

Original Research Article

Evaluation of high-fluorescence lymphocyte count as an early diagnostic marker of dengue infection using the Sysmex XN-350 hematology analyzer

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ABSTRACT

Background: Dengue is a major mosquito-borne viral infection prevalent in tropical and subtropical regions. Early diagnosis is essential for appropriate patient management. High-Fluorescence Lymphocyte Count (HFLC), generated by Sysmex XN-Series hematology analyzers, may provide additional information about lymphocyte activation during viral infections. The aim is to evaluate HFLC as an early diagnostic marker of dengue infection using the Sysmex XN-350 hematology analyzer.

Methods: This prospective observational case-control study was conducted in the Department of Pathology, GMERS Medical College and Hospital, Gandhinagar, from 1st August 2026 to 31st August 2026. The study included 100 participants, comprising 50 laboratory-confirmed dengue-positive patients and 50 dengue-negative controls. Complete blood count parameters, including hemoglobin, total leukocyte count, platelet count, lymphocyte percentage, and HFLC percentage, were analyzed. Diagnostic performance was evaluated using receiver operating characteristic (ROC) curve analysis.

Results: Dengue-positive patients showed significantly lower mean WBC counts (5140 ± 2033.70 vs. 6522 ± 1843.28 cells/ μ L; $p < 0.001$) and platelet counts ($208,340 \pm 143,548$ vs. $287,680 \pm 93,384$ cells/ μ L; $p = 0.002$) than controls. Lymphocyte percentage was significantly higher in dengue-positive patients ($40.16 \pm 16.93\%$ vs. $29.66 \pm 6.06\%$; $p < 0.001$). Mean HFLC percentage was significantly lower in dengue-positive patients ($9.16 \pm 7.73\%$ vs. $24.50 \pm 14.58\%$; $p < 0.001$). ROC analysis yielded an AUC of 0.807. At a cutoff of $\leq 20\%$, HFLC demonstrated 96.0% sensitivity, 58.0% specificity, 69.6% positive predictive value, and 93.5% negative predictive value.

Conclusions: HFLC may serve as a useful adjunctive screening parameter for suspected dengue infection. However, its moderate specificity necessitates interpretation alongside clinical findings, routine hematological parameters, and established dengue-specific diagnostic tests.

Keywords: Dengue, HFLC, Sysmex XN-350, Complete blood count, Diagnostic marker

INTRODUCTION

Dengue is an important mosquito-borne viral infection and represents a significant public health problem, particularly in tropical and subtropical regions of the world. The

disease is caused by the dengue virus (DENV), which belongs to the genus *Flavivirus* and the family *Flaviviridae*. Dengue virus is transmitted predominantly through the bite of infected female *Aedes aegypti* mosquitoes and, to a lesser extent, by *Aedes albopictus*.

Dengue infection affects individuals of all age groups and has a wide spectrum of clinical manifestations, ranging from asymptomatic infection and mild febrile illness to severe dengue associated with plasma leakage, hemorrhage, shock, and organ dysfunction. Dengue has expanded considerably in India over recent decades and continues to represent an important public health challenge because of changing epidemiology, recurrent outbreaks, urbanization, and the spread of mosquito vectors.^{1,2}

There are four major antigenically distinct serotypes of dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4. Infection with one serotype generally provides long-lasting immunity against that particular serotype but does not provide complete protection against infection with the remaining serotypes. Consequently, individuals living in dengue-endemic areas may experience more than one dengue infection during their lifetime. Secondary infection with a different serotype may be associated with an increased risk of severe disease in some patients. The clinical outcome of dengue infection is influenced by several factors, including the infecting serotype, previous dengue infection, host immune response, age, and underlying health conditions.

