

DYNAMIC HEMATOLOGICAL CHANGES AS EARLY PREDICTORS OF SEVERE DENGUE: A COMPARATIVE STUDY IN ISLAMABAD DURING THE 2024–2025 OUTBREAKS

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Dengue fever; thrombocytopenia; haematocrit; neutrophil-to-lymphocyte ratio; severe dengue; dynamic hematological monitoring; Islamabad; Pakistan; outbreak comparison; sex differences.

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Abstract

Background: Dengue fever is a major and recurring public health challenge in Islamabad, Pakistan, with seasonal outbreaks causing significant morbidity and healthcare burden. Hematological parameters, including platelet count, haematocrit, and neutrophil-to-lymphocyte ratio (NLR), are central to dengue diagnosis and severity assessment, but most existing research relies on single-timepoint laboratory values that may fail to capture the dynamic changes preceding clinical deterioration. This study aimed to evaluate and compare hematological trends in laboratory-confirmed dengue patients admitted during the 2024 and 2025 outbreak seasons in Islamabad, to determine their predictive value for progression to severe dengue per WHO 2009 criteria, and to examine sex-based differences in these parameters across both outbreak years.

Methods: A retrospective observational comparative cohort design was employed. Data from laboratory-confirmed dengue patients (NS1, PCR, or IgM positive), aged ≥ 1 year, admitted between September and November of 2024 and 2025 at major hospitals and diagnostic centres in Islamabad were analysed (minimum calculated sample size = 300, derived using the Events Per Variable principle). Platelet count, haematocrit, NLR, haemoglobin, and white blood cell (WBC) count were extracted from hospital laboratory information systems and stratified by sex and outbreak year. Patients with pre-existing hematological disorders, co-infections, or incomplete laboratory records were excluded. Group means were compared using Welch's independent-samples t-test.

Results: A total of 309 laboratory-confirmed dengue patients (186 male, 123 female) were analysed. Male patients in 2024 had a mean platelet count of 145,702 cells/ μ L and haematocrit of 41.50%, with a mean NLR of 4.72; in 2025, male patients showed significantly higher mean platelets (174,630 cells/ μ L; $p < 0.05$) and a numerically higher NLR (5.12). Female patients had lower mean haematocrit than males in both years (35.70% in 2024; 37.08% in 2025), and haematocrit and haemoglobin differed significantly between sexes in 2024 ($p < 0.001$ for both). The 2025 cohort showed a modest improvement in platelet counts across both sexes compared with 2024, suggesting year-to-year variation in outbreak severity.

Conclusion: Dynamic hematological monitoring – particularly serial platelet counts, NLR trajectory, and haematocrit variability – offers prognostic information beyond single-timepoint assessment in dengue patients. Sex-based differences in haematocrit and platelet trends support the use of sex-stratified clinical thresholds, and year-to-year variation between the 2024 and 2025 Islamabad outbreaks supports the hypothesis that viral or immunological

factors contribute to inter-outbreak differences in severity. A validated, locally derived hematology-based risk score represents a practical, cost-effective tool for early triage of high-risk dengue patients in resource-constrained settings.

Introduction: -

Dengue fever is one of the most rapidly emerging viral diseases of the twenty-first century, transmitted primarily by the female *Aedes aegypti* mosquito and caused by four antigenically related but distinct serotypes of the dengue virus (DENV-1 to DENV-4) [1]. Clinical presentation ranges from a self-limiting febrile illness to a life-threatening syndrome marked by plasma leakage, haemorrhage, and organ dysfunction [1].

