ORIGINAL ARTICLE

Factors Associated With Serum Ferritin Among Severe Dengue Patients at a Tertiary Hospital in Malaysia

Zety Wizana Rusman[1,2], Subashini C. Thambiah[1], Nur Shafini Che Rahim[2], Intan Nureslyna Samsudin[1], Siti Yazmin Zahari Sham[1], Malina Osman[3], Yin Ye Lai[1]

[1] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2] Department of Pathology, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia
[3] Department of Community Health, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: Severe dengue, which poses significant risks of both mortality and morbidity, lacks any specific antiviral therapy, underscoring the urgency for predictive biomarkers. Hyperferritinaemia observed in severe cases is thought to stem from excessive activation of monocytes and macrophages. This study aimed to determine factors associated with hyperferritinaemia in hospitalised patient with severe dengue. **Materials and methods:** A retrospective cross-sectional study was done among patients who were admitted to Hospital Kuala Lumpur with severe dengue in 2017 and 2018. Electronic patient demographic and laboratory data were recorded and analysed. **Results:** Hyperferritinaemia was observed in 69.2 % of the study population. Gender was significantly associated with ferritin status in severe dengue with significantly higher median ferritin levels in males compared to females. Thrombocytopaenia, raised aspartate transaminase (AST), alanine transaminase (ALT) and haematocrit (HCT) were significantly associated with hyperferritinaemia. Stepwise multilinear regression analysis found that only AST and HCT remained significantly associated with serum ferritin levels, explaining 59.5% of the variance (R= 0.595) in severe dengue patients. An increase of 1 U/L in AST is associated with a 9.54 µg/L increase in serum ferritin (95% CI: 8.03-11.04), while an increase of 1% in HCT corresponds to a 212.52 µg/L increase in serum ferritin (95% CI: 72.42-352.61). **Conclusion:** This pilot study found a lower prevalence of hyperferritinaemia compared to previous Asian studies. AST and HCT were independent predictors of serum ferritin in severe dengue patients, highlighting its importance as a biomarker for assessing dengue severity.
Malaysian Journal of Medicine and Health Sciences (2024) 20(SUPP11): 3-9. doi:10.47836/mjmhs20.s11.2

Keywords: Severe dengue, Ferritin, Liver enzymes, Platelets, Haematocrit

Corresponding Author:
Subashini C. Thambiah, MPath
Email: subashini@upm.edu.my
Tel : +60123923709

INTRODUCTION

Severe dengue is associated with high mortality and morbidity. Currently, there is no antiviral treatment for dengue. As such, there is an imminent need for biomarkers that can predict dengue severity. This is of utmost importance during the defervescence phase for those patients with non-severe infection that are anticipated to progress into severe dengue (1). This will in turn result in effective disease management.

Ferritin, an acute phase reactant, is produced and secreted by hepatocytes. During an acute phase response, its levels increase. However, ferritin can also be released by macrophages and cancer cells. Its functions include intracellular iron sequestration and storage abilities during immune activation, which is important for protection against microbial spread, oxidative damage, inflammation, and malignancy (2). Haemophagocytic lymphohistiocytosis (HLH) or macrophage activation syndrome is an uncommon occurrence of severe dengue and has been increasingly reported with markedly raised serum ferritin level (3-5).

Several studies have supported the role of ferritin as a predictor of severe dengue (3-5). However, there was only one study that demonstrated significant associations between hyperferritinaemia as a marker of dengue severity with thrombocytopaenia, elevated liver enzymes and coagulation disturbance (6). In Malaysia, studies regarding predictive biomarkers of dengue severity have been done. Ab-Rahman et al. demonstrated that ferritin and sCD136 were biomarkers for severe dengue

but did not show the association of serum ferritin with routine laboratory investigations i.e., platelets and liver enzymes (7). Md-Sani et al. also published a study on mortality prediction among patients with severe dengue hospitalised at Hospital Kuala Lumpur (HKL) in 2014. Their conclusion suggests that the optimal predictive model for mortality, established at the time of severe dengue diagnosis, incorporates serum bicarbonate and alanine transaminase (ALT) levels obtained at that juncture (8). However, serum ferritin was not included in their study. Hence, this study aimed to examine the relationship between serum ferritin levels and routine laboratory investigations done in dengue patients [platelets, haematocrit (HCT) and liver enzymes [ALT and aspartate transaminase (AST)] in predicting dengue severity, highlighting ferritin's importance as a prognostic marker. Identifying such predictive biomarkers is crucial for enabling early interventions and treatment strategies to reduce morbidity and mortality from dengue.