The clinical manifestations of dengue are highly variable. Patients commonly present with an acute onset of fever accompanied by headache, retro-orbital pain, myalgia, arthralgia, nausea, vomiting, weakness, and skin rash. In some patients, the febrile phase may be followed by a critical phase during which plasma leakage can occur. Patients who develop severe dengue may present with circulatory compromise, severe bleeding, shock, or significant organ involvement. Because the clinical manifestations of dengue may overlap with those of malaria, typhoid fever, leptospirosis, other viral infections, and bacterial febrile illnesses, clinical findings alone may not be sufficient to establish a definitive diagnosis. Therefore, early and accurate laboratory diagnosis is essential for appropriate patient management, monitoring, and identification of patients at risk of developing severe disease.³

Laboratory investigations play a central role in the diagnosis and monitoring of dengue infection. The diagnostic approach depends substantially on the day of illness at which the patient presents. During the early stage of infection, detection of viral components such as dengue non-structural protein 1 (NS1) antigen or viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) can provide evidence of acute dengue infection.

As the immune response develops, dengue-specific antibodies become detectable, and serological methods such as IgM and IgG testing become increasingly useful. Indian studies have demonstrated the usefulness of NS1 antigen detection during the early phase of illness and the complementary role of IgM testing during later stages of dengue infection.⁴⁻⁶

The complete blood count (CBC) is one of the most commonly performed laboratory investigations in patients with suspected dengue infection. CBC testing is inexpensive, rapidly available, and useful not only for the initial assessment of patients but also for monitoring disease progression. Typical hematological changes associated with dengue include leukopenia, relative lymphocytosis, thrombocytopenia, and changes in hematocrit. The platelet count is particularly important because thrombocytopenia is frequently observed during dengue infection and may become more pronounced during severe disease. Similarly, changes in hematocrit and platelet counts can provide useful information regarding disease progression and recovery.^{7,8} However, these conventional hematological abnormalities are not specific to dengue and may also occur in other infectious diseases. Therefore, there is a need to explore additional hematological parameters that may provide useful information during the early assessment of patients with suspected dengue infection.

Changes in lymphocyte populations are also observed during dengue infection as a result of immune activation. Activated and reactive lymphocytes may appear in peripheral blood during the development of the immune response. These cells can demonstrate morphological changes on peripheral blood smear examination; however, manual identification of reactive lymphocytes may be subjective and dependent on the experience of laboratory personnel. The development of automated hematology analyzers has created opportunities to investigate additional cellular parameters that may reflect changes occurring in leukocyte and lymphocyte populations during infection.

The Sysmex XN-Series hematology analyzers, including the Sysmex XN-350, provide automated complete blood count and leukocyte differential analysis using fluorescence-based flow cytometric technology. In addition to conventional CBC parameters, these analyzers provide additional information related to leukocyte populations. One such parameter is the High-Fluorescence Lymphocyte Count (HFLC). HFLC represents a population of lymphocytes demonstrating increased fluorescence characteristics during leukocyte differential analysis and may reflect activated or reactive lymphocyte populations occurring during immune stimulation.

The HFLC parameter is of particular interest in viral infections because viral infection can stimulate the immune system and result in the activation and proliferation of lymphocyte populations. Activated lymphocytes may demonstrate increased cellular RNA and other characteristics that result in stronger fluorescence signals during automated hematological analysis. Based on these fluorescence properties, the analyzer identifies and reports the HFLC parameter. HFLC can be expressed as HFLC%, representing the percentage of high-fluorescence lymphocytes and HFLC#, representing the corresponding absolute count. The potential usefulness of

HFLC in dengue lies in its ability to provide additional information during a routine CBC examination. Unlike specialized dengue-specific investigations, HFLC is generated as part of automated hematological analysis on compatible Sysmex XN analyzers. If HFLC demonstrates a consistent increase during dengue infection, it may provide an additional laboratory clue that could support the early suspicion of dengue, particularly when conventional CBC findings are nonspecific. A recent study from India specifically investigated HFLC generated by the Sysmex XN-350 in serologically confirmed dengue patients and evaluated its relationship with platelet count and disease recovery.⁹

However, HFLC is not specific to dengue because activated lymphocytes may also occur in other viral infections and inflammatory conditions. Therefore, HFLC should be considered an adjunctive hematological parameter rather than a standalone diagnostic test. Its diagnostic value needs to be evaluated against appropriate reference standards, including established dengue diagnostic methods such as NS1 antigen detection, RT-PCR, and dengue-specific IgM testing according to the stage of illness.