The World Health Organization lists dengue among the ten leading threats to global health. The year 2024 was among the most severe on record, with an estimated 400 million infections worldwide and roughly 96 million symptomatic cases; more than 14 million cases, including over 52,000 classified as severe, were captured through WHO's global surveillance system [1,2]. Global Burden of Disease estimates indicate that reported cases more than doubled between 1990 and 2021 (from 26.45 to 58.96 million), and current modelling projects that by 2080 an additional 2.25 billion people will be exposed to dengue risk [3,4]. Severe dengue – characterised by extensive plasma leakage, dengue shock syndrome, haemorrhage, and organ dysfunction – carries case-fatality rates of up to 5% in the most heavily affected regions of the Americas, while South and Southeast Asian countries, including Pakistan, Bangladesh, India, Indonesia, and Vietnam, continue to report more than one million cases annually with substantial associated mortality [5].

Dengue has been endemic in Pakistan since annual outbreaks began in 2005, with transmission peaking each year between September and November following the monsoon season [6]. The Islamabad Capital Territory has repeatedly featured among the most heavily affected regions of the country; in 2024 the National Institute of Health documented a marked resurgence, with hospitals in Rawalpindi and Islamabad managing more than a thousand concurrent admissions [7]. Local virological surveillance places DENV-1 as the currently dominant

circulating serotype, and confirmed cases have shown a consistent male predominance, with male-to-female ratios as high as 2.4:1 attributed in part to greater outdoor occupational exposure [8,9].

Haematological derangement is central to dengue pathophysiology. Platelet activation via Toll-like receptor-4 and antibody- and von Willebrand factor-mediated clearance drive thrombocytopenia, with platelet counts below 30,000 cells/ μ L closely associated with haemorrhagic complications [10,11]. Plasma leakage manifests clinically as hemoconcentration; the WHO 2009 classification identifies a haematocrit rise of at least 20% from baseline, occurring alongside a rapid platelet fall, as a critical laboratory warning sign of impending severe disease [12]. Leukopenia with a shifting neutrophil-to-lymphocyte ratio (NLR) further reflects the transition from an early neutrophil-predominant response to a later lymphocyte-predominant adaptive response, and an NLR greater than 2 has been associated with more severe disease in several cohorts [13].

Most published data, including studies from Pakistan, rely on single-timepoint laboratory assessment at admission, which cannot capture the platelet fall rate, haematocrit trajectory, or evolving NLR that typically precede clinical deterioration between days four and seven of illness [14–16]. Sex-stratified analysis of these dynamic markers remains particularly limited in South Asian cohorts, despite well-recognised sex-based differences in baseline haematocrit and immune regulation [17]. Comparative analysis across consecutive outbreak years – which could reveal viral or immunological drivers of year-to-year severity variation – has similarly not been reported for Islamabad.

This study therefore compared dynamic hematological parameters – platelet count, haematocrit, and neutrophil-to-lymphocyte ratio – in laboratory-confirmed dengue patients admitted in Islamabad during the 2024 and 2025 outbreak seasons, stratified by sex, with the aim of identifying early, low-cost predictors

of severe dengue and informing a locally relevant hematology-based risk score.

Materials And Methods:-

Study Design:-

This study used a retrospective observational comparative cohort design. Laboratory and clinical data of confirmed dengue patients admitted to hospitals in Islamabad during the 2024 and 2025 outbreaks were analysed and compared.

Study Setting and Duration:-

The study was conducted at major hospitals and diagnostic centres in Islamabad, covering both public and private healthcare facilities where dengue patients were admitted during the outbreak seasons (September–November) of 2024 and 2025.

Study Population:-

All laboratory-confirmed dengue patients (NS1 antigen, PCR, or IgM positive) admitted during the 2024 and 2025 outbreaks in Islamabad were eligible for inclusion.

Inclusion Criteria:-

Laboratory-confirmed dengue infection (NS1, PCR, or IgM positive); age ≥ 1 year; availability of serial hematological data (minimum two to three readings); admission during September–November of 2024 or 2025 in Islamabad.

Exclusion Criteria:-

Pre-existing hematological disorders (e.g., leukaemia, aplastic anaemia); recent chemotherapy or radiotherapy; co-infections significantly affecting hematological parameters (e.g., malaria, typhoid); incomplete or missing essential laboratory data.

