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Original Research Article

Analysis of essential hematological parameters and nutrient levels of dengue patients aiming for quick recovery and sustainable immunity

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ABSTRACT

Purpose: Dengue is one of the common public health issues globally where laboratory diagnostic test values of patients are primarily focused on hematological parameters. Micronutrients equally take a role in quick recovery, and active cum passive immunity is not mainly considered during and post-recovery of patients.
Materials and Methods: A cross-sectional study with a purposive sampling method was applied using pre-tested close-ended questionnaires and laboratory data (soft copies/hard copies) of participants admitted to hospitals or nursing homes within the North -24 Paraganas district from November 2021 to October 2022. 110 male and female patients of 40-60 years reported dengue fever (non-hospitalized cases), and dengue hemorrhagic fever (hospitalized cases) was considered based on 15 variables. STATA version 18.0 software analyzed data using multivariate regression and range plots.
Results: The Cronbach's alpha value was 0.72, and the consolidated probability value shows 0.01 ($p<0.05$), which denotes that the multivariate model was statistically significant and was rightly decided to include the relevant independent variables. Calcium, vitamin D, and total protein were statistically significant as P values were 0.01, the same for each. Similarly, p=0.05 was the same for Total Leukocyte count (TLC) and Alkaline phosphatase (ALP).
Conclusion: The study concludes that micronutrients like calcium, protein, vitamin D, etc., are often considered secondary recommendations in dengue treatment. A balanced, nutrient-rich diet for dengue patients is needed to maintain sustained immunity by extensively monitoring calcium, vitamin D, and total protein intake during and after recovery.

1. Introduction

Dengue viruses, which consist of four distinct yet related flaviviruses, are responsible for causing a significant emerging viral disease known as dengue fever (DF) and dengue hemorrhagic fever (DHF). DF is a sudden fever with nonspecific symptoms like headache, pain, nausea, vomiting, joint pains, weakness, and rash. It may cause anorexia, altered taste sensation, and mild sore throat.[1] DHF is defined by haemorrhaging and fluid leakage, frequently seen in cases of secondary Dengue virus (DENV) infection. Understanding the mechanisms that impact endothelial cell function is essential for developing preventive treatment approaches due to the involvement of serotype-cross reactive antibodies and mediators in the pathogenesis.[2] Dengue patients experience daily blood clotting fluctuations, mainly between days 3 and 8, showing a gradual decrease in white blood cells and platelets and increased blood concentration due to plasma leakage for DHF.[3] A systematic review and meta-analysis investigated the association between obesity and dengue severity, highlighting the varied results of studies

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suggesting obese patients with dengue infection have more severe manifestations.[4] Platelet (thrombocyte)activation, characterized by thrombocytopenia, is linked to various infections and sepsis, including dengue, and is a hallmark of many viral infections. The average platelet count should be 1.5 to 4.5 lakhs per microliter of blood for both sexes. Platelets play a crucial role in dengue disease, immunoregulation, activating upon infection and releasing cytokines from alpha granules, which regulate dengue virus infection.[5] Secondary dengue infection is linked to elevated lymphocytes (average 20-40% of WBCs), which shows1.0 to 4.8 thousand lymphocytes per microliter (μL) in the blood with atypical features. Such features indicate an augmented immune response due to secondary viral antigen exposure, leading to a more severe form of the disease.[6] Apart from thrombocytopenia, DF is characterized by peripheral blood parameters such as leucopenia (WBC < 5000 cells/mm3) and rising hematocrit (10-20%), which change the status of the illness over time. The average total leucocyte count (TLC) is 4 thousand to 11 thousand, and the hematocrit level should be 41% to 50% for males, while females should have 36% to 46%.[6,7] A study found that testing HbA1C is essential because diabetes may increase inflammatory markers and lower platelet counts in diabetic patients, potentially leading to severe dengue infection. However, the pathophysiology is still unknown in diabetes and other typical lifestyle disorders called comorbidities.[8] In a retrospective study conducted in a Thailand hospital, the dengue group showed gradually increasing hemoglobin and hematocrit levels from day 3 to 10, lower TLC (WBC), and platelet count. Studies found that patients with dengue had higher monocytes, atypical lymphocytes, and eosinophils than the control group. The neutrophil-to-lymphocyte ratio in the dengue group was higher initially but reversed later.[3,9] There is a significant relationship between the parameters of liver functions and dengue among Indian patients. A study conducted in India found that 74% of dengue patients had elevated transaminases, Serum glutamic oxaloacetic transaminase (SGOT), and Serum glutamic pyruvic transaminase (SGPT). SGOT, also called Aspartate aminotransferase (AST) and SGPT, is referred to as Alanine Transaminase (ALT). AST was more than double the mean value of ALT, contrasting with other viral hepatitis characterized by higher ALT values. The study also found a significantly elevated mean alkaline phosphatase (ALP) value in severe dengue.[10] Dengue shock syndrome (DSS) is nothing too different from DHF· A study showed that DHF/DSS patients had lower serum calcium levels than those with DF.[11] A study suggests that maintaining adequate vitamin D levels through diet or supplementation may provide adequate immune protection against severe dengue illness.[12,13] A study determined that 40% of subjects had a B12 level below 200 pg/L, potentially leading to severe thrombocytopenia, slower platelet count recovery, and prolonged hospitalization in dengue fever patients.[14] A study in India analyzed the serum proteome of dengue patients, revealing significant changes in several proteins with biological functions. The findings are the first to show human serum proteome alterations in dengue.[15] This study aimed to analyze essential hematological parameters and nutrient levels (as valid data) of DF and DHF patients seeking quick recovery and sustainable immunity. So, proper nutrition is essential to maintain such patients' immunity even after recovery.