A clinically relevant approach to evaluating HFLC is to compare confirmed dengue-positive patients with dengue-negative febrile patients. Such patients may have clinical features similar to dengue but do not have laboratory evidence of dengue infection. This comparison can provide useful information regarding the ability of HFLC to distinguish dengue from other causes of acute febrile illness. HFLC should also be evaluated in relation to conventional hematological parameters, particularly platelet count, WBC count, and lymphocyte percentage, to determine whether it provides additional information beyond routine CBC findings. The diagnostic performance of HFLC can be assessed using statistical methods such as receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) provides an overall measure of the ability of HFLC to discriminate between dengue-positive and dengue-negative individuals. An appropriate cutoff value can subsequently be derived from the study population, and its sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy can be evaluated.¹⁰

Therefore, the present study focuses on the evaluation of High-Fluorescence Lymphocyte Count (HFLC), generated by the Sysmex XN-350 hematology analyzer, as an early diagnostic marker of dengue infection. The study aims to evaluate HFLC% and HFLC# together with conventional CBC parameters in patients with confirmed dengue and appropriate control groups. It will also assess the relationship between HFLC and platelet count, WBC count, and lymphocyte percentage. By evaluating HFLC in conjunction with conventional hematological parameters and established dengue diagnostic tests, the study aims to determine whether this automated parameter can provide additional information for the early laboratory

assessment of dengue infection and whether it has sufficient diagnostic utility to be incorporated as an adjunctive parameter during routine hematological evaluation.

METHODS

Study design

This was a prospective observational case-control study conducted in the Department of Pathology at GMERS Medical College and Hospital, Gandhinagar. The study was designed to evaluate the diagnostic utility of High-Fluorescence Lymphocyte Count (HFLC) as an early marker of dengue infection using the Sysmex XN-350 automated hematology analyzer, along with routine complete blood count (CBC) parameters.

Study period

The study was conducted over a period from 1st august 2026 to 31st august 2026.

Study population

A total of 100 participants were included in the study. The study population consisted of: Group I – Dengue-positive group: 50 patients with laboratory-confirmed dengue infection. Group II – Control group: 50 individuals who were negative for dengue infection and fulfilled the control selection criteria.

Inclusion criteria

Dengue-positive group: patients clinically suspected of having dengue infection; Patients with laboratory-confirmed dengue infection based on the relevant dengue diagnostic test used in the study; Patients from whom a blood sample was available for CBC and HFLC analysis.

Control group: Individuals negative for dengue infection by the diagnostic test used in the study; Individuals from whom an adequate blood sample was available for hematological analysis.

Exclusion criteria

Patients with known hematological malignancies or chronic hematological disorders. Patients with previously diagnosed immunological disorders that could significantly alter lymphocyte morphology or count. Patients receiving chemotherapy or immunosuppressive therapy. Samples with inadequate volume, clotting, or significant pre-analytical errors.

Study methodology

After obtaining informed consent, venous blood samples were collected from all participants in appropriate EDTA-containing vacutainer tubes. Samples were processed

according to the laboratory's standard operating procedures. Complete blood count analysis was performed using the Sysmex XN-350 automated hematology analyzer. The following hematological parameters were recorded: hemoglobin (Hb), total leukocyte count (TLC), red blood cell count (RBC), platelet count, lymphocyte count/percentage and high-fluorescence lymphocyte count (HFLC). HFLC was assessed using the analyzer's automated differential leukocyte analysis, and the HFLC values obtained from the Sysmex XN-350 were recorded and compared between the dengue-positive and control groups. Dengue infection status was established using the laboratory diagnostic methods routinely employed at the study institution, including NS1 antigen and/or dengue-specific IgM/IgG testing, as applicable. The hematological findings of the dengue-positive group were subsequently compared with those of the control group to determine whether HFLC and other complete blood count (CBC) parameters differed significantly between the two groups.

Study groups

The participants were divided into two groups: Group I (n=50): Laboratory-confirmed dengue-positive patients. Group II (n=50): Dengue-negative control participants.