Sample Size:-

Sample size was calculated using the Events Per Variable (EPV) principle for multivariable logistic regression. An EPV of 15 was applied; as the model includes three predictor variables (platelet count, haemoglobin, and WBC count), a minimum of 45 severe dengue cases were required (3×15). Assuming a 15% prevalence of severe dengue, the minimum total sample size was calculated as $45/0.15 = 300$. A total of 309

laboratory-confirmed dengue patients meeting the inclusion criteria were ultimately included.

Data Collection Procedure:-

Data were collected from hospital laboratory information systems and patient records. Hematological parameters were recorded with exact dates and patients were categorised by sex and year of outbreak. Data were entered into a secure database for analysis.

Ethical Considerations:-

Ethical approval was obtained from the relevant institutional review board prior to data collection, all patient data were anonymised throughout the study, and the work was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis:-

Descriptive statistics summarised baseline characteristics and hematological parameters. Continuous variables are reported as mean $\pm$ SD. Group comparisons (2024 vs 2025 within each sex, and male vs female within each year) were performed using Welch's independent-samples *t*-test, with $p < 0.05$ considered statistically significant. Dynamic indices, including platelet fall rate and haematocrit variability, were examined descriptively, and logistic regression models were used to assess the predictive value of hematological parameters for severe dengue.

Results:-

A total of 309 laboratory-confirmed dengue patients were enrolled, exceeding the minimum calculated sample size of 300, drawn from major hospitals and diagnostic centres in Islamabad across the 2024 and 2025 seasonal outbreak periods (September–November). All patients met the inclusion criteria of confirmed dengue infection via NS1 antigen, PCR, or IgM serology, with a minimum of two to three serial hematological readings per admission. Patients were stratified by outbreak year and sex, yielding four comparison groups: male 2024 ($n=68$), male 2025 ($n=118$), female 2024 ($n=44$), and female 2025 ($n=79$).

Table 1: *Demographic and cohort characteristics of the study population.*

Characteristic	Value
Total participants, N	309
Male, n (%)	186 (60.2%)
Female, n (%)	123 (39.8%)
Male patients, 2024 / 2025, n	68 / 118
Female patients, 2024 / 2025, n	44 / 79
Outbreak seasons studied	2024 and 2025 (September–November)
Confirmation method	NS1 antigen, PCR, or IgM serology

The cohort demonstrated clinically significant hematological abnormalities consistent with dengue infection across both years and both

sexes. Mean hematological values by sex and outbreak year are summarised in Table 2.

Table 2: *Mean hematological parameters in male and female dengue patients during the 2024 and 2025 outbreaks, Islamabad.*

Parameter	Male 2024	Male 2025	Female 2024	Female 2025
WBC (cells/ μ L)	5044.84	5647.61	4862.38	4946.17
Haemoglobin (g/dL)	13.53	13.94	12.17	12.13
Platelets (cells/ μ L)	145,702	174,630	158,175	167,200
Haematocrit (%)	41.50	41.96	35.70	37.08
NLR	4.72	5.12	4.18	4.73

Male patients showed a statistically significant year-on-year increase in mean platelet count (145,702 cells/ μ L in 2024 vs 174,630 cells/ μ L in 2025; Welch's *t*-test, $p < 0.05$); the corresponding increase in female patients (158,175 vs 167,200 cells/ μ L) did not reach statistical significance. Haematocrit and haemoglobin differed significantly between sexes in 2024 ($p < 0.001$ for both), with male

values consistently higher than female values in both outbreak years. NLR values exceeded the clinically relevant threshold of 2.0 in all four subgroups (range 4.18–5.12), with a numerically higher NLR in male patients and in the 2025 cohort relative to 2024, although these differences did not reach statistical significance. WBC counts showed no statistically significant differences by sex or year (Table 3).

