Original Article

From Dengue Fever to Dengue Hemorrhagic Fever: Unveiling the Role of Micronutrients and Immune Modulators

Erum Amir[1], Amtul Quddos Latif[5], Shazia Fahmi[2], MS Fahmi[6], Shayan Zufishan[3] and Erum Khan[4]

ABSTRACT

Objective: The aim of this study was to analyze serum ferritin and Vitamin D levels as predictive indicators for disease severity in Dengue fever and dengue hemorrhagic fever.

Study Design: This was cross sectional study.

Place and Duration of Study: This study was conducted at the Clinical Pathology Department of Jinnah Post Graduate Medical Center (JPMC), Karachi, from November 2022 to February 2023.

Materials and Methods: This study included 200 individuals, recruited from the Medical OPD's and Dengue isolation ward with suspected Dengue with and without Warning signs. Serologically confirmed cases with dengue's clinical profile (fever, arthralgia/myalgia, retro-orbital pain, rashes, gastrointestinal and gingival bleeding and ascites), based on positive NS 1 antigen and Ig M antibody, study population was divided into two groups: Dengue fever and Dengue hemorrhagic fever. Samples of blood were collected from both categories, assessed for Serum Ferritin, Vitamin D and routine laboratory parameters. Data was analyzed by SPSS software Version 23.

Results: The study revealed that fever was the most prevalent clinical manifestation (100%) followed by arthralgia (58%), myalgia (65%), hepatosplenomegaly (59%), gastrointestinal bleeding (46%) in dengue with warning signs. Hematocrit (52.32 ±5.51), ferritin (1128.80±1190.73), cholesterol (189.51± 60.45) and low density lipoproteins (103.28±31.24) were found on higher edges whereas platelet count (47.51±23.19) and Vitamin D (7.92±2.50) levels declines much below the normal reference ranges.

Conclusion: Serum Ferritin and Vitamin D are promising severity markers in dengue and dengue hemorrhagic fever.

Key Words: Dengue fever, Dengue Hemorrhagic fever, Ferritin, Vitamin D, Hematocrit, Immune Modulators.

INTRODUCTION

Dengue is an emerging prevalent viral infection, caused by single-stranded RNA virus belonging to family Flaviviridae. Dengue virus (DENV) has four distinct serotypes with related antigenic determinants.

It can be transmitted primarily to host through bite of infected female mosquito Aedes aegypti and Aedes albopictus, highly adapted to urban settings. According to the World Health Organization (WHO), there are 50 million cases of dengue reported globally with primary burden stems predominantly from Asia, Africa and Middle East. The large dengue outbreaks in preceding years have had a profound effect on health care system in Pakistan[1-2]. Spectrum of clinical manifestations ranging from asymptomatic to severe forms of dengue as Dengue Hemorrhagic fever(DHF) and Dengue Shock Syndrome (DSS), leading to significant rise in morbidity and mortality[4-9]. Limited preventive and therapeutic options exists due to lack of specific antiviral treatment and vaccine. To address this, preliminary biomarkers are crucial for anticipating disease course and implementing timely interventions[6]. Prior studies revealed diverse hematological and biochemical markers, such as Ferritin linked to immune and endothelial activation, as well as Vitamin D, a potent immune modulator with implications on disease severity[1,5]. The aim of this study was to analyze serum ferritin and Vitamin D levels as predictive indicators for disease severity in Dengue fever and dengue hemorrhagic fever.

1. Department of Pathology / Anatomy[2] / Biochemistry[3] / Community Medicine & Health Sciences[4], Karachi Medical & Dental College, Karachi.