Assessment parameters

The primary study parameter include: high-fluorescence lymphocyte count (HFLC); secondary study parameters included: hemoglobin, total leukocyte count, platelet count and lymphocyte count/percentage. The diagnostic performance of HFLC for identifying dengue infection was assessed by comparing HFLC values between dengue-positive and control groups. Where appropriate, sensitivity, specificity, positive predictive value, negative predictive value, and area under the receiver operating characteristic (ROC) curve were calculated.

Statistical analysis

Data were entered into a computerized database and analyzed using appropriate statistical software. Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median with interquartile range (IQR), depending on the distribution of the data, while categorical variables were presented as frequencies and percentages. The distribution of continuous variables was assessed before selecting the appropriate statistical test. Comparisons between the dengue-positive and control groups were performed using the independent-samples t-test, while categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The diagnostic performance of HFLC was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the ROC curve (AUC), sensitivity, specificity, and optimal cut-off value were determined where applicable. A $p < 0.05$ was considered statistically significant.

RESULTS

Demographic characteristics of the study population

A total of 100 participants were included in the study, comprising 50 dengue-positive patients and 50 controls. The demographic characteristics of the study population are presented in Table 1. The mean age of dengue-positive patients was 28.98 ± 9.11 years, compared with 32.38 ± 11.18 years in the control group. The difference in mean age between the two groups was not statistically significant ($p = 0.099$). The sex distribution was identical in both groups, with males accounting for 72.0% and females for 28.0% of participants. No, statistically significant difference in sex distribution was observed between the groups ($p = 1.000$). These findings indicate that the dengue-positive and control groups were comparable with respect to age and sex.

Table 1: Demographic characteristics of the study population.

Characteristic	Dengue positive (n=50)	Control (n=50)	Total (n=100)	P value
Age, years (Mean $\pm$ SD)	28.98 ± 9.11	32.38 ± 11.18	30.68 ± 10.27	0.099
Male, N (%)	36 (72.0)	36 (72.0)	72 (72.0)	1.000
Female, N (%)	14 (28.0)	14 (28.0)	28 (28.0)	—

Comparison of hematological parameters between dengue-positive patients and controls

The mean WBC count was significantly lower in dengue-positive patients (5140 ± 2033.70 cells/ μ l) compared with controls (6522 ± 1843.28 cells/ μ l; $p < 0.001$). In contrast, the lymphocyte percentage was significantly higher in the dengue-positive group ($40.16 \pm 16.93\%$) than in controls ($29.66 \pm 6.06\%$; $p < 0.001$).

The mean HFLC percentage was significantly lower among dengue-positive patients ($9.16 \pm 7.73\%$) compared with controls ($24.50 \pm 14.58\%$; $p < 0.001$). The mean hemoglobin concentration did not differ significantly between dengue-positive patients (12.69 ± 2.25 g/dl) and controls (12.88 ± 1.89 g/dl; $p = 0.662$). Platelet counts were significantly lower in dengue-positive patients ($208,340 \pm 143,548$ cells/ μ l) compared with controls ($287,680 \pm 93,384$ cells/ μ l; $p = 0.002$).

Table 2: Comparison of hematological parameters between dengue-positive patients and controls.

Parameter	Dengue Positive (n=50), Mean $\pm$ SD	Control (n=50), Mean $\pm$ SD	P value
WBC (cells/ μ l)	5140 $\pm$ 2033.70	6522 $\pm$ 1843.28	<0.001
Lymphocyte (%)	40.16 $\pm$ 16.93	29.66 $\pm$ 6.06	<0.001
HFLC (%)	9.16 $\pm$ 7.73	24.50 $\pm$ 14.58	<0.001
Hemoglobin (g/dl)	12.69 $\pm$ 2.25	12.88 $\pm$ 1.89	0.662
Platelet count (cells/ μ l)	208,340 $\pm$ 143,548	287,680 $\pm$ 93,384	0.002

Distribution of HFLC values between dengue-positive patients and controls

Among dengue-positive patients, 48 of 50 participants (96.0%) had an HFLC value of $\leq$ 20%, whereas only 2

patients (4.0%) had an HFLC value $>$ 20%. In contrast, among controls, 21 participants (42.0%) had HFLC values $\leq$ 20%, while 29 participants (58.0%) had values $>$ 20%. The distribution demonstrates that lower HFLC values were more frequently observed among dengue-positive patients than among controls.

