was observed in 21.5% of patients, while thrombocytopenia occurred in 62.1%. Leukopenia showed a significant association with DWS (χ^2=5.481, p = 0.019; Cramér's V = 0.13), with adjusted residuals highlighting its stronger link to DWS (AR = 3.40). Thrombocytopenia, though prevalent, was more common in milder cases (DWWS). Logistic regression confirmed leukopenia's association with severity (OR = 0.9999, p = 0.011), while thrombocytopenia paradoxically correlated with slightly increased severity odds (OR = 1.000005, p = 0.023). Leukopenia was significantly associated with symptoms such as joint pain, nausea, and rash, while thrombocytopenia was linked to retro-orbital pain and abdominal discomfort. No significant associations were observed between these hematological abnormalities and sex or age group.

Conclusion This study highlights leukopenia as an early marker of dengue severity in Koshi province, while challenging thrombocytopenia's assumed role in disease progression. Despite limitations from convenience

*Correspondence:
Birendra Prasad Gupta
birendraphd@gmail.com

Full list of author information is available at the end of the article

sampling and single-timepoint data, this study provides actionable insights for risk stratification in regional dengue management. Future longitudinal studies are needed to validate these associations and refine multivariable risk models.

Clinical trial registration details Clinical trial number: not applicable.

Keywords Dengue, Leukopenia, Thrombocytopenia, Severity of dengue, Eastern Nepal

Introduction

Dengue fever, a mosquito-borne viral disease caused by the dengue virus, is a significant public health concern in tropical and subtropical regions, including Nepal [1–4]. The disease is caused by four antigenically distinct serotypes of dengue virus (DENV 1–4) belonging to the family *Flaviviridae* and genus *Flavivirus*. Dengue viruses are transmitted to human by the bite of *Aedes aegypti* and *Aedes albopictus*. Dengue infections range from asymptomatic cases to severe manifestations such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can result in life-threatening complications [1].

Dengue fever is characterized by febrile illness with severe headache, retro-orbital pain, myalgia or arthralgia, nausea or vomiting, skin rashes while DHF is characterized by high fever lasting for 2 to 7 days, hemorrhagic manifestations due to plasma leakage, and thrombocytopenia. Severe plasma leakage can lead to circulatory failure and shock, a condition known as DSS, which may result in death. Hematological abnormalities such as Leukopenia, defined as a reduction in leukocyte count (< 4,000 cells/mm^3), often indicates disease progression and are associated with plasma leakage and severe dengue. Similarly, Thrombocytopenia, characterized by a reduction in platelet count (< 150,000 cells/mm^3) serves as a key marker of bleeding risk and dengue severity [5, 6].

Dengue was first detected in Nepal in 2004 in a foreigner in Chitwan in 2004 and subsequently the larger outbreak occurred in 2006 and 2010 [7, 8]. Thereafter, dengue outbreak reported annually in different parts of the country. In 2019, Sunsari district faced its worst ever outbreak of dengue, with over 3000 cases and it has been reported annually in 2022 and 2023 in different districts of Koshi Province of Eastern Nepal [9, 10]. In 2024, Nepal reported 34,385 dengue cases, with Koshi Province accounting for 2,067 cases (6% of the national burden). Recent genomic studies have identified DENV-1 (genotype-V), DENV-2 (Cosmopolitan-IVa), and DENV-3 (genotype-III) as circulating serotypes during the 2022 outbreak, with phylogenetic links to strains from India and China [11]. These serotypes, particularly DENV-2 and DENV-3, are associated with more severe clinical manifestations and hematological abnormalities, including leukopenia and thrombocytopenia [12–14].

Despite repeated outbreaks, Nepal's public health system lacks robust diagnostic facilities, leading to dengue diagnoses based primarily on clinical symptoms [15]. This reliance on symptom-based diagnosis rather than laboratory confirmation limits early detection of severe cases and delays appropriate management [16]. Previous studies have shown that, leukopenia and thrombocytopenia are prevalent in 25–82% and 40–89% of dengue cases, respectively [17–21]. While prior studies in Nepal have predominantly examined hospitalized or severe dengue cases in urban settings such as Kathmandu, data remain scarce for outpatient populations particularly in high-burden rural provinces like Koshi, where distinct DENV serotypes (e.g., DENV-1 and DENV-3) circulate and resource limitations complicate early severity detection [19, 21].

This study aims to determine the prevalence and clinical significance of leukopenia and thrombocytopenia among the laboratory confirmed dengue patients attending medical care at four hospitals in Koshi Province, Nepal. These two parameters were selected due to their established roles as key prognostic indicators in dengue, with leukopenia often preceding severe manifestations and thrombocytopenia being a critical marker for bleeding risk and disease severity. These parameters are routinely monitored in clinical practice for dengue management and are critical for identifying patients at risk of complications, particularly in outpatient settings. By providing comprehensive data from an understudied region, this work aims to strengthen evidence-based approaches for dengue monitoring and early intervention in similar resource-constrained settings.

Methods

Study design and setting

A cross-sectional study was conducted in four tertiary care hospitals in Sunsari district, Koshi Province Nepal, from September to December 2022, during a seasonal dengue outbreak.

The study sites included Bijayapur Hospital (Dharan), Sunkoshi Laboratory (Dharan), B.P. Koirala Institute of Health Sciences (Dharan) and Itahari Municipal Hospital (Itahari). Figure 1 illustrates the geographical locations of the study sites.