MATERIALS AND METHODS

Study population

This retrospective, cross-sectional study was conducted in the Pathology Department, HKL using data of patients admitted with severe dengue from 1st January 2017 to 31st December 2018. This hospital was selected as it is a tertiary healthcare centre serving large community areas in the Klang Valley. The study employed a convenient sampling approach. The sample size was determined utilising the following formula:

$$n = \frac{Z^2_{1-a/2}p(1-p)}{d^2}$$

$$n = \frac{1.96^2 \text{ X } 0.07(1-0.07)}{0.05^2}$$

$$n = 101 \text{ patients}$$

where z = confidence level at 95% (standard value of 1.96); α = level of significance; p = incidence of severe dengue among all dengue cases = 7% (9); d = margin of error at 5% (standard value of 0.05).

The study cohort comprised adult individuals aged 18 years and older, admitted to the medical or dengue wards in HKL diagnosed as severe dengue by the attending clinician with confirmed dengue-seropositive results (positive NS-1 Antigen, dengue IgM and dengue IgG in combination or positive on its own). Inclusion criteria also included availability of serum ferritin, ALT, AST, platelets and HCT results. Clinical diagnosis of the disease was based on the 3rd Edition of the Malaysian Clinical Practice Guidelines (CPG) Management of Dengue Infection in Adults (10). Confirmed cases of chronic inflammatory disease, patients with recent blood transfusion, iron metabolic disorder, liver cirrhosis, platelet disorders and red cells disorders were excluded.

Data collection

Data of 120 patients who had been notified as severe dengue in HKL from 1st January 2017 to 31st December 2018 were obtained from the Public Health Unit of HKL. Patient demographic data (age, gender, and ethnicity) was extracted and documented into the Proforma. Laboratory data on admission, encompassing serum ferritin, liver enzymes (ALT, AST), platelets, and HCT were obtained electronically. Access to the Proforma was restricted to researchers to uphold patient confidentiality. Each patient was assigned a unique code number for identification purposes. Approval for this study was obtained from the Director of HKL [CRC HKL Registration Number: HCRC. IIR-2017-12-269] and the Malaysian Research Ethical Committee (MREC) Ministry of Health [ref letter: (5) KKM/NIHSEC/P18-78(6)], (NMRR 17-2809-38412).

Laboratory investigations

Serum ferritin was measured by electrochemiluminescence immunoassay method on the automated Cobas 6000 analyser (Roche Diagnostics GmbH, Mannheim, Germany), while serum ALT and AST activities were measured by kinetic UV method on the automated biochemistry analyser Cobas 8000 (Roche Diagnostics GmbH, Mannheim, Germany). Platelets and HCT were measured by fluorescent flow cytometry on the automated haematology analyser Sysmex XN-1000. Hyperferritinaemia in this study was defined as serum ferritin of more than 500 µg/L, according to diagnostic criteria of HLH (11). All methods followed the standard operating procedures of the Chemical Pathology Laboratory at HKL and met both internal quality control and external quality assessment standards. Laboratory analyses were closely monitored to ensure precision and accuracy.

Data analysis

Statistical analyses were conducted using the IBM SPSS Statistics software package, Version 25.0, by IBM Corp. in Armonk, NY. A significance level of $p < 0.05$ was considered statistically significant. Non-parametric methods were employed for data analysis. Categorical variables were presented as frequencies and percentages, while continuous variables were described using median and interquartile range (IQR). The association between demographic factors and laboratory parameters with hyperferritinaemia was assessed using Chi-square test or Fisher's exact test and Mann–Whitney U test for categorical and continuous variables, respectively. Linear regression analysis was performed to examine the linear relationship of significant variables with serum ferritin among study population. Subsequent multilinear

regression analysis was done to determine independent predictors of serum ferritin.