5. Department of Clinical Pathology, Jinnah Postgraduate Medical Centre, Karachi.

6. Department of Ophthalmology, Baqai Medical University, Karachi.

Correspondence: Dr. Erum Amir, Assistant Professor of Pathology, Karachi Medical & Dental College, Karachi.
Contact No: 03159833392
Email: erumamir09@gmail.com

MATERIALS AND METHODS

The Clinical Pathology Department at Jinnah Postgraduate Medical Center (JPMC), Karachi, conducted a cross sectional, hospital–based observational study during dengue epidemics from November 2022 to February 2023. The study encompassed a total of 200 dengue cases , categorized into two groups : Group one enrolled 100 individuals with suspected dengue from the OPD, while Group two comprised 100 consecutive DHF/DSS patients admitted to dengue isolation ward based on WHO 2009 clinical criteria .Eligibility criteria : both genders, aged between 18 to 50 years, febrile presentation, exclusion criteria : other febrile illness (typhoid, malaria), fever > two weeks, chronic illness, hematological malignancies and bleeding diathesis. Informed written consent collected from the guardian or patients prior to enrollment. Structured data collection sheet was used for systematic documentation of pertinent clinical and demographic characteristics.Serologically all samples were assessed for NS-1 antigen and Ig M antibody by utilizing Enzyme linked immunosorbent assay (ELISA) in matches sera by Dengue NS1 Rapid Test Cassette-Citest for Immunoglobulin M. Antigen NS1 was identified by Dengue NS1 Rapid Test Cassette-Citest. Serologically positive cases were categorized as: Dengue without Warning signs and Dengue with Warning sign. For hematological parameters including hematocrit, Total leucocyte count, and platelet count 5 ml of sample was collected in EDTA containing vaccutainer, thoroughly mixed ,analyzed on SYSMEX XN 1000 ™ auto analyzer . Lipid profile (cholesterol,LDL,HDL), hs CRP were evaluated by collecting 3 ml of blood in vaccutainers with lithium heparin . Plasma was analyzed after centrifugation on Beckmen Coulter AU 5800 . Serum Ferritin and Vitamin D levels were measured on LIAISON XS-DiaSorin autoanalyzer by Chemiluminescence technique. Data analysis was performed by Statistical package for social sciences (SPSS) Version 23.Categorical variables were summarized as frequencies and percentages while Continuous variables as mean and standard deviation. A two tailed probability value of <0.05 (95% CI) was accepted as level of statistical significance. Difference between two groups was analyzed by independent sample t-Test or Mann Whitney U TEST.

RESULTS

Present study recruited total of 200 serologically confirmed patients of dengue infection, 100 each from OPD and Dengue isolation ward of JPMC. Individuals were categorized into Dengue fever and Dengue Hemorrhagic fever/Dengue Shock syndrome groups . Fever was prevalent in both groups whereas Dengue with warning signs characteristically shown hemorrhage and leakage of plasma (Table 1). Younger population (aged 26 to 35 years) predominantly females, had dengue fever . While older age groups (45 to 55 years) with a higher proportion of males, commonly exhibited Dengue with warning signs (Table 2).

Table No. 1: Comparative analysis of Clinical profile between Dengue Fever and Dengue Hemorrhagic Fever

Clinical profile	Dengue fever	Dengue hemorrhagic fever/Dengue shock syndrome
Fever	100	100
Vomiting	7	13
Jaundice	03	10
Myalgias	65	90
Arthralgias	58	58
Drowsiness	44	44
Diarrhea	04	12
Gastrointestinal bleeding	6	22
Positive tourniquet test	0	35
Gingival bleeding	0	46
Hepatosplenomegaly	0	59
Pleural effusion	0	0.9
Ascites	0	11

Table No. 2: Baseline Characteristics of the Patients with Dengue and Dengue hemorrhagic fever

Age Distribution of the patients		
Patient's Age	Dengue Fever (n=100)	Dengue Hemorrhagic Fever (n=100)
18 to 25 yrs	19	04
26 to 35 yrs	41	10
36 to 45 yrs	28	25
46 to 55 yrs	6	55
>55 yrs	12	6
Gender distribution of the dengue patients		
	Dengue fever	Dengue hemorrhagic fever
Females	43	69
Males	57	21

Table 3 revealed a significant rise in Hematocrit in DHF/DSS (52.32 ± 5.51743 %) with considerably low platelet counts (47.510 ± 23.19) compared to dengue fever without warning signs. Table 4 indicated inverse relationship between Serum cholesterol (189.51 ± 60.45), LDL (103.28 ± 31.24) and HDL (56.115 ± 44.85) and dengue severity in DSS/DHF .