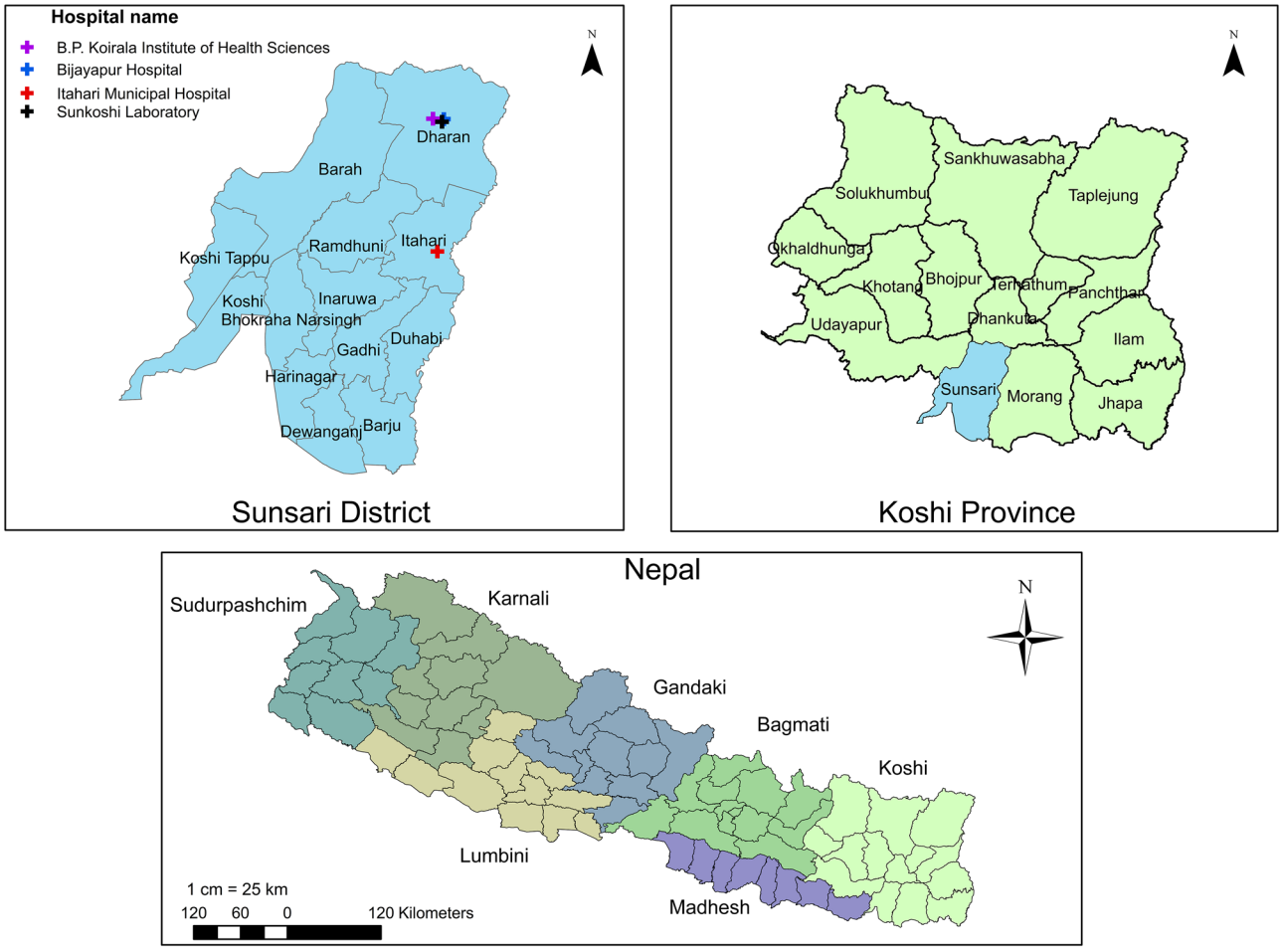

Fig. 1 **A**. Map of data collection sites for study in Sunsari district. **B**. Map of data collection sites for study in Sunsari. **C**. Map of data collection sites for study in Sunsari district district

Study population and patient enrollment

Patients presenting to the Outpatient Department (OPD) of participating hospitals with a history of fever for ≤72 h and clinical symptoms suggestive of dengue were screened for enrollment. Dengue was confirmed through NS1 antigen or IgM/IgG positivity. A total of 325 patients meeting these criteria were enrolled after providing written informed consent themselves or through their guardians. Patients with suspected dengue who did not have upper respiratory symptoms were considered eligible. Patients were excluded, if they had localizing symptoms suggesting an alternative diagnosis (e.g., pneumonia, otitis), known HIV infection, chronic illnesses (e.g., liver or kidney disease, malignancy), were on immunosuppressive therapy, or were pregnant.

A convenience sampling approach was used to enroll all eligible patients presenting to hospital outpatient departments (OPDs) during the study period. While this method ensured timely data collection during the outbreak, it introduces potential selection bias, as only patients who sought hospital care, typically those with moderate to severe symptoms were included. This may underrepresent mild/asymptomatic dengue cases managed at home, limiting generalizability to the broader population. A post-hoc power calculation was performed using G*Power 3.1 [22], based on prevalence estimates of leukopenia (64.68%) and thrombocytopenia (40.48%) from a Nepalese study [17]. Using a chi-square test with a 5% type I error rate and effect sizes derived from observed associations (Cohen's $w = 0.229$ for leukopenia, $w = 0.417$ for thrombocytopenia), the sample size of 325 patients provides 91.4% power for leukopenia and 99.9% power for thrombocytopenia to detect associations with disease severity, ensuring adequate statistical validity.

The research was conducted in accordance with the Declaration of Helsinki and ethical approval was obtained from the Nepal Health Research Council, Kathmandu, Nepal (Approval No. 678). Confidentiality was maintained through data anonymization, with personal identifiers replaced by sample codes.

