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PRELIMINARY EVALUATION OF THE DIAGNOSTIC VALUE OF HFLC IN DIFFERENTIATING DENGUE FEVER FROM OTHER FEBRILE ILLNESSES

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ABSTRACT

Dengue fever (DF) remains a primary global health concern. High Fluorescence Lymphocyte Count (HFLC) reflects the immune response to infection by quantifying antibody-secreting cells (ASC), such as plasmablasts/plasma cells. ASCs are known to increase markedly in dengue compared to other febrile illnesses (OFIs).

Objective: This study aimed to preliminarily evaluate the diagnostic value of HFLC in distinguishing dengue fever from other febrile illnesses.

Methods: A cross-sectional study was conducted on 317 patients presenting with fever on admission. Clinical features and laboratory parameters, including HFLC# and HFLC%, were collected. Patients were categorized into the dengue infection (DI) group (n = 267) and the OFI group (n = 50).

Results: The DI group had significantly higher median HFLC# (0.11 G/L) and HFLC% (5.2%) compared to the OFI group (0.01 G/L and 0.20%, respectively; $p < 0.001$). ROC analysis showed good diagnostic performance, with AUCs of 0.794 (HFLC#) and 0.842 (HFLC%). A cut-off value of 0.025 G/L for HFLC# yielded 70.4% sensitivity and 78.0% specificity. For HFLC%, a threshold of 0.75% resulted in 70.8% sensitivity and 88.0% specificity.

Conclusion: HFLC demonstrates potential as a rapid and accessible marker for differentiating dengue fever from other febrile illnesses in clinical practice.

Keywords: Dengue fever, HFLC, diagnosis.

1. INTRODUCTION

Dengue fever (DF) is an acute infectious disease caused by the Dengue virus (DENV), transmitted primarily by Aedes mosquitoes, especially Aedes aegypti. Globally, approximately 50 million cases of Dengue are reported annually [1]. In Vietnam, from 1999 to 2020, there were 1,844,407 reported cases of Dengue fever, including 1,250 fatalities [2]. Currently, the diagnosis of Dengue fever primarily relies on clinical manifestations and serological tests such as NS1 antigen and IgM antibody assays. Although crucial for confirming Dengue infection, these methods have limitations. In particular, the clinical symptoms of Dengue during the febrile phase often overlap with those of other acute febrile illnesses, such as bacterial infections, malaria, typhoid fever, leptospirosis, and, more recently, COVID-19. During outbreak peaks, healthcare systems may become overwhelmed, and shortages of diagnostic test kits further complicate definitive diagnosis [3]. These challenges underscore the need for improved diagnostic approaches to distinguish Dengue fever from other febrile illnesses during the early stages.

In addition to conventional complete blood count (CBC) parameters, modern automated hematology analyzers can provide novel indices that have been investigated for their utility in diagnosing infectious diseases at the time of hospital admission. One such index is the High Fluorescent Lymphocyte Count (HFLC), which reflects a population of lymphocytes with high fluorescence intensity [4]. HFLC has been shown to represent antibody-secreting B cells, plasmablasts/plasma cells. Therefore, HFLC may indicate the host's immune response to infection, including DENV

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infection. However, the diagnostic utility of HFLC in Dengue fever has not been previously studied. This study aims to: (1) compare HFLC values between Dengue fever and other febrile illnesses; (2) initially assess the potential diagnostic value of HFLC in differentiating Dengue fever from other febrile illnesses at the time of hospital admission.

2. MATERIALS AND METHODS

2.1 Study Population

This study included 317 patients who were hospitalized in the Department of Infectious Diseases, 103 Military Hospital, from June 2022 to December 2024.

- Inclusion criteria:

(1) Patients presenting with fever as an initial symptom;

(2) Patients who underwent a CBC test using the Sysmex XN-1000 hematology analyzer on the day of admission;

(3) Patients who had an NS1 antigen test within the first five days of symptom onset and/or IgM/IgG testing after day 5.