RESULTS

Table I shows the demographics and laboratory parameters of the study subjects. Most subjects were female (70.0%), Malay (73.3%) between 18 to 59 years old (89.2%), with a median age of 29 (IQR = 25) years old. Among non-Malays, Indians were the second highest ethnicity (16.7%) followed by Chinese (5.8%) and others (4.2%) [data not shown]. Majority of the subjects were Malaysian. The median levels of serum ferritin, AST and ALT were above the reference interval. The median and mean for platelet and HCT, respectively were below the reference interval.

Table I: Demographic factors and laboratory parameters of study population

Demographic factors		(n=120) N (%)
Age (years)		
29 (25)*		
18 to 59 years		107 (89.2)
60 years and above		13 (10.8)
Sex		
Male		36 (30.0)
Female		84 (70.0)
Race		
Malay		88 (73.3)
Non-Malay		32 (26.7)
Nationality		
Malaysian		115 (95.8)
Non-Malaysian		5 (4.2)
Laboratory parameters	**Results**	**Reference interval*****
Ferritin (µg/L)	1189.00 (2707.90)*	Male: 30 – 400 Female: 13 – 150
Platelet (10^3/µL)	92.50 (91.00)	150 – 410
AST (U/L)	96.00 (151.00)*	<51
ALT (U/L)	67.50 (116.00)*	<42
HCT (%)	35.40 ± 5.08**	Male: 40 – 50 Female: 36 – 46

AST: Aspartate transaminase; ALT: Alanine transaminase; HCT: Haematocrit; *Median (IQR); **Mean ±SD
*** Reference interval used in Hospital Kuala Lumpur

The association of demographics and laboratory parameters with serum ferritin status is demonstrated in Table II. Hyperferritinaemia was observed in 69.2% of the study population. The median levels of ALT, AST and mean HCT were significantly higher in the hyperferritinaemia group compared to the normoferritinaemia group. The median platelet, however, was significantly lower in the hyperferritinaemia group. Gender was significantly associated with ferritin status in severe dengue (p=0.002) [Table II] with significantly higher median ferritin levels in males compared to females (p=0.001) [Table III].

Table II: Association of demographic factors and laboratory parameters with serum ferritin

Variables		Hyperferritinaemia (n=83) n (%)	Normoferritinaemia (n=37) n (%)	$\chi^{2\,a}$	p value^
Age (years)	18 – 59	31 (24)* 73 (88.0)	24 (19)* 34 (91.9)	0.105	0.746
	≥60	10 (12.0)	3(8.1)		
Race	Malay	65 (78.3)	23 (62.2)	3.414	0.065
	Non-Malay	18 (21.7)	14 (37.8)		
Gender	Male	32 (38.6)	4 (10.8)	9.380	**0.002**
	Female	51 (61.4)	33 (89.2)		
Nationality	Malaysian	78 (94.0)	37 (100.0)		0.322
	Non-Malaysian	5 (6.0)	0 (0.0)		
Laboratory parameters	**Hyperferritinaemia (n=83)**	**Normoferritinaemia (n=37)**	**z[b]**	**p value^**	**Reference interval*****
Platelet (10^3/µL)	61 (73)*	132 (84)*	-4.919	0.001	150 – 410
AST (U/L)	134 (254)*	38 (38)*	-5.791	0.001	<51
ALT (U/L)	99 (166)*	25 (29)*	-6.104	0.001	<42
HCT (%)	36 ± 6**	34 ± 6**	-2.404	0.016	Male: 40 – 50 Female: 36 – 46

AST: Aspartate transaminase; ALT: Alanine transaminase; HCT: Haematocrit; *Median (IQR); **Mean ± SD; [a]Chi square test (χ^2); [b]Mann-Whitney test (z); ^statistical significance at p <0.05
***Reference interval used in Hospital Kuala Lumpur

Table III: Association of serum ferritin with gender

	Male (n=36) Median (IQR)	Female (n=84) Median (IQR)	z[a]	p value[b]	Reference interval[c]
Ferritin (µg/L)	2000.0 (10028.3)	794.9 (1690.1)	-3.941	0.001	Male: 30 – 400 Female: 13 – 150

[a]Mann-Whitney test (z), [b]statistical significance at p <0.05
[c]Reference interval used in Hospital Kuala Lumpur