Data collection

A structured case report form (CRF) was developed for this study that was used to collect the following data at enrollment of study participants. CRF collected demographic data, clinical features, medical history, dengue confirmation test, baseline laboratory results (e.g., CBC, liver function tests), and treatment details (e.g., medications, fluid therapy). Trained research assistants conducted face-to-face interviews using a standardized protocol under the supervision of the principal investigator.

Laboratory procedures

Dengue fever was confirmed using rapid diagnostic test (RDT) kits, primarily SD BIOLINE Dengue Duo NS1 Ag + Ab Combo, which detects NS1 antigen and IgG/IgM antibodies across all clinical stages. In some settings, Dengucheck Combo (EU, CE-certified) was also used for confirmation. Following diagnosis, hematological and biochemical analyses were conducted using standard laboratory instruments at the respective hospitals. The DIASYS Response 910 automated biochemistry analyzer was used for clinical chemistry assessments. All facilities followed standardized protocols for blood sample collection, handling, and analysis. Equipment calibration and reagent validation were conducted regularly in accordance with hospital laboratory standards.

Disease classification

Patients were classified using the 2009 World Health Organization (WHO) dengue case classification as dengue without warning signs (DWWS) and dengue with warning signs (DWS) [6]. The 2009 WHO dengue classification system was applied in accordance with Nepal's national clinical guidelines and to ensure consistency with regional studies in South Asia that predominantly utilize this framework. Those categorized as DWWS presented with fever and at least two additional features, such as nausea or vomiting, rash, aches and pains, leukopenia, a positive tourniquet test, and had laboratory-confirmed dengue, while maintaining adequate oral intake and urine output. In contrast, patients classified as DWS exhibited at least one clinical warning sign such as, abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy or restlessness, liver enlargement (> 2 cm), or hematological changes such as increased hematocrit with concurrent platelet count ≤ 100,000/mm^3. The presence of certain comorbidities (e.g., pregnancy, infancy, advanced age, diabetes, renal failure) or social factors (e.g., living alone or far from a hospital) also qualified patients for the DWS category [6].

Thrombocytopenia was characterized by a platelet count below 150,000 cells/mm^3. The condition was further categorized as mild (> 100,000–<150,000), moderate (> 50,000–100,000), or severe (< 50,000) [23]. Leukopenia was defined as a total white blood cell (leukocyte) count of lower than 4,000 cells/mm^3 [5].

Data management and analysis

Data were entered into Microsoft Excel and analyzed using Python (v3.9) with libraries including pandas, SciPy, and statsmodels etc. Patients with missing laboratory results were excluded from statistical analyses to ensure data completeness. Continuous variables, such as leukocyte and platelet count, were tested for normality using the Shapiro-Wilk test; since they were non-normally distributed, they were reported as medians with interquartile ranges (IQR). Categorical variables, including dengue severity categories (DWWS, DWS) and presence of leukopenia or thrombocytopenia, were presented as frequencies and percentages.

Associations between hematological parameters and dengue severity were determined using chi-square (χ^2) tests, with post-hoc adjusted residuals to identify group specific differences. Effect sizes were quantified using Cramer's V, where values of 0.1, 0.3, and 0.5 represents small, moderate, and large effects, respectively. In addition, chi-square or fisher's exact test were also used to explore associations between leukopenia and thrombocytopenia with key demographic and clinical characteristics.

Binary logistic regression was performed to model the relationship between hematological parameters (leukocyte and platelet counts) and disease severity, adjusting for age, sex, and comorbidities (diabetes). Continuous variables (leukocyte and platelet count) were included in the models without categorization. No subgroup analysis or sensitivity analysis was conducted in this study. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated, and statistical significance was set at $p < 0.05$.

Results

Patients' demographics and clinical characteristics

A total of 325 patients with laboratory-confirmed dengue were enrolled across four health facilities from September to December 2022. Sociodemographic characteristics of the study population are summarized in Table 1. Gender distribution was nearly equivalent (52.9% male, 47.1% female). Age was categorized into three groups as: Child (≤ 14 years), Adult (15–59 years), and Elderly (≥ 60 years). The adult group constituted the majority at 73.5% ($n = 239$) followed by children (12.6%) and elderly individuals (13.9%). Among adults ≥ 20 years ($n = 259$), body mass index (BMI) classifications using WHO criteria indicated that the majority had a normal BMI (63.7%), while 25.9% were overweight (Table 1).

Table 1 Baseline characteristics of patients (*N* = 325)

Baseline characteristics	Frequencies	Percentage (%)
Age, median (IQR)	36 (23–51)	-
Child (≤ 14 years)	41	12.6
Adult (15 to 59 years)	239	73.5
Elderly (≥ 60 years)	45	13.9
Gender		
Male	172	52.9
Female	153	47.1
BMI for Adults (≥ 20 years, *n* = 259)		
Healthy weight (18.5–24.9)	165	63.7
Overweight (25–29.9)	67	25.9
Underweight (< 18.5)	18	6.9
Obese (30 ≤)	9	3.5

Table 2 Clinical characteristics of patients (*N* = 325)