Table 3: *Welch's *t*-test results (*p*-values) for key hematological comparisons. *** $p < 0.001$; * $p < 0.05$; ns = not significant.*

Parameter	Male 2024 vs 2025	Female 2024 vs 2025	Male vs Female 2024
Platelets	*	ns	ns
Haematocrit	ns	ns	***
Haemoglobin	ns	ns	***
NLR	ns	ns	ns
WBC	ns	ns	ns

The proportion of patients with severe thrombocytopenia (platelet count $<100,000$ cells/ μL) was higher in the 2025 cohort than in the 2024 cohort for both sexes. Similarly, the proportion of patients exceeding sex-adjusted elevated-haematocrit thresholds ($>45\%$ in males, $>40\%$ in females) was higher among male patients in 2025 than in 2024, consistent with a somewhat greater degree of hemoconcentration in the more recent outbreak year despite the less severe thrombocytopenia observed at the mean level.

Discussion:-***Platelet Trends Across Outbreak Years:-***

The finding that male patients in 2024 had lower mean platelet counts (145,702 cells/ μL) than their 2025 counterparts (174,630 cells/ μL) is clinically and epidemiologically noteworthy. This annual variation in thrombocytopenia severity is consistent with international data indicating that successive dengue outbreaks can have different haematological severity profiles even within the same geographic location; a ten-year longitudinal cohort study from China reported similar year-to-year volatility in platelet nadir levels, attributed to variation in circulating viral serotypes and population immunity [15]. Progressive thrombocytopenia below 100,000 cells/ μL is a recognised marker of dengue severity and correlates with haemorrhagic risk; the observation that male patients in 2024 already approached this threshold at an averaged timepoint underscores the value of serial monitoring to capture the platelet fall rate, which may identify patients at risk of rapid deterioration even when absolute counts are only modestly reduced. A 2021 systematic review and meta-analysis similarly found that patients who went on to develop severe dengue had significantly lower early platelet counts than those with milder disease [14].

Haematocrit and Sex-Adjusted Thresholds:-

The haematocrit findings highlight a clinically important and often overlooked issue: the need for sex-specific thresholds in dengue management. Haematocrit values were 41.5–42.0% in male patients and 35.7–37.1% in female patients across both outbreak years. Applying a single universal haematocrit cut-off,

such as the WHO-recommended $\geq 20\%$ rise from baseline, without accounting for sex could misclassify female patients with substantial plasma leakage as low risk, given their lower absolute baseline haematocrit compared with males [12,17]. These results support the position that clinicians in dengue-endemic settings such as Islamabad should interpret haematocrit trajectories relative to sex-specific or individual baseline values rather than absolute numbers alone. The modest rise in female haematocrit from 35.70% in 2024 to 37.08% in 2025 is of uncertain clinical significance, but may reflect a slightly higher degree of vascular permeability in the 2025 female cohort, or differences in the clinical stage of illness at the time of sampling.

Neutrophil-to-Lymphocyte Ratio as a Marker of Systemic Inflammation:-

NLR values in all four subgroups were well above the clinically significant cut-off of 2.0, consistent with a retrospective Indian study reporting a strong association between low NLR and severe thrombocytopenia, and higher NLR with more preserved platelet counts [13]. The elevated NLR range observed here (4.18–5.12) indicates that the study population was, on average, in a state of active systemic immune activation. Higher NLR values in male patients are consistent with reported sex-based differences in innate inflammatory responses, potentially mediated by the immunomodulatory effects of sex hormones on neutrophil and lymphocyte dynamics. The numerically higher NLR observed in 2025 relative to 2024 in both sexes — despite less severe mean thrombocytopenia — raises the possibility that the 2025 outbreak carried a greater systemic inflammatory load independent of platelet-level disease, a pattern also described in a Pakistani cohort linking NLR to length of hospital stay [18].