2. Materials and Methods

2.1. Study area

The study was conducted in West Bengal, the entire north-24 Paraganas district, and considered laboratory test reports and questionnaire responses of patients with dengue fever and dengue hemorrhagic fever.

2.2. Study duration

The study from November 2022 to January 2024 took 15 months; 13 months were spent on data collection and 2 months on data analysis and report preparation.

2.3. Study design

A cross-sectional study with a purposive sampling method was applied using a study tool for data collection. The tool was pre-tested close-ended questionnaires apart from laboratory data (soft copies/hard copies) of participants admitted to three multi-specialty hospitals or five nursing homes within north-24 Paraganas districts in West Bengal.[16,17] The 'Informed Consent' form was filled out and submitted with a physical signature from each participant before the data collection. The study was conducted without involving, drawing, or testing direct blood samples and not any experimented/randomized control trial, so no ethical number was applicable.[18] 'Diet Fitness' (certificate no.- last four digits: XX8282), a private healthcare institute with a valid municipal license under the 'human nutrition category' run by the nutritionist (the lead author) and other researchers conducted altogether. The initiative to carry out this study was primarily based on secondary data for the well-being of dengue patients, healthcare providers, and other stakeholders.

2.4. Data collection

A total of 110 male and female patients of 40-60 years reported dengue fever (non-hospitalized cases) and dengue hemorrhagic fever (hospitalized cases) were considered from November 2021 till October 2022 typically based on the laboratory test reports of Hba1c, lymphocyte and total leukocyte counts, hematocrit, hemoglobin, liver functions parameters such as Alkaline phosphatase (ALP), Aspartate

aminotransferase (AST) and Alanine transaminase (ALT). Most importantly, the study exclusively included nutritional parameters such as calcium, total protein, vitamin D, and vitamin B12, often correlated with hematological and biochemical parameters. All data was collected from the patients after recovery individually at the diet clinic once they visited for diet consultation; hence, data was mixed and unavailable in private or public portals. The study was based on purposive sampling, so primary data was collected as per choice and convenience using a pre-tested close-ended questionnaire.

2.5. Data analysis

In this study, we used statistical software, STATA version 18.0, for the data analysis. The study analyzed the linear relationship using Multivariate Regression among all the 14 predictors (covariances) concerning platelet count, which was the biomarker of both types of dengue. Graphical and regression analyses were conducted to assess the relationship among the variables in response to platelet counts. The reliability test Cronbach's alpha was done due to the presence of nutrition-related primary data collected from the patients.[19]

3. Results

Post-data analysis, the study found 62 DF and 11 DHF females and 28 DF and 9 DHF males with statistically significant and non-significant mixed test results based on analysis of all 15 variables. Figure 1 shows the graphical analysis of the numbers of male vs. female participants per the dengue statuses on the 7^{th} day from the detection and diagnosis of DF or DHF. The Cronbach's alpha value was used to test the internal consistency of coded responses against ten questions on a 5-point Likert scale at the opinion pattern assigned to each participant after their recovery. Clinic visits, WhatsApp apps, telephone calls, and emails were the data-sharing tools that they used for their interests and convenience. The study found that Cronbach's alpha was 0.72.