Clinical characteristics	Prevalence, *n* (%)	Severity, % (Mild/Moderate/Severe)	Duration, % (Short*/ Moderate**)
Fever	322 (99.1)	–	26.1 / 73.9
Headache	175 (53.8)	19.4 / 80.6 / 0	64 / 36
Muscle Pain	183 (56.3)	3.8 / 79.2 / 17	16.4 / 83.6
Joint Pain	129 (39.7)	6.2 / 80.6 / 13.2	22.5 / 77.5
Retroorbital Pain	76 (24.6)	36.8 / 63.2 / 0	67.1 / 32.9
Rash	18 (5.5)	100 / 0 / 0	88.9 / 11.1
Chest Pain	19 (5.8)	94.7 / 5.3 / 0	89.5 / 10.5
Gastrointestinal symptoms			
Vomiting	43 (13.2)	–	100 / 0
Nausea	73 (22.5)	63 / 37 / 0	91.8 / 8.2
Diarrhea	31 (9.5)	61.3 / 38.7 / 0	83.9 / 16.1
Abdominal Pain	21 (6.5)	38.1 / 61.9 / 0	100 / 0

*Short duration: 1–3 days; Moderate duration: 4 – 7 days. **Severity graded as mild, moderate, or severe based on WHO 2009 guidelines

At enrollment, vital signs indicated a median temperature of patients was 37.2 °C (IQR 36.7–38.3), reflecting the febrile nature of dengue, with a median systolic blood pressure of 120 mmHg (IQR 110–130) and diastolic of 80 mmHg (IQR 70–90). The median pulse rate was 74 beats per minute (IQR 70–78), and the median respiratory rate was 20 breaths per minute (IQR 18–24), both within typical ranges for this cohort. The most common presenting symptom among dengue patients was fever (99.1%), followed by headache (53.8%), myalgia (56.3%), and arthralgia (39.7%). Gastrointestinal symptoms, including nausea (22.5%) and vomiting (13.2%), were frequently reported. When stratified by age group, vomiting was more commonly reported among children (22.0%) compared to adults (12.1%) and elderly individuals (11.1%). Nausea was most frequent among adults (24.3%), followed by elderly (22.2%) and children (12.2%). Rashes were reported in 18 participants (5.5%), all of which were mild in severity. Among those with rashes, Majority (88.9%) had rash onset within three days of fever onset (short duration). All cases were erythematous, and 77.8% also exhibited maculopapular features. Detailed clinical characteristics are presented in Table 2.

Most symptoms were of moderate severity, with fever and muscle pain lasting longer (73.9% and 83.6% moderate duration, respectively) (Table 2). No cases of clinically significant bleeding manifestations (e.g., epistaxis, gum bleeding, gastrointestinal bleeding, petechiae), edema, ascites, hepatomegaly (> 2 cm), pleural effusion, or neurological complications were observed among patients.

Diagnostic methods, disease severity, and treatment

Laboratory confirmation of dengue was primarily achieved through NS1 antigen testing (65.2%), followed by IgG serology (39.4%,). IgM positivity was rare (0.6%). Using WHO 2009 criteria, participants were classified as dengue without warning signs (DWWS) (60%) or dengue with warning signs (DWS) (40%); no cases met the criteria for severe dengue (SD). Consequently, associations between hematological markers and progression to SD could not be assessed. (Fig. 2)

Symptomatic medications (e.g., antipyretics, analgesics) were administered to nearly all patients (96.3%), while intravenous (IV) fluid therapy was required in a small subset (5.5%). These cases were managed in the OPD and received fluids as supportive care for early dehydration from fever, nausea, or vomiting.

Prevalence of leukopenia and thrombocytopenia

The prevalence of leukopenia was found as 21.5% (70/325), with the highest occurrence observed in the adult age group (22.6%), followed by the elderly (20.0%) and children (17.1%), as shown in Table 3. The median leukocyte count was 5400 cells/mm^3 (IQR: 4160–7100), which falls within the normal range, indicating that leukopenia was not the predominant finding in the overall population.

Thrombocytopenia was more common, identified in 62.1% (202/325) of patients. Among those with thrombocytopenia, mild severity was the most frequent (58.0%), followed by moderate (36.1%) and severe cases (5.9%). The adult age group exhibited the highest prevalence of thrombocytopenia (74.7%), followed by the elderly (14.4%) and children (10.9%). The median platelet count was 130,000 cells/mm^3 (IQR: 99,000–170,000), reflecting a tendency toward thrombocytopenia in the study population. Figure 3 further illustrates the distribution of leukocyte and platelet counts stratified by dengue severity categories (DWWS and DWS).