- Exclusion criteria:

Patients with hematopoietic disorders, autoimmune diseases, or who were on immunosuppressive therapy were excluded.

- Study duration:

From June 2022 to December 2024 at the Department of Infectious Diseases, 103 Military Hospital.

2.2. Study Design and Methods

Study design: Cross-sectional retrospective study.

Sample size: Convenience sampling.

Patients were diagnosed with Dengue fever based on the WHO 2009 guidelines. For evaluating the diagnostic value of HFLC, patients were divided into two groups: those with Dengue infection (DI group) and those with other febrile illnesses (OFI group).

Clinical characteristics and laboratory results on admission (including etiological diagnostics, CBC, and biochemical tests) were collected. HFLC values were recorded as both absolute count (HFLC#) and relative percentage (HFLC%). The reference values for HFLC# and HFLC% were 0.00 G/L and 0.00%, respectively.

Data analysis:

Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

2.3 Ethical Considerations

The study protocol was approved by the Ethics Committee of 103 Military Hospital (Decision No. 89/HĐĐĐ dated August 19, 2024). Data usage and publication were authorized by the Hematology and Blood Transfusion Center of the hospital. The authors declare that they have no conflict of interest.

3. RESULTS

3.1 General Characteristics of the Study Population

Table 1. Baseline Characteristics of Study Participants

Characteristics			
Total (n=317)	**DI (n=267)**	**OFI (n=50)**	**p-value**
Age, median (IQR)			
46 (33 – 64)	43 (32– 62)	60 (44 – 69)	<0,001
Female, n (%)			0,145
144 (45,4)	126 (47,2)	18 (36,0)	
Male, n (%)			
173 (54,6)	141 (52,8)	32 (64)	
Days of fever on admission, median (IQR)			
4 (3 – 5)	4 (3 – 5)	2 (2 – 4,5)	<0,001

The median age of patients in the DI group (43 years) was significantly lower than that of the OFI group (60 years). The number of febrile days at admission was significantly higher in the DI group (4 days) compared to the OFI group (2 days). No statistically significant difference was observed in the gender distribution between the two groups.

Table 2. Selected Clinical and Laboratory Characteristics

Characteristics			
Total (n=317)	**DI (n=267)**	**OFI (n=50)**	**p-value**
Flushed skin/Rash, n (%)			
160 (50,5)	143 (53,6)	17 (34,0)	0,011

Characteristics			
Total (n=317)	DI (n=267)	OFI (n=50)	p-value
Headache, n (%)			
230 (72,6)	205 (76,8)	25 (50,0)	< 0,001
Nausea/ Vomiting, n (%)			
113 (35,6)	96 (36,0)	17 (34,0)	0,791
Myalgia/ Arthralgia, n (%)			
256 (80,8)	232 (86,9)	24 (48,0)	< 0,001
WBC (G/L), median (IQR)			
4,44	4,10	8,28	< 0,001
(3,19 – 6,88)	(2,92– 6,11)	(5,64 – 11,18)	
NEU (G/L), median (IQR)			
2,31	2,05	6,63	< 0,001
(1,42 – 4,01)	(1,32 – 3,16)	(3,81 – 9,37)	
LYMP (G/L), median (IQR)			
1,05	1,12	0,89	0,003
(0,66 – 1,82)	(10,68 – 1,96)	(0,44 – 1,25)	
MONO (G/L), median (IQR)			
0,49	0,46	0,64	0,002
(0,29 – 0,73)	(0,28 – 0,96)	(0,42 – 0,93)	
HGB (g/L), median (IQR)			
140,00	142,00	119,00	< 0,001
(129,00 – 156,00)	(132,00 – 158,00)	(107,75 – 139,00)	
HCT (L/L), median (IQR)			
0,41	0,42	0,36	< 0,001
(0,38 – 0,45)	(0,39 – 0,46)	(0,33 – 0,40)	
PLT (G/L), median (IQR)			
57,00	39,00	179,50	< 0,001
(18,50 – 148,50)	(17,00 – 116,00)	(120,50 – 211,00)	