The average levels of serum ferritin showed substantial rise 1128.80 ± 1190.73 ng/ml in DHF/DSS, on contrary Ferritin levels in DF were(160.10 ± 353.18) ,within normal range of serum ferritin in adults. DHF/DSS

category demonstrated severely lower (7.09± 2.50 ng/ml) than normal ranges of Vitamin D (>20.0 ng /ml). (Table 5).

Table No. 3: Comparative Evaluation of Hematological parameters in patients of Dengue fever and Dengue Hemorrhagic fever

Hematological profile	DENGUE fever Mean (Standard deviation)	Dengue Hemorrhagic fever Mean (Standard deviation)	P value
Hematocrit (%)	45.8673 (3.45408)	52.32 (5.51743)	< 0.001 *
Total leucocyte count /cumm	3.0551 (1.008)	3.0870 (1.00641)	0.824
Platelet count /cumm	73.37 (28.88)	47.510 (23.19)	< 0.001*

Table No.4: Comparative analysis of Lipid profile in Dengue and Dengue hemorrhagic fever

Lipid profile parameters	Dengue fever Mean (Standard deviation)	Dengue Hemorrhagic fever Mean (Standard deviation)	P value
Serum HDL mg/dl	17.68 (19.18)	56.115 (44.85)	< 0.001*
Serum Cholesterol mg/dl	70.39 (43.68)	189.51 (60.45)	< 0.001 *
Serum LDl mg/dl	50.5316 (19.50)	103.28 (31.24)	< 0.001 *

Table No. 5: Comparative analysis of serum Ferritin, Vitamin D and hs CRP in Dengue and Dengue hemorrhagic fever

Ancillary Biochemical Parameter	Dengue fever Mean (Standard deviation)	Dengue Hemorrhagic fever , Mean (Standard deviation)	P value
hs CRP mg/L	11.45 (19.57)	9.45 (17.71)	0.452
Serum Vitamin D (ng/ml)	20.46 (17.02)	7.92± 2.50	< 0.001*
Serum Ferritin (ng/ml)	160.10 (353.18)	1128.80 (1190.73)	< 0.001 *

DISCUSSION

Dengue poses a serious threat worldwide, the global incidence of dengue has witnessed significant growth over the years. Henceforth it becomes a pervasive global concern, spreading across approximately more than 100 countries. Situated in tropical and sub tropical region. In contrast to the self limiting DF as febrile illness, DHF has potential to escalate into fatal disease. DHF is characterized by plasma leakage, bleeding tendency and liver impairment .Prompt and precise diagnosis and management of this phase is vital for enhancing patient's prognosis[5].

Mostly individuals with DF were between 26 to 35 years with female prevalence. Our results were in line with Murmu AR et al where majority of DF patient belongs to same age group[10]. Young adults engage in education or employment could be the logical reason behind this higher frequency of dengue infection. Contrary to that majority of the patients of Dengue with warning signs are elderly individuals with greatest number of males. As the age progresses the immune system becomes weak, further augments the risk of complications. In our study we observed that DF has mild to moderate disease presentation in majority of population. While patients of DHF/DSS has more aggressive clinical profile, with bleeding diathesis and plasma leakage[7].

The abrupt decline of mean platelet count was observed in this study, presumed as the potential cause of gingival and gastrointestinal bleeding in DHF/DSS. Thrombocytopenia is consistent and diagnostic feature of DHF/DSS, and raises the red flag for occurrence of plasma leakage on the other hand it's occasionally seen in DF. Similar correlation was reported in study showed inverse relationship between platelet count and bleeding tendencies[7]. More than 50 % of the patients in present research showed increased Hematocrit, >20 % of normal value for adults (45%), outlined WHO criteria for distinguishing DHF/DSS from DF, utilized during dengue outbreak[11-13].