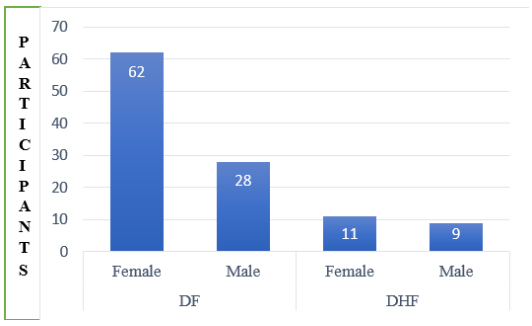

Figure 1: Graphical analysis of participants with DF and DHF

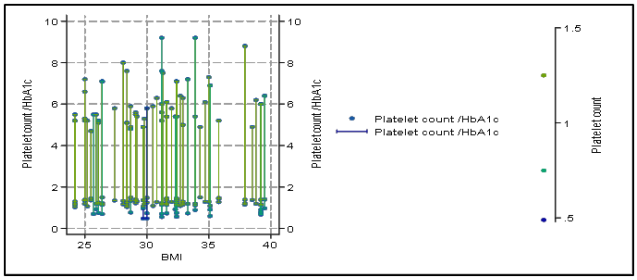

Figure 2: Range plot considering BMI vs. Platelets vs. Y2 variable Hba1c

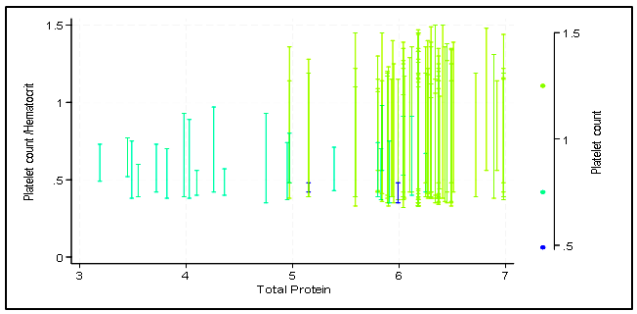

Figure 3: Range plot considering total Protein vs. Platelets vs. Y2 variable Hematocrit

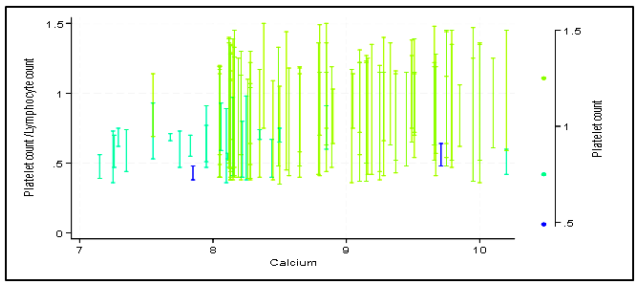

Figure 4: Range plot considering calcium vs. Platelets vs Y2 variable Lymphocyte

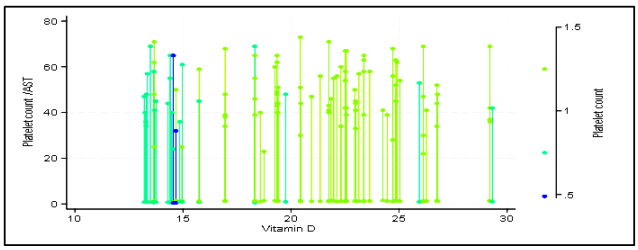

Figure 5: Range plot considering vitamin D vs. Platelets vs Y2 variable AST

Table 1: Descriptive statistics of the study variables: Mean, Standard Deviation, and Values

Variable	Mean	SD.	Min.	Max.
Age	50.06	6.20	40	60
BMI	31.29	4.28	24.2	39.5
HbA1c	6.12	1.08	4.7	9.2
Platelet count/μL of blood	1.14	0.25	0.48	1.5
Lymphocyte count/μL of blood	0.50	0.11	0.35	0.75
Total Leukocyte count/μL of blood	3.96	0.71	3.1	6.2
Haematocrit (%)	0.40	0.05	0.32	0.56
Haemoglobin (g/dL)	11.18	2.16	7.8	17.3
ALP (IU/L)*	160.67	30.70	109	225
ALT (IU/L)	37.74	7.58	23	55
AST (IU/L)	48.56	12.24	22	73
Calcium (mg/dL)	8.71	0.76	7.15	10.2
Vitamin D (IU)	20.38	4.55	13.21	29.32
Total Protein (g/dL)	5.89	0.83	3.19	6.98
Vitamin B12 (pg/mL)**	306.45	75.78	167.74	453.55