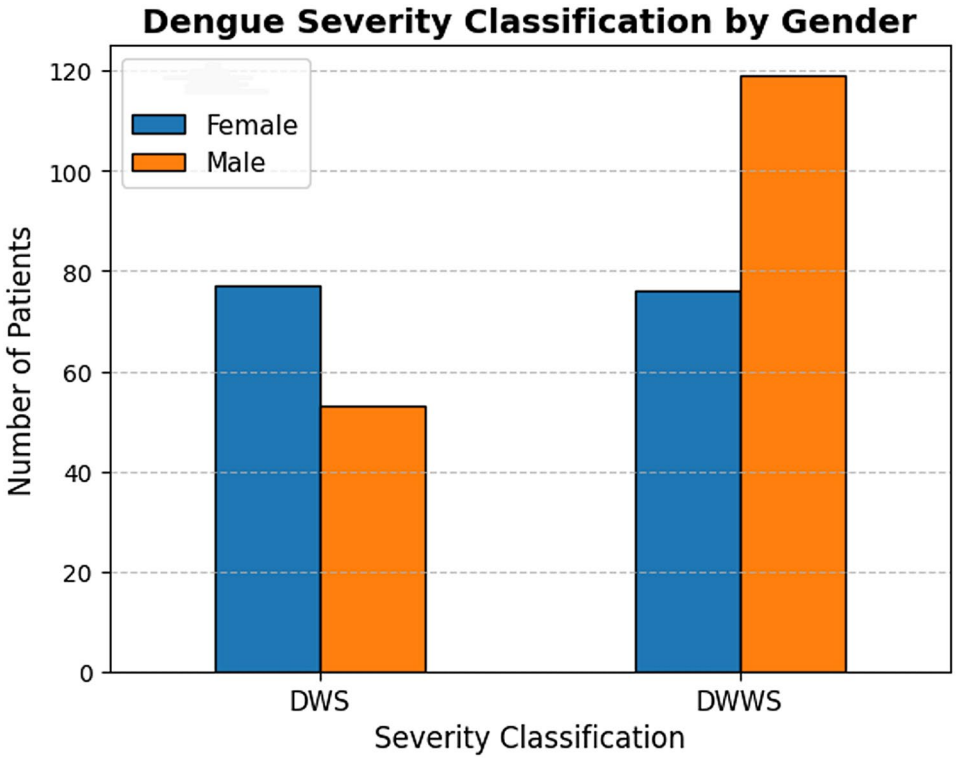

Fig. 2 Severity classification based on gender

Table 3 Prevalence of leukopenia and thrombocytopenia ($N = 325$)

Hematological Parameter (cells/mm^3)	Frequencies	Percentage (%)
Leukocyte count, Median (IQR)	5400 (4160–7100)	
Leukopenia	70	21.5
Normal	255	78.6
Leukopenia by age group ($n = 70$)		
Child (≤ 14 years)	7	10
Adult (15 to 59 years)	54	77.1
Elderly (≥ 60 years)	9	12.9
Platelet count, Median (IQR)	130,000 (99000–170000)	
Thrombocytopenia	202	62.1
Normal	123	37.9
Severity of Thrombocytopenia ($n = 202$)		
Mild	117	58
Moderate	73	36.1
Severe	12	5.9
Thrombocytopenia by age group ($n = 202$)		
Child (≤ 14 years)	22	10.9
Adult (15 ≤ to ≥ 59 years)	151	74.7
Elderly (≤ 60 years)	29	14.4

Further analysis was conducted to examine associations between leukopenia and thrombocytopenia with key demographic and clinical characteristics (Table 4). Neither leukopenia nor thrombocytopenia showed significant association with age group or gender. However, certain clinical features exhibited notable differences. Leukopenia was significantly more frequent among patients reporting joint pain (32.6% vs. 14.3%, $p = 0.0002$), muscle pain (26.2% vs. 15.5%, $p = 0.0279$), retroorbital pain (31.6% vs. 18.5%, $p = 0.023$), and rash (50.0% vs. 19.9%, $p = 0.0057$). Similarly, thrombocytopenia was significantly more prevalent among those experiencing headache (68.6% vs. 54.7%, $p = 0.0138$), vomiting (41.9% vs. 65.2%, $p = 0.0055$), nausea (45.2% vs. 67.1%, $p = 0.0011$), and abdominal pain (86.4% vs. 60.4%, $p = 0.028$). These findings highlight potential clinical indicators that may be associated with hematological abnormalities in dengue patients (Table 4).

Association of leukopenia and thrombocytopenia with dengue severity

A Chi-square test revealed a significant association between dengue severity and both leukopenia ($\chi^2 = 5.481$, $p = 0.019$, $df = 1$) and thrombocytopenia ($\chi^2 = 4.714$, $p = 0.030$, $df = 1$). These findings indicate that both lower white blood cell and platelet counts are linked to increased dengue severity. The detailed results of the Chi-square test are presented in Table 4. Although

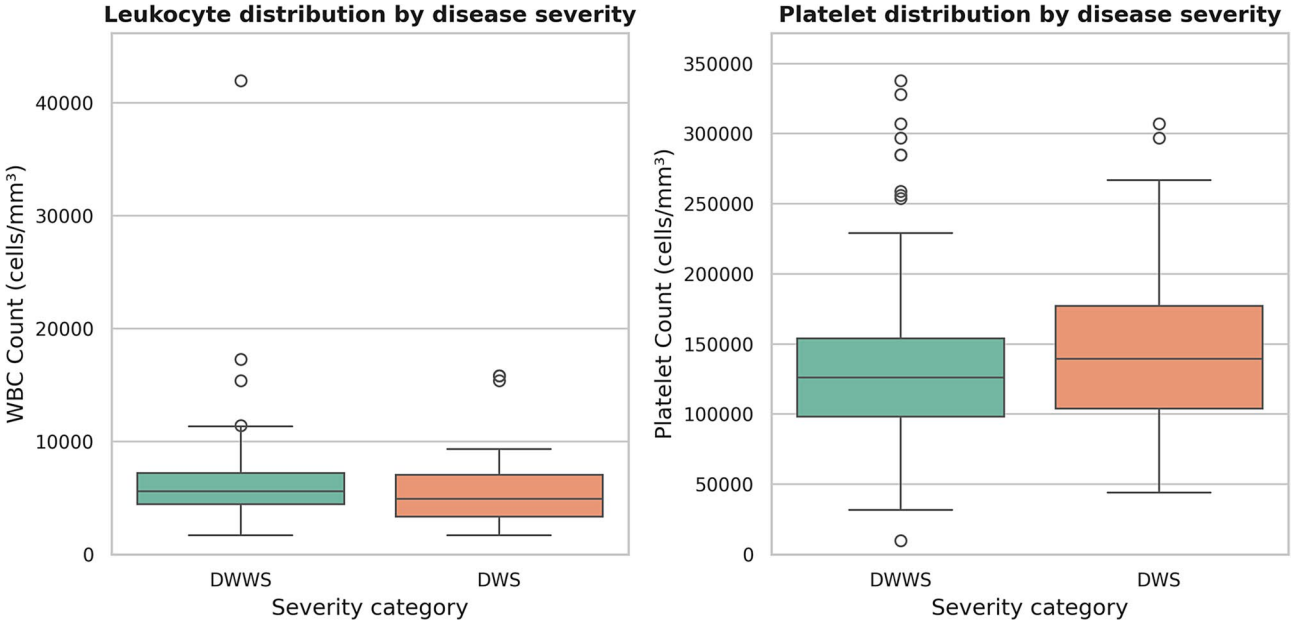

Fig. 3 Distribution of leukocyte and platelet based on severity categories (DWWS and DWS)