Significant rise in Cholesterol, high and low density lipoproteins mainly observed in patients with DHF/DSS. Changes in lipid profile facilitates the entry of virus by replication complex and modifying lipid micro environment.[11,8,] lipid availability and composition levels both in cell and tissues is crucial for successful replication of DENV[4].

Vitamin D is key predictor of disease progression in dengue, by promoting Macrophage activation leading to hold on viral replication. DHF/DSS associated with cytokine storm release from T lymphocytes and Macrophages in response to high viral load. The integrated activity of innate and cell mediated immune system generate immediate protective response.[14-16] Studies have highlighted the role of Vitamin D in modulating both arms of immune system consecutively through Vitamin D receptors on surface of immune cells. Vitamin D deficiency would lead to upregulation of Interleukin 6 expression with monocyte activation facilitated by TNF α[17]. This inflammatory mediators induced dysregulated immune response might lead to detrimental "cytokine storm" eventually cause Multiple organ failure and derange coagulation cascade[19]. Present study disclosed Vitamin D deficiency in dengue with Warning signs (DHF/DSS) portrayed Vitamin D as key player in immune modulation.

Infection triggers an acute phase response as systemic manifestation of inflammation. It is characterize specifically by production of acute phase proteins (APP's). The systemic response engrosses the rise in positive APP's and fall in negative APP's. Ferritin classified as positive APP. The cytokine induced Alterations in APP's predominantly attributed to their synthesis in liver parenchyma. Transcriptional up gradation of H chain of DENV is notably induced by TNF α and IL 6 α[18]. Serum ferritin was extensively elevated in DHF throughout the course of disease as compared to DF patients. In Current study significant boost in serum levels were observed in DHF, concordant with other local and international studies [5].

CONCLUSION

It's worth noting in present study that Ferritin is key player in inflammatory response induced by TNF and IL6, proved it to be a promising predictive marker of disease severity in dengue infection. Further insights need to explore the functions of Ferritin as APP. Immune system respond inappropriately in Vitamin D deficiency , increase the risk of disease progression which could be fatal . In future more follow up studies should be conducted to establish the role of Vitamin D supplements as good prognostic marker.

Limitations & Strengths of the Study: Small Sample size. Lack of serial sampling is basic restraints in this study. Larger spectrum of patients from different hospital set ups could strengthen our results.

Author's Contribution:

Concept & Design of Study:	Erum Amir
Drafting:	Amtul Quddos Latif, Shazia Fahmi
Data Analysis:	MS Fahmi, Shayan Zufishan, Erum Khan
Revisiting Critically:	Erum Amir, Amtul Quddos Latif
Final Approval of version:	Erum Amir