Note: *IU/L -International units per liter; dL- desi Liter; **pg/mL – picograms/mL

Table 2: Multivariate regression model: Platelet vs. 14 covariances

Platelet count	Coefficient	Standard. error	t	P>t	[95% conf.	Interval]
Age	0.00	0.00	-0.81	0.42	-0.01	0.00
BMI	0.00	0.00	-0.60	0.55	-0.01	0.01
HbA1c	-0.01	0.02	-0.48	0.63	-0.04	0.02
Lymphocyte count	-0.28	0.15	-1.95	0.05	-0.57	0.00
Total Leukocyte count	0.03	0.02	1.38	0.17	-0.01	0.08
Haematocrit	-0.25	0.32	-0.79	0.43	-0.88	0.38
Haemoglobin	-0.01	0.01	-0.82	0.42	-0.02	0.01
ALP	0.00	0.00	-4.90	0.00	0.00	0.00
ALT	0.00	0.00	-0.97	0.33	-0.01	0.00
AST	0.00	0.00	-1.97	0.05	-0.01	0.00
Calcium	0.06	0.02	2.51	0.01	0.01	0.11
Vitamin D	0.01	0.00	2.72	0.01	0.00	0.02
Total Protein	0.07	0.02	2.90	0.01	0.02	0.12
Vitamin B12	0.00	0.00	0.18	0.86	0.00	0.00
Consolidated	1.08	0.39	2.78	0.01	0.31	1.84

Table 1 shows the descriptive statistics of all study variables, considering their mean, standard deviation (SD), minimum, and maximum values. The average age of patients was 50 years, body mass index (BMI) was 31, and platelet count was 1.14 per microliter (μL) of blood for both patients. Mean BMI was class -1 obesity as per the WHO-Asian BMI classification. Further, HbA1c, lymphocytes, ALP, and AST lab values were observed more than the biological reference interval (BRI). Conversely, total protein and vitamin D were less, and TLC, hemoglobin, and calcium were on the borderline of BRI.

As per the hypothesis, multivariate regression was performed to test the linear relationship between the dependent variable, i.e., platelet, and 14 independent variables (Table 2). The study found that lymphocyte count, ALP, AST, calcium, vitamin D, and total protein (all 6) were statistically significant, and the rest were non-significant. Table 2 shows the multivariate regression model within platelet and other covariances. The consolidated probability value shows 0.01 ($p<0.05$), which denotes that the model was statistically significant and was rightly decided to include the relevant independent variables.

Table 3 shows Pearson's correlation with the illustrated matrix. The matrix found mixed responses with platelets and other variables. There were some negative correlations and partially positive correlations. ALP was negatively correlated with platelets, whereas calcium, vitamin D, and total protein were positively correlated with nearby value 1. Figure 2 shows the two-way graphical analysis using range plot 'range spike w/cap' based on platelet on Y1 and Hba1c Y2 axis adding on the right and BMI on X axis. The cap size was small, and the color variable was selected for this range plot. Figure 3 was illustrated for analyzing the total protein concerning platelets (X-axis),

Table 3: Correlation matrix of all variables in the study

Variable	BMI	HbA1c	Platelets	Lc	TLC	Hc	Hb	ALP	ALT	AST	Ca	Vit-D	TP	Vit-B12
BMI	1													
HbA1c	0.25	1												
Platelets	-0.07	-0.14	1											
Lc	-0.04	-0.13	-0.00	1										
TLC	0.04	-0.02	0.05	0.14	1									
Hc	0.09	-0.04	-0.15	-0.07	-0.10	1								
Hb	0.06	-0.02	0.06	-0.06	0.14	-0.13	1							
ALP	0.12	0.09	-0.63	-0.03	0.12	0.15	0.01	1						
ALT	-0.10	-0.11	-0.04	-0.05	-0.09	0.02	0.15	0.01	1					
AST	0.01	-0.01	-0.06	0.02	0.00	0.02	-0.17	-0.12	-0.30	1				
Ca	-0.04	-0.25	0.42	0.20	0.14	-0.10	0.15	-0.24	0.04	0.07	1			
Vit-D	0.10	-0.07	0.51	0.04	0.00	0.01	0.26	-0.39	0.00	0.00	0.30	1		
TP	0.03	-0.11	0.61	0.12	0.00	-0.09	0.04	-0.51	-0.06	-0.07	0.38	0.45	1	
Vit-B12	-0.05	0.04	0.17	0.21	0.17	-0.16	0.16	-0.13	-0.07	0.18	0.28	0.16	0.19	1

and Y2 added variable as hematocrit. Figure 4 shows the graphical status of calcium examining platelets and added variable lymphocytes. Finally, Figure 5 shows the graphic status of vitamin D (X) concerning platelets and the Y2 variable as AST. All these graphs show the relationship and statuses of the dependent variable (main parameter) platelet and the independent variable or covariance (predictor), mainly the daily essential nutrients, which were the common influencing factors for DF/DHF patients' immunity.