All variables that exhibited significant associations with serum ferritin were incorporated into a simple linear regression analysis, as detailed in Table IV. Notably, while gender demonstrated a statistically significant association with serum ferritin (p <0.05), it was not included in this regression analysis as male subjects (n = 36) were much lesser in number compared to females (n = 84) and its inclusion in the prediction model created an unstable model with large confidence interval (CI) [(95% CI: 1131.88-4199.07) [data not shown]. Single

linear regression analysis showed significant association of all laboratory parameters with ferritin. However, subsequent stepwise multiple linear regression analysis revealed that only AST and HCT remained significantly associated with serum ferritin levels, explaining 59.5% of the variance (R= 0.595) in severe dengue patients. AST (Beta = 0.741) has a greater influence on serum ferritin than HCT (Beta = 0.177) [data not shown]. An increase of 1 U/L in AST is associated with a 9.54 µg/L increase in serum ferritin (95% CI: 8.03-11.04), while an increase of 1% in HCT corresponds to a 212.52 µg/L increase in serum ferritin (95% CI: 72.42-352.61) (Table IV).

Table IV: Factors associated with serum ferritin levels among study population

Variables	SLR			MLR		
	b	95% CI	p value*	b	95% CI	p value*
Platelet (10^3/µL)	-19.056	-34.35 – -3.77	0.015	-	-	-
ALT (U/L)	9.962	6.32 – 13.60	0.001	-	-	-
HCT (%)	261.972	48.39 – 475.55	0.017	212.516	72.42 – 352.61	0.003
AST (U/L)	9.662	8.11 – 11.21	0.001	9.536	8.03 – 11.04	0.001

SLR; simple linear regression; MLR; multiple linear regression; *statistical significance at $p<0.05$; $R^2=0.595$

DISCUSSION

In this study, 69% of study subjects were defined as hyperferritinaemia that is a serum ferritin level of more than 500 µg/L (12). This prevalence was relatively low compared to previous studies among Pakistan (95%) (13) and Indian populations (100%) (14). Likewise, a study conducted in Aruba Caribbean Island revealed an elevated prevalence of hyperferritinaemia, reaching 90.5% among patients with dengue infection, in contrast to a mere 9.5% among individuals with other febrile conditions. These results indicate a robust specificity of 88% and an odds ratio (OR) of 6, indicating that hyperferritinaemia could potentially serve as a discriminatory marker between dengue and other febrile illnesses (6). The discordance in prevalence may be explained by a higher proportion of females in our study compared to previous ones.

There was significant difference between gender, AST, ALT, platelets and HCT between hyperferritinaemia and normoferritinaemia groups (Table II). Although the study population was predominantly females, majority of males (32/36; 88.8%) had hyperferritinaemia compared to females (51/84; 60.7%) with males having significantly higher median value of serum ferritin (2000 µg/L) compared to females (795 µg/L) (Table III). Baseline ferritin levels are known to be higher in males than in females as evident by the gender-specific reference intervals used in this study. Serum ferritin is the reflection of a person's body iron storage. Its lower levels in females, especially in the reproductive age group is due to menstrual blood loss and increment of iron utilisation during pregnancy (15).

The Malaysian CPG on Management of Dengue Infection in Adults concluded that there is no correlation between platelet count and dengue severity (10). However, various studies found that lower platelet count was significantly associated with dengue haemorrhagic fever (16, 17) but not an independent predictor of it (16). In this study, the median platelet count was significantly lower in the hyperferritinaemia group compared to the normoferritinaemia group. This finding was comparable to other studies (1, 6). Nevertheless, in this study, while a reduced median platelet count was observed in the hyperferritinaemia cohort, individuals with normal ferritin levels also manifested thrombocytopaenia, albeit with a comparatively elevated median level in comparison to the hyperferritinaemia cohort. This indicates that thrombocytopaenia is a common finding in dengue infected cases, irrespective of severity (10). Dengue virus suppresses bone marrow progenitor cell function thus inhibiting platelet production. Dengue infection also can cause peripheral platelet consumption due to disseminated intravascular coagulation and platelet destruction secondary to anti-platelet antibody and activation of complement system (18).