Table 3: Distribution of participants according to HFLC cutoff value.

HFLC category	Dengue positive (n=50)	Control (n=50)	Total (n=100)
HFLC $\leq$ 20%	48 (96.0%)	21 (42.0%)	69 (69.0%)
HFLC $>$ 20%	2 (4.0%)	29 (58.0%)	31 (31.0%)
Total	50 (100%)	50 (100%)	100 (100%)

Diagnostic performance of HFLC for dengue infection

The diagnostic performance of HFLC percentage for differentiating dengue-positive patients from controls was evaluated using receiver operating characteristic (ROC) curve analysis. The ROC analysis demonstrated an area

under the curve (AUC) of 0.807, indicating good discriminatory performance of HFLC percentage for differentiating dengue-positive patients from controls. An HFLC cutoff value of $\leq$ 20% demonstrated a sensitivity of 96.0% and a specificity of 58.0%. The positive predictive value was 69.6%, while the negative predictive value was 93.5%.

Table 4: Diagnostic performance of HFLC (%) for detection of dengue infection.

Diagnostic parameter	Result
Optimal HFLC cutoff	$\leq$ 20%
Area under the ROC curve (AUC)	0.807
Sensitivity	96.0%
Specificity	58.0%
Positive predictive value (PPV)	69.6%
Negative predictive value (NPV)	93.5%

Diagnostic classification using the HFLC cutoff

Among the 50 dengue-positive patients, 48 were correctly classified as HFLC-positive, while 2 had HFLC values

above the cutoff. Among the 50 controls, 29 were correctly classified as HFLC-negative, whereas 21 had HFLC values $\leq$ 20%.

Table 5: Diagnostic classification according to HFLC cutoff value of $\leq$ 20%.

HFLC result	Dengue positive	Control
HFLC $\leq$ 20%	48	21
HFLC $>$ 20%	2	29
Total	50	50

The selected HFLC cutoff demonstrated high sensitivity for identifying dengue-positive patients but moderate specificity for correctly identifying controls.

DISCUSSION

Demographic characteristics of the study population

The present study demonstrated that the dengue-positive and control groups were comparable with respect to age and sex distribution. The mean age of dengue-positive patients was 28.98 ± 9.11 years, while males constituted 72.0% of the study population. These findings are broadly consistent with previous studies reporting a predominance of dengue infection among young adults and males.

Gupta et al. reported a higher prevalence of dengue-positive cases among males (68.6%) than females (31.4%).¹¹ Ukey et al also observed a higher prevalence of dengue infection among males and reported that infection was common among individuals aged 15–30 years in Central India.¹²

These observations are comparable with the present study, in which the mean age of dengue-positive patients was approximately 29 years and males constituted 72% of participants. The predominance of young adults may be related to increased occupational, environmental, and outdoor exposure to mosquito vectors. Importantly, the dengue-positive and control groups in the present study were statistically comparable with respect to age and sex, thereby minimizing the potential confounding effects of these demographic variables on the comparison of hematological parameters, including HFLC percentage.

Comparison of hematological parameters between dengue-positive patients and controls

The present study demonstrated significant hematological differences between dengue-positive patients and controls. The mean WBC count was significantly lower in dengue-positive patients (5140 ± 2033.70 cells/ μ L) compared with controls (6522 ± 1843.28 cells/ μ L; $p < 0.001$). This finding is consistent with previous studies identifying leukopenia as a common hematological manifestation of dengue infection. Progressive leukopenia followed by thrombocytopenia has been reported as an important pattern of hematological change during the course of dengue illness.

The lymphocyte percentage was significantly higher among dengue-positive patients ($40.16 \pm 16.93\%$) than among controls ($29.66 \pm 6.06\%$; $p < 0.001$). This finding may reflect alterations in leukocyte distribution and immune activation associated with dengue virus infection, including the presence of activated and atypical lymphocytes.