the Chi-square tests indicated significant associations between dengue severity and the presence of leukopenia as well as thrombocytopenia, Cramér's V values of 0.13 (leukopenia) and 0.12 (thrombocytopenia) indicate small effect sizes, suggesting modest but clinically relevant associations with dengue severity (Table 5). These findings highlight the clinical relevance of these hematological parameters in assessing dengue severity.

Further, the Post-hoc Adjusted Residuals (AR) analysis provides additional insights into the specific relationships between hematological parameters and dengue severity (Table 6). Adjusted residuals greater than 2 indicate significant differences between observed and expected counts, with positive values suggesting that the observed count exceeds the expected count, and negative values indicating the opposite. The adjusted residuals analysis indicates that leukopenia is more strongly associated with DWS showing a significant positive residual (AR = 3.4) and a negative residual (AR = -2.78) for DWWS. Conversely, thrombocytopenia was more prevalent in DWWS, with a positive residual (AR = 1.78) when present and a negative residual (AR = -2.28) when absent. These patterns suggest that leukopenia may be a stronger indicator of DWS, while thrombocytopenia is more characteristic of DWWS, providing valuable insights for clinical assessment. Logistic regression analysis was performed to quantify the associations between hematological parameters and dengue severity (Table 6).

Logistic regression revealed a statistically significant association between dengue severity and both leukocyte count (coefficient = -0.0001, $p = 0.011$) and platelet count (coefficient = 0.000005, $p = 0.023$). age and comorbidities such as diabetes were considered as confounders in the analysis (Table 7). Lower leukocyte counts were associated with higher odds of severe dengue (OR = 0.9999, 95% CI: -0.0001 to -0.00003), consistent with the known role of leukopenia as a marker of disease progression. Conversely, higher platelet counts were associated with a slight increase in the odds of severe dengue (OR = 1.000005, 95% CI: 0.000001 to 0.000009). Variance inflation factor (VIF) analysis confirmed no multicollinearity among the predictor variables (VIF < 2 for all) (Table 7).

The model demonstrated a statistically significant fit (LLR p-value = 0.0048), but its low explanatory power (pseudo-R-squared = 0.024) indicates that leukocyte and platelet count alone explain only a small proportion of the variance in dengue severity (Table 7). These results highlight the need for multivariable models incorporating additional predictors to better understand the determinants of dengue severity.

Discussion

This cross-sectional study investigated the prevalence and clinical significance of leukopenia and thrombocytopenia among 325 dengue patients presented to a OPD of four hospitals in Eastern, Nepal. The findings demonstrated that leukopenia was present in 21.5% of patients, while thrombocytopenia was observed in 62.1%, with mild thrombocytopenia being the most common (58%). Both hematological abnormalities were significantly associated with dengue severity, as demonstrated by chi-square tests (leukopenia: $\chi^2 = 5.481$, $p = 0.019$, Cramér's V = 0.13; thrombocytopenia: $\chi^2 = 4.714$, $p = 0.03$, Cramér's

Table 4 Association of leukopenia and thrombocytopenia with demographic and clinical characteristics ($N = 325$)

Variable	Category	Leukopenia *n* (%)	Thrombocytopenia *n* (%)	*p*-value (L)	*p*-value (T)
Age group	Adult (15–59)	54 (22.6)	151 (63.2)	0.7031	0.4806
	Elderly (≥ 60)	9 (20)	29 (64.4)		
	Child (≤ 14)	7 (17.1)	22 (53.7)		
Gender	Male	36 (20.9)	111 (64.5)	0.7885*	0.3614*
	Female	34 (22.2)	91 (59.5)		
BMI category	Healthy weight	40 (24.2)	106 (64.2)	0.2872	0.9719
	Overweight	17 (25.4)	42 (62.7)		
	Extreme (Underweight & Obese)	3 (11.1)	17 (63)		
Fever	Yes	69 (21.4)	199 (61.8)	0.5182*	0.2921*
	No	1 (33.3)	3 (100)		
Headache	Yes	42 (24)	120 (68.6)	0.3027	0.0138
	No	28 (18.7)	82 (54.7)		
Muscle pain	Yes	48 (26.2)	114 (62.3)	0.0279	1.0000
	No	22 (15.5)	88 (62)		
Joint pain	Yes	42 (32.6)	84 (65.1)	0.0002	0.4375
	No	28 (14.3)	118 (60.2)		
Retroorbital pain	Yes	24 (31.6)	46 (60.5)	0.0230	0.8422
	No	46 (18.5)	156 (62.7)		
Rash	Yes	9 (50)	15 (83.3)	0.0057*	0.0977
	No	61 (19.9)	187 (60.9)		
Chest pain	Yes	4 (21.1)	9 (47.4)	1.0000*	0.2603
	No	66 (21.6)	193 (63.1)		
Vomiting	Yes	9 (20.9)	18 (41.9)	1.0000	0.0055
	No	61 (21.6)	184 (65.2)		
Nausea	Yes	14 (19.2)	33 (45.2)	0.6925	0.0011
	No	56 (22.2)	169 (67.1)		
Diarrhea	Yes	3 (9.7)	14 (45.2)	0.1445	0.0634
	No	67 (22.8)	188 (63.9)		
Abdominal pain	Yes	9 (40.9)	19 (86.4)	0.0309*	0.0280
	No	61 (22.8)	183 (60.4)		