The significantly lower median platelet in hyperferritinaemia group in this study might be explained by the relationship between coagulation and inflammatory processes in severe dengue. In severe dengue, antibody independent enhancement and overactivation of T cells are hypothesised as the cause of excessive generation of cytokines (19). Hypercytokinaemia stimulates overwhelming production of serum ferritin from hepatocytes and activated macrophages (20) and contributes to overactivation of coagulation and fibrinolysis processes that occur in severe dengue (18). Activation of coagulation process leads to binding of von Willebrand factors (vWF) to platelet surface that further activate platelet aggregation and peripheral platelet consumption for thrombus formation. Apart from platelets, other coagulation markers such as thrombin anti-thrombin complex and vWF were also shown to be significantly associated with hyperferritinaemia in dengue cases (6).

Research indicates a significant association between elevated liver enzymes and severe dengue (6, 16, 17, 21). Direct virus invasion and dysregulated immune response lead to hepatocellular damage and subsequent release of serum ferritin as well as AST and ALT from hepatocytes (22). Dengue is a notable aetiology of acute viral hepatitis in dengue endemic regions (16). Raised ALT and AST levels have been linked with bleeding (23).

Mechanisms of liver injury in dengue infection include direct virus invasion and replication in hepatocytes, hypotensive episodes, which lead to ischaemic injury, immunology injury due to dysregulated immune response and hepatotoxic effect of medications such as herbal remedies and acetaminophen (10). Our study revealed that although both AST and ALT were significantly associated with serum ferritin level, the independent predictor for hyperferritinaemia among study subjects was only AST. A study by Lee et al. also demonstrated AST as an independent predictor of dengue haemorrhagic fever (24). Many studies found consistent findings of higher AST elevation compared to ALT in severe dengue patients (16, 25, 26). ALT is primarily expressed by hepatocytes whereas AST is also expressed by other organs like skeletal muscles, heart, kidney, brain and red blood cells (22). During dengue illness, dengue virus might cause acute damage not only to hepatocytes but also to other non-hepatic tissues that express AST. This may explain the finding of higher AST than ALT during dengue infection. As a result of this non-hepatic tissue damage, individuals with elevated AST levels were more inclined to be categorised as severe dengue based on the 2009 WHO dengue classification (16).

To our knowledge, no previous study has demonstrated an association between HCT and serum ferritin in severe dengue patients. A study in Thailand found that the mean HCT level was significantly higher in severe dengue compared to non-severe dengue cases (27). Rising HCT is a marker of plasma leakage. Severe plasma leakage is one of the diagnostic criteria of severe dengue diagnosis (10). On the other hand, hyperferritinaemia is commonly found in severe dengue patients secondary to dysregulated macrophage activation (12). Hence, the significant association between these two parameters indirectly indicate severe dengue. HCT was shown to be a predictor of severe dengue in studies in Thailand (OR= 1.34, $p<0.05$) (27) and India (OR=3.14, $p<0.05$) (28). Our study appears to be the first to demonstrate HCT as an independent predictor of hyperferritinaemia in severe dengue patients. Dysregulated activation of monocytes, macrophages and T lymphocytes in severe dengue has been shown to generate excessive amount of cytokines. This hypercytokinaemia is an important contributing factor to both excessive production of serum ferritin and endothelial dysfunction (29). Endothelial dysfunction leads to increased capillary permeability and subsequently increased plasma leakage, which is reflected by the rising HCT (29). HCT as an independent predictor of hyperferritinaemia indirectly explains the pathophysiology of hypercytokinaemia in severe dengue.

This research presents certain limitations. As it is retrospective in nature, not all severe dengue cases included data on serum ferritin. Moreover, serum ferritin was not tested for every dengue patient in this study; it was typically ordered by clinicians when HLH was suspected or as part of routine investigation for anaemia. This could explain the predominance of female subjects with a lower median ferritin level. Additionally, the utilisation of different severity classifications for dengue in this study compared to previous research could introduce a potential confounding factor in the interpretation and comparison of results. Nevertheless, this study marks the first attempt in Malaysia to assess the associated factors of hyperferritinaemia in severe dengue cases. To enhance the validity of our findings, the sample size should be increased by including participants from multiple centres to better represent the Malaysian population. Notably, our study defined severe dengue based on the classification outlined in the latest Malaysian CPG (10), which aligns with the 2009 WHO Dengue Classification and Level of Severity. This approach contrasts with previous studies that relied on the 1997 WHO Dengue Classification, potentially overlooking important clinical features of severe dengue cases due to its incomplete coverage of the disease spectrum. Further prospective cohort studies are warranted to elucidate the role of serum ferritin as a prognostic marker, aid in risk stratification, and guide treatment strategies for severe dengue patients.