A notable finding of the present study was the significantly lower HFLC percentage among dengue-positive patients ($9.16 \pm 7.73\%$) compared with controls ($24.50 \pm 14.58\%$; $p < 0.001$). This demonstrates a significant association between HFLC percentage and dengue status in the present study population. However, this finding differs from studies by Raharjo and Hadi, who reported increased HFLC values among patients with dengue hemorrhagic fever, and from studies demonstrating higher HFLC values in severe dengue compared with non-severe dengue. Such differences may be attributable to variations in disease severity, day of illness, characteristics of the control population, and differences in the analytical interpretation of HFLC parameters. Therefore, the diagnostic significance of HFLC should be interpreted in relation to the clinical phase and severity of dengue infection.¹³

Hemoglobin levels did not differ significantly between dengue-positive patients and controls ($p = 0.662$), indicating that hemoglobin concentration was not significantly associated with dengue status in the present study population. In contrast, platelet counts were significantly lower among dengue-positive patients ($208,340 \pm 143,548$ cells/ μ L) compared with controls ($287,680 \pm 93,384$ cells/ μ L; $p = 0.002$). This finding is consistent with the well-established occurrence of thrombocytopenia in dengue infection.

Overall, the present study confirms significant alterations in WBC count, lymphocyte percentage, platelet count, and HFLC percentage among dengue-positive patients. Although the observed direction of HFLC change differs from some previous reports, the highly significant difference between dengue-positive patients and controls supports further investigation of HFLC as a potential adjunctive hematological marker for dengue infection.

Distribution of HFLC values between dengue-positive patients and controls

In the present study, the distribution of HFLC values differed markedly between dengue-positive patients and controls. Using an HFLC cutoff value of $\leq 20\%$, 48 of 50 dengue-positive patients (96.0%) had HFLC values within this category, compared with 21 of 50 controls (42.0%). Conversely, only 4.0% of dengue-positive patients had HFLC values $> 20\%$, whereas 58.0% of controls were classified in this category. These findings suggest that an HFLC value of $\leq 20\%$ was strongly associated with dengue positivity in the present study population.

Previous studies evaluating HFLC and related high-fluorescent cell parameters have demonstrated variable findings. In contrast, Ningombam et al. Reported the diagnostic utility of an HFLC% cutoff $> 5.2\%$ for identifying dengue-positive patients, while Raharjo and Hadi reported increased HFLC values in patients with dengue hemorrhagic fever.¹⁴

Therefore, differences in HFLC distribution between studies may be influenced by the day of illness, disease severity, characteristics of the comparison group, sample selection, and differences in the analytical interpretation of HFLC parameters. The present findings suggest that HFLC $\leq 20\%$ may have potential utility as an adjunctive screening parameter for dengue infection; however, further prospective studies involving larger populations and standardized sampling at different stages of illness are required to validate this cutoff.

Diagnostic performance of HFLC for dengue infection

In the present study, ROC curve analysis demonstrated that HFLC had good discriminatory ability for differentiating dengue-positive patients from controls, with an area under the ROC curve of 0.807. An optimal cutoff value of $\leq 20\%$ was identified. At this cutoff, HFLC demonstrated a sensitivity of 96.0%, specificity of 58.0%, positive predictive value of 69.6%, and negative predictive value of 93.5%.

The high sensitivity observed in the present study indicates that HFLC may be useful as an adjunctive screening parameter for identifying patients with suspected dengue infection. Furthermore, the high negative predictive value suggests that an HFLC value outside the dengue-associated cutoff category may help reduce the likelihood of dengue infection in the studied population. However, the moderate specificity and positive predictive value indicate that HFLC should not be used as a stand-alone confirmatory diagnostic test and should be interpreted together with clinical findings and established laboratory investigations.

Hong et al evaluated HFLC as a predictor of severe dengue and reported an AUC of 0.833 for HFLC%, with a cutoff value of 13.15%, sensitivity of 70.6%, and specificity of 80.5%. The AUC reported in the present study (0.807) was comparable to their findings. However, the present study demonstrated substantially higher sensitivity but lower specificity. This difference may be explained by differences in the study objectives and populations, as the present study evaluated dengue-positive patients versus controls, whereas Hong et al evaluated severe versus non-severe dengue.¹⁵

Overall, the present findings suggest that HFLC has potential utility as a rapid adjunctive hematological screening marker for dengue infection. Its high sensitivity and negative predictive value are particularly valuable for screening purposes. Nevertheless, because of its moderate specificity, HFLC should be used in combination with clinical assessment and established dengue-specific diagnostic tests such as NS1 antigen and serological testing.