4. Discussion

Various studies show that DF and DHF are more prevalent globally among people aged 40-60. However, associated comorbidities affect those below 40 and above 60 years equally. As per the CDC, dengue cases and deaths have risen disproportionately among adults over 50 since 1990, with Asia accounting for most cases and the fastest-aging region. Children under 15 have seen declines.[20] However, the current study's main objective was to determine the linear relationship between platelets and other blood parameters based on available secondary data. Similarly, primary data was collected through 10 selective questions to get responses about participants' consideration of nutrients in daily diet during and post-disease. The study analyzed the common nutrients needed for the patients' daily diet, even during treatment and post-recovery. Hence, BMI, a parameter of comorbidity and nutrition parameters like calcium, vitamin D, and total protein, was considered for the multivariate and range plot analyses. The probability (P) value represents the probability of the null hypothesis being true, with a P value greater than 0.05 indicating the test hypothesis's rejection or no effect observed. In this study, six variables were statistically significant in the multiple linear regression model. The study found that despite primary data collected from the questionnaire, data reduction was not mandatory by principal component analysis (PCA) or exploratory factor analysis (EFA) because values were mainly less than 0.3. The previous study by the National Center for Vector Borne Diseases Control presented reports of the highest dengue cases in Telangana, Karnataka, and Maharashtra, with Kerala reporting 20 deaths in 2022. West Bengal and Delhi were worst hit, with over 500 cases and 800 new infections due to nonstop rain in 2020.[21] This study is mainly concerned with the common micronutrients often in crisis. Nutrients are required in proportionate amounts per recommended dietary (RDA) values. Proper nutrition with all the right nutrients can provide innate and specific immunity through active and passive ways. Consuming plenty of fluids, such as oral rehydration solutions, soups, citrus fruit juices, coconut water, and drinking water, is needed to manage hydration and prevent dehydration during high fever and vomiting. Lemon water is a cost-effective source of vitamin C, while pomegranates,

apples, oranges, and mixed juices are also beneficial. Homemade soups with spinach, beetroot, and tomato are suitable for hydration. Beetroot contains high amounts of vitamins A, B9, C, manganese, and iron, which boost erythrocytes and prevent anemia.[21,22] Sweet lime juice benefits dengue patients due to its bioactive compounds, including ascorbic acid, B vitamins, amino acids, and polyphenols.[23] In the present study, vitamin D was statistically significant. Vitamin D regulates calcium and phosphorous metabolism, is crucial for bone development, and acts as an immunomodulator, targeting immune cells, thereby increasing the risk of immune system issues and infection amelioration.[24,25] Calcium's role in platelet aggregation is underexplored in dengue therapeutic studies, necessitating further research to understand its potential in modulating cardiac dysfunction, immunopathogenesis, and dengue-related platelet abnormalities.[26]The choice of host protein is crucial in dengue treatment, with specialized pathways like necroptosis or cytokine production preferred over essential processes like protein synthesis or RNA processing. Understanding serotype-specific differences in NS5-host protein interactions can help fine-tune drug usage and potentially open new avenues for effective viral therapy and clinical management.[27]Overall, micronutrient consideration in the daily diet provides quick recovery and sustainable immunity during and after viral infections, including severe dengue cases.

5. Conclusions

Diagnostic parameters are carefully considered with primary importance for dengue fever and hemorrhagic patients, but micronutrients as essential parameters often come secondary in the dengue treatment process. Like other hematological parameters, micronutrients such as total protein, calcium, and vitamin D are crucial. Similarly, active and passive immunity should be sustainable even after the recovery of DHF/DSS, keeping well-balanced micronutrients. So, the current study concludes that a calculated nutrient-rich diet is highly recommended for patients to avoid recurrent dengue or other viral infections besides medication or physician's advice. Moreover, calcium, vitamin D, and total protein should be monitored during and after the recovery of all dengue patients and incorporated into the diet, preferably through natural sources or supplementation.

6. Source of Funding

None.

7. Conflict of Interest

None.

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