CONCLUSION

The prevalence of elevated ferritin levels among severe dengue cases in this study stood at 69.2%, a figure relatively lower than reported in prior Asian studies. A notable gender disparity was observed, with males exhibiting higher median ferritin levels compared to females. Among severe dengue patients, only AST and HCT emerged as independent predictors of serum ferritin. These results highlight the significance of serum ferritin as a biomarker for assessing the severity of dengue infection.

ACKNOWLEDGEMENT

The authors would like to thank the Director General of Health Malaysia for his permission to publish this article.

REFERENCES

1. Soundravally R, Agieshkumar B, Daisy M, Sherin J, Cleetus CC. Ferritin levels predict severe dengue. Infection. 2015; 43(1): 13–19. doi:10.1007/s15010-014-0683-4 https://doi.org/10.1007/s15010-014-0683-4
2. Maria, Alida, and Margaretha Viljoe. 'Acute Phase Proteins: Ferritin and Ferritin Isoforms'. Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins, InTech, 3 Oct. 2011; Crossref, https://doi.org/10.5772/20586
3. Srichaikul T, Punyagupta S, Kanchanapoom T, Chanokovat C, Likittanasombat K, Leelasiri A. Hemophagocytic syndrome in Dengue

hemorrhagic fever with severe multiorgan complications. Journal of the Medical Association of Thailand. 2008; 91(1): 104–109.
4. Rueda E, Méndez A, González G. Sнndrome hemofagocнtico asociado con dengue hemorr6gico [Hemophagocytic syndrome associated with dengue hemorrhagic fever]. Biomedica. 2002; 22(2):160–166. https://doi.org/10.7705/biomedica.v22i2.1155
5. Tan LH, Lum LC, Omar SF, Kan FK. Hemophagocytosis in dengue: comprehensive report of six cases. Journal of Clinical Virology. 2012; 55(1): 79–82. https://doi.org/10.1016/j.jcv.2012.06.005
6. van de Weg CA, Huits RM, Pannuti CS, et al. Hyperferritinaemia in dengue virus infected patients is associated with immune activation and coagulation disturbances. PLoS Neglected Tropical Diseases. 2014; 8(10): e3214. https://doi.org/10.1371/journal.pntd.0003214
7. Ab-Rahman HA, Rahim H, AbuBakar S, Wong PF. Macrophage Activation Syndrome-Associated Markers in Severe Dengue. International Journal of Medical Sciences. 2016; 13(3): 179–186. https://doi.org/10.7150/ijms.13680
8. Md-Sani SS, Md-Noor J, Han WH, et al. Prediction of mortality in severe dengue cases. BMC Infectious Diseases. 2018; 18(1): 232. https://doi.org/10.1186/s12879-018-3141-6
9. Mohd-Zaki AH, Brett J, Ismail E, L'Azou M. Epidemiology of dengue disease in Malaysia (2000-2012): a systematic literature review. PLoS Neglected Tropical Diseases. 2014; 8(11): e3159. https://doi.org/10.1371/journal.pntd.0003159
10. Mustafa M, Mohamad Nor AT, Mat Jelani A, et al, Clinical Practice Guidelines Management of Dengue Infection in Adults (3rd edition). Malaysia Health Technology Assessment Section (MaHTAS), Medical Development Division, Ministry of Health Malaysia. 2015.
11. Rosado FG, Kim AS. Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. American Journal of Clinical Pathology. 2013; 139(6): 713–727. https://doi.org/10.1309/AJCP4ZDKJ4ICOUAT
12. Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatric Blood & Cancer. 2007; 48(2): 124–131. https://doi.org/10.1002/pbc.21039
13. Nadeem M, Shafiq MM, Manzoor MS, Ahmed SI. Serum ferritin: an indicator of disease severity in patients with dengue infection. Journal of Rawalpindi Medical College. 2016; 20(3): 165–167.