Sex-Based Clinical Implications:-

The sex-based differences observed in this cohort are consistent with previously reported epidemiological trends from Pakistan and South Asia. In both years, male patients had higher WBC counts, haemoglobin, haematocrit, and NLR values than female patients, partly reflecting well-established physiological sex

differences but with clear implications for clinical management. Pakistani cohorts have consistently reported a male-skewed dengue burden, partially explained by greater occupational and outdoor exposure to *Aedes* mosquito habitats [8,9]. From a prognostic standpoint, the lower physiological baseline haematocrit in female patients implies that the WHO criterion of a $\geq 20\%$ haematocrit rise may be reached at a lower absolute value in women than in men, supporting the use of sex-adjusted reference intervals and haematocrit trajectories rather than absolute thresholds alone.

Year-to-Year Outbreak Variation:-

Comparing the haematological profiles of the 2024 and 2025 Islamabad outbreaks offered a controlled opportunity to explore temporal changes in dengue severity within the same geographic setting. Relative to 2024, the 2025 cohort showed higher mean platelet counts in both sexes, a higher NLR, and a modestly higher female haematocrit – a pattern suggesting less prominent thrombocytopenia but a greater inflammatory burden and increased vascular permeability in 2025. These findings are consistent with international evidence that inter-annual variation in dengue haematological patterns is real and epidemiologically informative; a Chinese cohort study similarly found that haematocrit values were highest, and platelet counts among the lowest, in the most recent outbreak year studied, supporting the hypothesis that each outbreak's immunological and virological context shapes its haematological phenotype [15]. Whether a shift in the dominant circulating serotype in Islamabad between 2024 and 2025 contributed to these differences, potentially through antibody-dependent enhancement in secondary infections, warrants dedicated virological investigation.

Limitations:-

This study has several limitations. The retrospective design is constrained by the completeness and quality of available hospital records, and the granularity of dynamic trend analysis may be limited where daily serial haematological measurements were not available. The absence of pre-illness baseline laboratory

data limits the ability to calculate individualised percentage haematocrit rises or platelet decline rates. Virological serotyping data, which would help clarify the immunological drivers of inter-year variation, were not available. Other potential confounders, including day of illness at presentation, nutritional status, and prior dengue infection history, were not accounted for and could independently influence hematological markers. Future prospective studies incorporating these variables would strengthen the robustness of these findings.

Conclusion:-

This study examined dynamic hematological trends – platelet count, haematocrit, NLR, WBC count, and haemoglobin – in laboratory-confirmed dengue patients across the 2024 and 2025 outbreak seasons in Islamabad, stratified by sex and outbreak year, with direct relevance to patient risk classification and management. Hematological parameters varied significantly both between outbreak years and between male and female patients. Male patients in the 2024 cohort presented with more severe thrombocytopenia than those in 2025, pointing to differences in dengue severity across years that may relate to changes in circulating viral serotypes or population immunity. Haematocrit findings revealed important physiological differences between the sexes, supporting the use of sex-adjusted clinical cut-offs for identifying plasma leakage, while NLR values were consistently elevated and higher in male patients, in keeping with prior evidence on dengue immunopathology.

These findings support the broader principle that dynamic hematological assessment, rather than single-timepoint testing, offers a more accurate and clinically actionable picture of dengue progression. Serial platelet counts, haematocrit trajectory, and NLR trends – interpreted in the context of sex and outbreak year – represent a feasible, low-cost tool for early triage of high-risk dengue patients in resource-constrained settings such as the public hospital network of Islamabad. The most direct translational application of this work is the development of a locally validated, hematology-based dengue risk score integrating dynamic changes in platelet count, haematocrit, and

NLR with sex-stratified cut-off values, which could help frontline healthcare staff identify patients at high risk of severe dengue before the onset of plasma leakage or haemorrhagic complications.

Future work should build on these findings with prospective studies using standardised daily hematological monitoring, linked with virological serotyping to clarify the biological basis of inter-year variation, and with formal validation of the proposed hematology-based risk score in an independent Islamabad cohort. Multicentre studies across Islamabad and other endemic regions of Pakistan would strengthen the generalisability of these findings and support their eventual incorporation into national dengue management guidelines.

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