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Iqtadar S, Khan A, Mumtaz SU, Livingstone S, Chaudhry MNA, Raza N, et al. Vitamin D Deficiency (VDD) and Susceptibility towards Severe Dengue Fever—A Prospective Cross-Sectional Study of Hospitalized Dengue Fever Patients from Lahore, Pakistan. Trop Med Infect Dis 2023;8:43.
2. Asghar, R.M., Ashraf, R.R., Saheel, K., Hussain, A. An Evaluation of Haematological Changes in Paediatric Dengue Fever Patients at a Tertiary Care Hospital Rawalpindi during 2019 Outbreak. J Rawalpindi Med College 2021;25(2):208-212.
3. Tabassum S, Naeem A, Nazir A, Naeem F, Gill S, Tabassum S. Year-round dengue fever in Pakistan, highlighting the surge amidst ongoing flood havoc and the COVID-19 pandemic: a comprehensive review. Ann Med Surg (Lond) 2023;85(4):908-912.
4. Panwar A, Kumar R, Goel R, Aggarwal S, Saraswat S, Bansal P, et al. Severe dengue in children associates with dysregulation of lipid homeostasis, complement cascade and retinol transport. Clin Transl Med 2023;13(6):e1271.
5. van de Weg CAM, Huits RMHG, Pannuti CS, Brouns RM, van den Berg RWA, et al. Hyperferritinaemia in Dengue Virus Infected Patients Is Associated with Immune Activation and Coagulation Disturbances. PLoS Negl Trop Dis 2014;8(10):e3214.
6. Rojekar MV, Kumavat V, Panot J, Rojekar AA, Lalla P. Diagnosis Embattled - Correlation of Serum Ferritin Lipid Profile with Severity of Dengue in Paediatric Population. Int J Med Reviews Case Reports 2021;5(2):12-16.
7. Kularatnam GA, Jasinge E, Gunasena S, Samaranayake D, Senanayake MP, Wickramasinghe VP. Evaluation of biochemical and haematological changes in dengue fever and dengue hemorrhagic fever in Sri Lankan children: a prospective follow up study. BMC Pediatr 2019;19:1-9.
8. Lima WG, Souza NA, Fernandes SOA, Cardoso VN, Godói IP. Serum lipid profile as a predictor of dengue severity: A systematic review and meta-analysis. Rev Med Virol 2019;29(5):e2056.
9. Jayarajah U, Madarasinghe M, Hapugoda D, Dissanayake U, Perera L, Kannangara V, et al. Clinical and biochemical characteristics of dengue infections in children from Sri Lanka. Global Pediatr Health 2020;7:2333794X20974207.
10. Murmu AR, Mubarak R. Correlation between serum ferritin levels and severity of dengue feverin tertiary care center: an observational study Int J Res Med Sci 2021;9(6):1735-1740.
11. Koh C, Islam MN, Ye YH, Chotiwan N, Graham B, Belisle JT, et al. Dengue virus dominates lipid metabolism modulations in Wolbachia-coinfected Aedes aegypti. Commun Biol 2020;3(1):518.
12. Patel MI, Patel A, Patel A, Patel S, Padsala S. Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases. Int J Research Med Sciences 2018;6(6):2099-105.
13. Tyagia BK, Selvia SK, Chellaswamya V, Arorab NK, Shepardc DS, Halasac YA, et al. Association of dengue symptoms with haematological parameters: a retrospective study of 10 hospitals in India. Dengue 2016;39:62.

14. George T, Jakribettu RP, Yesudas S, Thaliath A, Pais ML, Abraham S, Baliga MS. Clinico-haematological parameters in dengue in adults: a retrospective study from a tertiary care hospital. IJAR 2018;4(6):88-91.

15. Moras E, Achappa B, Murlimanju BV, et al. Early diagnostic markers in predicting the severity of dengue disease. 3 Biotech 2022;12:268.

16. Mehboob R, Munir M, Azeem A, Naeem S, Ahmad FJ. Low Platelet Count Associated with Dengue Hemorrhagic Fever. Int J Advances Chem (IJAC) 2015;1(1):31.

17. Mirza WA, Zhang K, Zhang R, Duan G, Khan MSN, Ni P. Vitamin D deficiency in dengue fever patients' coinfected with H. pylori in Pakistan. A case-control study. Front Public Health 2022; 10:1035560.

18. Suresh SC, Hanumanthaiah R, Ramakrishna C, Sandeep R, Narasimhasetty PS, Ramakrishna V, et al. Serum Ferritin As a Prognostic Indicator in Adult Dengue Patients. Am J Trop Med Hyg 2020; 104(3):1072-1078.

19. Finkelstein JL, et al. Micronutrients, Immunological Parameters, and Dengue Virus Infection in Coastal Ecuador: A Nested Case-Control Study in an Infectious Disease Surveillance Program. J Infectious Diseases 2020;221(1):91–101.