14. Petchiappan V, Hussain TM, Thangavelu S. Can serum ferritin levels predict the severity of dengue early?: an observational study. International Journal of Research in Medical Sciences. 2019; 7(3): 876–881. https://doi.org/10.18203/2320-6012.ijrms20190940
15. Lacher DA, Hughes JP, Carroll MD. Biological variation of laboratory analytes based on the 1999–2002 National Health and Nutrition Examination Survey. National health statistics reports; no 21. Hyattsville, MD: National Center for Health Statistics. 2010.
16. Lee LK, Gan VC, Lee VJ, Tan AS, Leo YS, Lye DC. Clinical relevance and discriminatory value of elevated liver aminotransferase levels for dengue severity. Plos Neglected Tropical Diseases. 2012; 6(6): e1676. https://doi.org/10.1371/journal.pntd.0001676
17. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue in the early febrile phase: viremia and antibody responses. The Journal of Infectious Diseases. 1997; 176(2): 322–330. https://doi.org/10.1086/514048
18. Azeredo EL, Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in dengue: interrelationship between virus and the imbalance between coagulation and fibrinolysis and inflammatory mediators. Mediators of Inflammation. 2015; 2015: 313842. https://doi.org/10.1155/2015/313842
19. Lei HY, Huang KJ, Lin YS, Yeh TM, Liu HS, Liu CC. Immunopathogenesis of Dengue Haemorrhagic Fever. American Journal of Infectious Diseases. 2008; 4(1): 1–9. https://doi.org/10.3844/ajidsp.2008.1.9
20. Durbin AP, Vargas MJ, Wanionek K, et al. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. Virology. 2008; 376(2): 429–435. https://doi.org/10.1016/j.virol.2008.03.028
21. Wang WK, Chen HL, Yang CF, et al. Slower rates of clearance of viral load and virus-containing immune complexes in patients with dengue hemorrhagic fever. Clinical Infectious Diseases. 2006; 43(8): 1023–1030. https://doi.org/10.1086/507635
22. Samanta J, Sharma V. Dengue and its effects on liver. World Journal of Clinical Cases. 2015; 3(2): 125–131. https://doi.org/ 10.12998/wjcc.v3.i2.125
23. Parkash O, Almas A, Jafri SW, Hamid S, Akhtar J, Alishah H. Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia). BMC Gastroenterology. 2010; 10: 43. https://doi.org/10.1186/1471-230X-10-43
24. Lee MS, Hwang KP, Chen TC, Lu PL, Chen TP. Clinical characteristics of dengue and dengue hemorrhagic fever in a medical center of southern Taiwan during the 2002 epidemic. Journal of Microbiology, Immunology, and Infection. 2006; 39(2): 121–129. https://europepmc.org/article/med/16604244
25. Sudha K, Dushyanth B, Prabhu A, Souparnika.

Biochemical Profile Alterations in Dengue Patients. International Journal of Pharmaceutical Sciences Review and Research. 2017; 32: 170–172.
26. Diwakar NT, Madhu G. Role of serum ferritin and serum aminotransferases in predicting the severity in dengue fever with thrombocytopenia. Journal of Evolution of Medical and Dental Sciences. 2018; 7(50): 5378–5382. https://doi.org/10.14260/jemds/2018/1190
27. Pongpan S, Wisitwong A, Tawichasri C, Patumanond J, Namwongprom S. Development of dengue infection severity score. ISRN Pediatrics. 2013; 2013: 845876. https://doi.org/10.1155/2013/845876
28. Agrawal VK, Prusty BS, Reddy CS, Reddy GK, Agrawal RK, Bandaru VC. Clinical profile and predictors of Severe Dengue disease: A study from South India. Caspian Journal of Internal Medicine. 2018; 9(4): 334–340. https://doi.org/10.22088/cjim.9.4.334
29. Malavige GN, Gomes L, Alles L, et al. Serum IL-10 as a marker of severe dengue infection. BMC Infectious Diseases. 2013; 13: 341. https://doi.org/10.1186/1471-2334-13-341