Headache and musculoskeletal pain were significantly more prevalent in the DI group (76.8% and 86.9%, respectively) compared to the OFI group (50.0% and 48.0%). No significant difference was found in the prevalence of nausea or vomiting. Significant differences in hematological parameters were observed. The median WBC count was significantly lower in the DI group (4.10 G/L) compared to the OFI group (8.28 G/L). Similar patterns were noted in neutrophil and monocyte counts. Conversely, lymphocyte counts were significantly higher in the DI group. In terms of red blood cell indices, both hemoglobin and hematocrit levels were significantly elevated in the DI group. Median platelet count was markedly lower in the DI group (39.00 G/L) versus the OFI group (179.50 G/L).

3.2 Characteristics of HFLC in Dengue Infection and Its Diagnostic Value

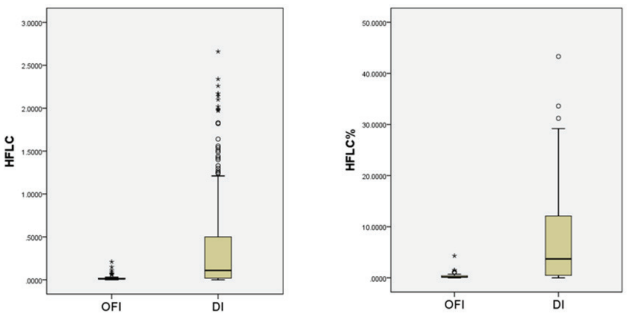

Figure 1. Comparison of HFLC# (left) and HFLC% (right) between the DI and OFI groups

Patients in the DI group had significantly higher HFLC# values than those in the OFI group (0.11 G/L vs. 0.01 G/L; $p < 0.001$). Likewise, the HFLC% was significantly higher in the DI group (5.20%) compared to the OFI group (0.20%) ($p < 0.001$) (Figure 1).

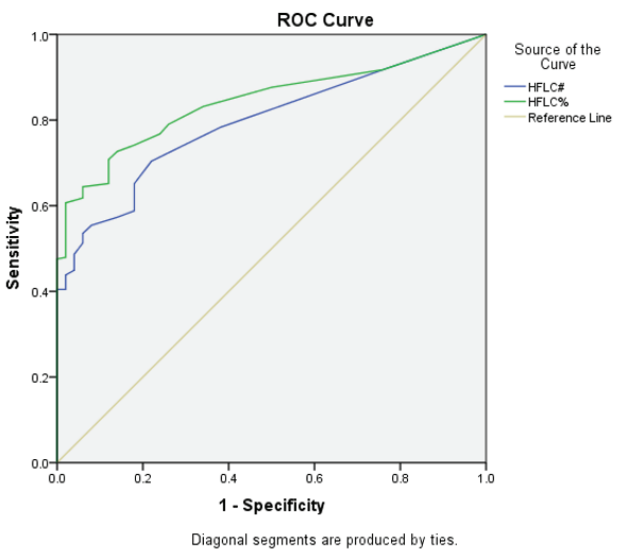

Figure 2. ROC curves of HFLC# (green) and HFLC% (light green) for differentiating Dengue from other febrile illnesses

ROC curve analysis revealed that HFLC# and HFLC% had strong discriminatory power in differentiating dengue infection from other febrile illnesses, with AUCs of 0.794 and

0.842, respectively (Figure 2). The optimal cut-off for HFLC# was 0.025 G/L, yielding a sensitivity of 70.4% and a specificity of 78.0%. For HFLC%, a threshold of 0.75% gave a sensitivity of 70.8% and a specificity of 88.0%.

4. DISCUSSION

4.1 Characteristics of Patients with Dengue and Other Febrile Illnesses

The DI group was significantly younger than the OFI group and presented to the hospital after a longer duration of fever. These findings are consistent with the demographic profile of dengue cases. Clinical symptoms such as headache and myalgia/arthralgia were more prominent in the DI group, which is in line with findings from Jenny G.H. Low et al. (2011), who also identified these symptoms as key features differentiating dengue from other febrile illnesses [5]. Dengue has also been referred to as "breakbone fever," due to its typical presentation of intense headache, retro-orbital pain, and diffuse musculoskeletal pain [1,6].