Values are presented as n (%). P values were calculated using the chi-square test unless otherwise indicated. Fisher's exact test was used when expected cell counts were < 5, as denoted by an asterisk (*). Extreme BMI includes underweight (< 18.5 BMI) and obese (≥ 30 BMI) categories

Table 5 Associations of leukopenia and thrombocytopenia with dengue severity

Hematological Parameter	Severity of Dengue (DWWS, DWS)	Chi-square (χ^2)	*p*-value*	Cramér's V
Leukopenia	Yes / No	5.481	0.0192	0.13
Thrombocytopenia	Yes / No	4.714	0.0299	0.12

* Level of Significance: $p < 0.05$ indicates statistical significance, Degrees of Freedom (df): 1

Table 6 Adjusted residuals for leukopenia and thrombocytopenia vs. severity classification

Hematological Parameter	Severity classification	AR* (Leukopenia)	AR* (Thrombocytopenia)
Leukopenia	DWS	3.40 (Y), -1.78 (N)	-
	DWWS	-2.78 (Y), 1.46 (N)	-
Thrombocytopenia	DWS	-	-2.18 (Y), 2.79 (N)
	DWWS	-	1.78 (Y), -2.28 (N)

'Y' = Yes, 'N' = No *

V = 0.12) and logistic regression adjusted for age and diabetes (leukocyte count: OR = 0.9999, $p = 0.011$; platelet count: OR = 1.000005, $p = 0.023$). Additionally, no significant association was found between these hematological abnormalities and demographic factors such as age and gender; however, leukopenia was significantly more common among patients with joint pain, muscle pain, retroorbital pain, and rash, while thrombocytopenia was more frequent among those with headache, vomiting, nausea, and abdominal pain. Notably, leukopenia was strongly associated with DWS as indicated by high adjusted residuals (3.40), suggesting its potential role as a marker for identifying patients at higher risk of complications. However, the unexpected positive association between platelet count and severity contradicts the well-established link between thrombocytopenia and severe dengue, warranting further investigation.

Leukopenia and thrombocytopenia in dengue fever stem from complex virus-host interactions. Leukopenia results primarily from bone marrow suppression by the dengue virus, which inhibits leukocyte production, compounded by immune-mediated peripheral destruction via cytokines (e.g., TNF-α, IL-6) and sequestration in organs like the spleen [24]. Thrombocytopenia arises from suppressed megakaryopoiesis, immune-mediated platelet destruction by antiplatelet antibodies and complement activation, and platelet activation by nonstructural protein 1 (NS1) via Toll-like receptor 4, leading to aggregation and clearance [25, 26]. Inflammatory mediators and disseminated intravascular coagulation further consume platelets, exacerbating thrombocytopenia [25]. These mechanisms explain leukopenia's significant association with dengue with warning signs (DWS) in our study ($\chi^2 = 12.5$, $p = 0.002$), reflecting heightened immune activation. Thrombocytopenia's non-significant association (OR = 0.999, $p = 0.78$) may relate to early sample collection, as platelet declines typically peak later [27].

This study addresses critical gaps in the regional understanding of dengue's hematological profile in Nepal, particularly outside the Kathmandu Valley, where data remain sparse despite recurrent outbreaks [17, 28]. While prior studies in Nepal have reported leukopenia

Table 7 Logistic regression analysis of hematological parameters associated with dengue severity

Variable	Coefficient	Std. Error	z-value	*p*-value	Odds Ratio (OR)	95% CI
Leukocyte	-0.0001	0.00005	-2.53	0.011	0.9999	-0.0001 to -0.00003
Platelet	0.000005	0.000002	2.28	0.023	1.000005	0.000001 to 0.000009

Pseudo R-squared: 0.024, Log-Likelihood: -213.38, LLR p-value: 0.0048

and thrombocytopenia prevalence rates of 25–82% and 40–89%, respectively, these estimates vary widely due to differences in study settings, diagnostic criteria, and regional disparities [17–19, 28]. In Nepal, a 2025 Lalitpur study reported leukopenia in 64.7% and thrombocytopenia in 40.5% of dengue cases, with disease severity association [17]. While a 2024 Madhesh province study found leukopenia in 87.2% and thrombocytopenia in 66.2% of patients linked to warning signs like abdominal pain underscoring geographical heterogeneity in hematological manifestations [28].

Internationally, a 2022 outbreak study in Bangladesh observed thrombocytopenia in 61% and noted leukopenia as common in severe dengue, with symptoms like headache (84%), vomiting (75%), and joint pain (75%) [29]. A 2021 Bangladesh study reported thrombocytopenia in 66.1% and leukopenia as a common feature, with bleeding manifestations in pediatric patients ($p = 0.009$ for melena) and GIT symptoms like abdominal pain (86.5%) and vomiting (69.6%) [30]. Our findings leukopenia in 21.5% and thrombocytopenia in 62.1% align with these trends but further refine the clinical significance of these markers by linking leukopenia to dengue with warning signs (adjusted residual = 3.40) and thrombocytopenia to milder presentations. By integrating chi-square tests, logistic regression, and adjusted residuals analysis, this study advances methodological rigor compared to earlier descriptive works, providing actionable insights for risk stratification in resource-limited tertiary care settings.