Diagnostic classification using the HFLC cutoff

In the present study, application of an HFLC cutoff value of $\leq 20\%$ correctly identified 48 of 50 dengue-positive patients, corresponding to a sensitivity of 96.0%. Among the control group, 29 of 50 participants had HFLC values $> 20\%$, corresponding to a specificity of 58.0%. Thus, the selected cutoff demonstrated high sensitivity but moderate specificity for differentiating dengue-positive patients from controls.

The findings of the present study may be compared with those of Chhabra et al who evaluated HFLC obtained from the Sysmex XN-1000 hematology analyzer for rapid screening of dengue infection. Their study included dengue-positive patients, dengue-negative febrile patients, and healthy controls. Using an HFLC% cutoff of 1.75, they reported a sensitivity of 52% and specificity of 90%. Compared with their findings, the present study demonstrated substantially higher sensitivity but lower specificity. These differences may be attributable to differences in the HFLC cutoff values, patient populations, comparison groups, and the clinical phase of dengue at the time of sample collection.¹⁶

Overall, the HFLC classification in the present study demonstrated that the majority of dengue-positive patients fell within the HFLC $\leq 20\%$ category. The high sensitivity indicates that this cutoff may be useful for screening patients with suspected dengue infection. However, because 21 control participants were also classified as HFLC $\leq 20\%$, the moderate specificity indicates that HFLC should be interpreted as an adjunctive hematological marker and used in combination with clinical findings and dengue-specific diagnostic tests.

CONCLUSION

The present prospective observational case-control study evaluated the utility of High-Fluorescence Lymphocyte Count (HFLC) obtained from the Sysmex XN-350 hematology analyzer as an early adjunctive diagnostic marker of dengue infection. A total of 100 participants were included, comprising 50 laboratory-confirmed dengue-positive patients and 50 dengue-negative controls. The dengue-positive and control groups were comparable with respect to age and sex distribution. Significant differences were observed in several hematological parameters between the two groups. Dengue-positive patients demonstrated significantly lower WBC counts and platelet counts, along with significantly higher lymphocyte percentages, confirming the characteristic hematological alterations associated with dengue infection.

A significant difference in HFLC percentage was also observed between the dengue-positive and control groups. In the present study, the mean HFLC percentage was significantly lower among dengue-positive patients compared with controls. Using a cutoff value of HFLC

≤20%, 96% of dengue-positive patients were correctly identified, resulting in a sensitivity of 96.0%. The specificity was 58.0%, while the positive predictive value and negative predictive value were 69.6% and 93.5%, respectively. ROC curve analysis demonstrated an AUC of 0.807, indicating good discriminatory ability of HFLC percentage for differentiating dengue-positive patients from controls. These findings suggest that HFLC has potential value as a rapid adjunctive hematological screening parameter for dengue infection.

The high sensitivity and negative predictive value observed in this study indicate that HFLC may be particularly useful during the initial laboratory assessment of patients with suspected dengue. However, the moderate specificity indicates that HFLC should not be used as a standalone confirmatory diagnostic test.

Therefore, HFLC should be interpreted in conjunction with clinical findings, routine CBC parameters, and established dengue-specific diagnostic tests such as NS1 antigen detection and serological testing. Since HFLC is generated during routine automated hematological analysis without requiring an additional specialized investigation, it may provide useful supplementary information in laboratories equipped with Sysmex XN-Series analyzers.

In conclusion, HFLC percentage demonstrated good diagnostic potential as an adjunctive marker for dengue infection in the present study. Further studies involving larger sample sizes, dengue-negative febrile controls, and standardized assessment according to the day and severity of illness are recommended to validate the observed cutoff value and establish the clinical role of HFLC in the early diagnosis and monitoring of dengue infection.

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