4.2. Compare HFLC values between 2 groups and diagnostic Value of HFLC in Differentiating Dengue from Other Febrile Illnesses

Our study found that both HFLC# and HFLC% were significantly elevated in patients with dengue infection compared to those with other febrile illnesses (Figure 1). The difference in HFLC between the two groups may be attributed to several immunological factors. As previously described by Linssen et al. (2007), HFLC reflects a population of activated B lymphocytes or plasma cells secreting antibodies [7]. These cells exhibit high fluorescence intensity due to increased RNA content.

The proportion and absolute number of plasma cells are markedly higher in dengue infection. Wrammert et al. (2012) reported that plasmablasts constituted 47% of CD19+ B cells in dengue patients, compared to less than 10% in those with influenza or yellow fever [8]. Hence, the increase in HFLC reflects a robust humoral immune response specific to dengue infection, distinguishing it from other febrile diseases.

ROC analysis further supports the clinical utility of HFLC in the diagnosis of dengue. HFLC%, in particular, demonstrated high specificity (88%) and acceptable sensitivity (70.8%), making it a promising biomarker for rapid differentiation of dengue infection from other febrile conditions. This could provide a cost-effective and accessible tool for early triage and management, especially in endemic regions.

5. CONCLUSION

HFLC values (both HFLC# and HFLC%) were significantly higher in dengue patients compared to those with other febrile illnesses. These parameters showed good diagnostic performance and may serve as a rapid, automated marker to support early dengue diagnosis. Further research is needed to validate their clinical utility in larger populations.

REFERENCES

[1] Whitehorn, J. & Farrar, J. Dengue. Br. Med. Bull. 95, 161–173 (2010). doi:10.1093/bmb/ldq019.

[2] Ha, V. H. ., Ha, T. T. T. ., Thai, P. Q. ., Nghia, N. D. ., Nam, V. S. ., & Duong, T. N. . (2022). Sốt xuất huyết dengue tại Việt Nam giai đoạn 1999 – 2020. Vietnam J. Prev. Med. 32, 9–15 (2022). doi:https://doi.org/10.51403/0868-2836/2022/603.

[3] Kularatne, S. A. M. Dengue fever. BMJ 351, h4661 (2015). doi:10.1136/bmj.h4661.

[4] Oehadian, A. et al. New parameters available on Sysmex XE-5000 hematology analyzers contribute to differentiating dengue from leptospirosis and enteric fever. Int. J. Lab. Hematol. 37, 861–868 (2015). doi:10.1111/ijlh.12422.

[5] Low, J. G. H. et al. The Early Clinical Features of Dengue in Adults: Challenges for Early Clinical Diagnosis. PLoS Neglected Tropical Diseases vol. 5 (2011). doi:10.1371/journal.pntd.0001191.

[6] Chen, L. H. & Wilson, M. E. Dengue and chikungunya infections in travelers. Curr. Opin. Infect. Dis. 23, 438–444 (2010). doi:10.1097/QCO.0b013e32833c1d16.

[7] Linssen, J. et al. Identification and quantification of high fluorescence-stained lymphocytes as antibody-synthesizing/secreting cells using the automated routine hematology analyzer XE-2100. Cytometry B. Clin. Cytom. 72, 157–166 (2007). doi:10.1002/cyto.b.20150.

[8] Wrammert, J. et al. Rapid and massive virus-specific plasmablast responses during acute dengue virus infection in humans. J. Virol. 86, 2911–2918 (2012). doi:10.1128/JVI.06075-11.

[9] Lee, H. et al. Comparison of Six Commercial Diagnostic Tests for the Detection of Dengue Virus Non-Structural-1 Antigen and IgM/IgG Antibodies. Ann. Lab. Med. 39, 566–571 (2019). doi:10.3343/alm.2019.39.6.566.