This study findings confirm that leukopenia and thrombocytopenia are important markers of dengue severity, but their role varies regionally. In our study, leukopenia was strongly linked to dengue with warning signs (DWS), unlike a 2023 Coastal India study where thrombocytopenia was the main severity predictor [31]. This difference may arise from distinct dengue virus strains (DENV-1 and DENV-3 in Nepal) or immune responses in our population [11]. While thrombocytopenia was common (62.1%), matching Nepal's 2022 outbreak rates [32], its unexpected association with higher severity odds (OR = 1.000005) contradicts traditional understanding. This paradox may be attributed to several methodological factors. First, the cross-sectional design may have captured patients in early stages of illness before platelet decreased resulting in relatively higher platelet counts in severe cases who presented early. Second, patients with more concerning symptoms might have been referred to higher tertiary care earlier, introducing referral bias. Third, platelet counts may not decline uniformly in all severe cases and can be influenced by individual variability or coexisting conditions. A 2016 Indian study similarly noted this paradox, proposing that platelet levels might drop later in the illness, after initial hospital visits [33]. Notably, despite widespread thrombocytopenia, no severe dengue cases occurred in study cohort, aligning with a study in Kathmandu where thrombocytopenia alone did not predict severe outcomes particularly in the early stages of the disease [20]. Clinically, this implies leukopenia is a more actionable early marker for DWS in Nepal, while thrombocytopenia may require context (e.g., timing, co-occurring symptoms like abdominal pain). These insights underscore the need for comprehensive assessment models that incorporate additional clinical parameters, such as liver enzyme levels and specific serological markers, to improve early identification and management of patients at risk for severe dengue.

From a clinical perspective, these findings have important implications for patient management in resource-limited settings like Eastern Nepal, where laboratory resources and hospital beds are often constrained during outbreaks. The strong association of leukopenia with DWS suggests that leukocyte count (an inexpensive and widely available test) can serve as an early marker to prioritize monitoring or referral for patients at risk of complications. In contrast, the high prevalence but variable predictive value of thrombocytopenia indicates that platelet count should be interpreted alongside clinical features rather than used in isolation to guide admission decisions. This approach aligns with WHO recommendations that emphasize clinical context over numerical thresholds. Implementing such a strategy could improve risk stratification and resource allocation, ensuring that high-risk patients receive timely care while avoiding unnecessary hospitalizations in mild cases.

This study's primary strength lies in its focused examination of leukopenia and thrombocytopenia among dengue patients in Eastern, Nepal, providing valuable insights into their prevalence and clinical associations in a region with limited prior data. The cross-sectional design enabled a comprehensive snapshot of hematological parameters at a specific disease stage, facilitating the identification of significant correlations between these markers and disease severity. Although our logistic regression analysis revealed statistically significant associations between leukopenia and dengue severity, the

model's low explanatory power (pseudo-R^2 = 0.024) indicates that these hematological parameters alone explain only a small portion of the variation in clinical outcomes. The study's cross-sectional nature also limits the ability to observe dynamic changes in blood counts over time, which could offer a more pattern understanding of disease progression. Additionally, the timing of sample collection relative to disease onset was not recorded, which is a critical factor influencing hematological parameters particularly platelet counts that tend to decline later in the illness. This may partially explain the unexpected association observed between platelet count and severity. The absence of severe dengue cases limited insights into progression to severe disease. Convenience sampling and recruitment from four urban-centric tertiary hospitals may have introduced selection bias, as milder or asymptomatic cases managed at home were underrepresented and findings may not be generalizable to other populations. We have identified regional serotypes (DENV-1/DENV-3) as potential contributors to hematological variability, serotype-specific data were not systematically collected, limiting pathophysiological interpretation. Future multivariate longitudinal and multicenter studies are needed to validate these findings, account for disease phase during sample collection, and explore hematological trends over time across diverse clinical settings in Nepal.

Conclusion

This study highlights the significant prevalence of leukopenia and thrombocytopenia among dengue patients presented to a hospital's OPD in Eastern, Nepal and their associations with disease severity. Leukopenia as a significant marker was strongly linked with dengue with warning signs (DWS) (adjusted residual = 3.40; $p = 0.019$, Cramér's V = 0.130), reinforcing its potential as an early indicator of disease progression. In contrast, while thrombocytopenia was common, its unexpected positive association with disease severity raises questions and suggests that isolated platelet counts may not reliably predict severe outcomes, especially without considering other clinical variables or timing of sample collection. From a clinical perspective, these findings emphasize the potential utility of leukocyte counts as an accessible marker for early triage in resource-limited settings, where advanced diagnostic capacity may be unavailable. These results underline the need for contextualized, multivariable risk assessment models incorporating clinical symptoms and other laboratory markers to guide decision-making. Future longitudinal studies are essential to track dynamic hematological changes over time and validate these associations across broader, diverse patient populations in Nepal and beyond.

Abbreviations

AR	Adjusted Residual
CI	Confidence Interval
DENV	Dengue Virus
DWWS	Dengue Without Warning Signs
DWS	Dengue With Warning Signs
OPD	Out Patient Department
OR	Odds Ratio

Supplementary Information

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Supplementary Material 1

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Author contributions

BPG, SU designed the study, review and finalize the manuscript. CW analyzed, interpreted the data and draft the manuscript. VPG, AKS, SC and ED review the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics committee of Nepal Health Research Council, Kathmandu, Nepal (Approval No. 678). Informed consent was obtained from all participants or their guardians, and patient confidentiality was maintained through data anonymization.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Virology Society Nepal, Kathmandu, Nepal
[2]BP Koirala Institute of Health Science, Dharan, Nepal
[3]Global Clinical Research, Kathmandu, Nepal
[4]Sona Polyclinic, Birgunj, Nepal

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