



CORRELATION OF SERUM CALCIUM WITH SEVERITY OF DENGUE AMONG DENGUE PATIENTS ADMITTED IN A TERTIARY CARE CENTRE OF EASTERN INDIA

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ABSTRACT

The virus that spreads by vectors most quickly in the globe is dengue fever. If dengue infection is not treated quickly and effectively, the morbidity and fatality rate is very high. Nearly 80% of dengue infections had low calcium levels, which are particularly common in severe cases. Nevertheless, there is a dearth of research on calcium homeostasis in dengue infection, While existing recommendations overlook the importance of blood calcium in dengue management, our research demonstrates that serum calcium levels are directly tied to how severely the virus impacts the patient.

Materials and methods: This is a cross-sectional study conducted in the Department of Medicine, IMS and SUM Hospital, Bhubaneswar, Odisha. The study is included 150 patients diagnosed with Dengue disease by NS1 or Dengue IgM. All the patients were categorised into 4 groups named DF, DHF 1, DHF 2, DHF 3 and DHF 4. Again, patients were classified in to 3 groups such as mild, moderate and severe hypocalcaemia based on serum calcium level.

Observation: The study population (mean age of 39.1 years with 12.44 SD) exhibited a 2:1 male-to-female distribution. Clinical observations revealed a progressive decline in serum calcium as disease severity increased: patients with Dengue Fever (DF) averaged 8.93 mg/dL, while those with DHF grades 1–2 and DHF grade 3 showed lower levels of 8.35 mg/dL and 7.98 mg/dL, respectively. With an average platelet count of 1.4 lakh/mm³ and a hematocrit of 44.27%, the data confirmed a strong negative correlation ($r = -0.892$) between calcium concentration and the severity of the infection.

Conclusion: The severity of dengue infection and serum calcium level were found to be significant negative correlation in the current study. Thus, blood calcium level may be used as Identifying this specific biomarker could streamline the diagnosis and treatment of high-risk dengue patients, though additional studies are necessary to verify its effectiveness.

KEYWORDS: Serum Calcium, Hypocalcemia, Dengue, Severity of Dengue, Correlation.

Introduction

Dengue fever has evolved into a critical public health crisis across tropical regions, particularly within Southeast Asia and the Americas[1]. While the *Aedes* mosquito-borne illness was identified as early as 1780, global cases have surged dramatically since the 1960s[2]. The clinical spectrum of the virus ranges from mild, undifferentiated fever to life-threatening conditions like Dengue Shock Syndrome (DSS) [3]. Currently, clinicians rely on a combination of physical symptoms and serological testing for diagnosis. Recent research, however, has explored the prognostic value of serum calcium levels. Specifically, hypocalcemia has been linked to poor outcomes, potentially due to its role in the complement system, endothelial dysfunction, and the plasma leakage characteristic of severe infections[4].

Methodology

This is a cross-sectional observational study conducted at IMS and SUM hospital, a tertiary health care centre in eastern India at Bhubaneswar, Odisha.

Inclusion Criteria

All cases of dengue diagnosed by NS1 or Dengue IgM.

Exclusion Criteria

- Autoimmune diseases
- Malignancy
- Using of medical treatment affecting WBC count such as hematopoietic systemic disorders
- Pregnancy
- Age<16yrs
- Dengue in the presence of other infectious diseases.

Sample Size

Based on the statistical analysis, mysamplesizewas150.

Statistical Analysis

Normally distributed continuous variables were expressed as mean with standard deviation (SD). Categorical variables were expressed in numbers and percentage. Data were analysed using IBM SPSS statistics software (version 26.0, Chicago, IL, USA).

Study Tools and Technique

The study protocol was approved by the ethical committee of IMS &SUM hospital before the beginning of the study. The written informed consent was obtained from all the patients who participated in the study. All the patients diagnosed with dengue fever from the Outpatient as well as Inpatient departments were included in our study. All patients were examined for their vitals and symptoms, and subjected to Platelet count, Hematocrit, Calcium, Liver function tests, renal function tests and a USG abdomen at admission. They were stratified to Dengue fever, Dengue hemorrhagic fever 1, Dengue hemorrhagic fever 2, Dengue hemorrhagic fever 3 and Dengue hemorrhagic fever 4. The data were analysed to correlate hypocalcemia with severity of Dengue fever.

Grading of Hypocalcemia

mild-8.0–8.39mg/dL

moderate-7.5–7.99mg/dL

severe-under7.5 mg/dL

Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee of IMS and SUM Hospital, Bhubaneswar, Odisha prior to commencement of the study. Written informed consent was obtained from all participants. Confidentiality and anonymity of patient data were strictly maintained throughout the study in accordance with ethical guidelines and Declaration of Helsinki (revised in 2024).

Operational Definition of Severity of Dengue Fever

DF/DHF	Grade	Symptoms	Labs
DF		Fever with two or more of the ff: headache, retro-orbital pain, myalgia, arthralgia	Leukopenia Thrombocytopenia $\leq 100,000$ No evidence of plasma loss
DHF	I	Above signs plus positive tourniquet test	Thrombocytopenia $<100,000$ Hct rise $>20\%$
DHF	II	Above signs plus spontaneous bleeding	Thrombocytopenia $<100,000$ Hct rise $>20\%$
DHF	III	Above signs plus circulatory failure (weak pulse, hypotension, restlessness)	Thrombocytopenia $<100,000$ Hct rise $>20\%$
DHF	IV	Profound shock with undetectable blood pressure and pulse	Thrombocytopenia $<100,000$ Hct rise $>20\%$

Results

A total of 150 patients were analysed in our study. The mean age was found as 39.1 ± 12.44 years with a range between minimum 18 years to maximum 80 years. Among the 150 patients, 46 were females and 104 were males. A total of 121 patients were diagnosed with NS1 antigen positive, and other 29 with Ig M antibody to Dengue. The descriptive analysis for demographic profile and clinical parameters were depicted below in Table 1.

The mean BMI of the population was 21.97. The heart rate ranged from 59 to 148 beats per minute. The mean systolic BP was 117.43 and the mean diastolic BP was 72.29. The

mean calcium at admission was 8.717. The mean platelet count at admission was 1,40,300/cubic mm and mean hematocrit was 44.27. Out of 150 patients, around 89 patients had normal calcium levels, 30 patients had mild hypocalcemia, 21 patients had moderate hypocalcemia and 10 of them had severe hypocalcemia (Figure 1).

To further simplify, 35 females (76.10%) had normal calcium levels, 6 (13%) had mild hypocalcemia, 5 (10.90%) had moderate hypocalcemia and no female had severe hypocalcemia. 54 males (51.9%) had normal calcium levels, 24 (23.1%) of them had mild hypocalcemia, 16 (15.40%) of them had moderate hypocalcemia and 10 (9.60%) of them had severe hypocalcemia.

Table 1: Demographic Profile and Clinical parameters of all patients

	Minimum	Maximum	Mean	Std. Deviation
Age	18	80	39.1	12.444
BMI	18.9	29	21.971	1.8103
HR	59	148	89.26	24.603
SBP	78	142	117.43	15.653
DBP	38	90	72.29	10.99
Calcium	7.2	10.7	8.717	0.8708
Hct	33	56	44.27	6.033
Hb	9.2	15.1	12.482	1.2579
TLC	2.9	14.2	7.523	3.2137
Platelet	21	430	140.3	79.641
Urea	7	42	16.03	7.165
Creatinine	0.4	2.8	1.348	0.4734

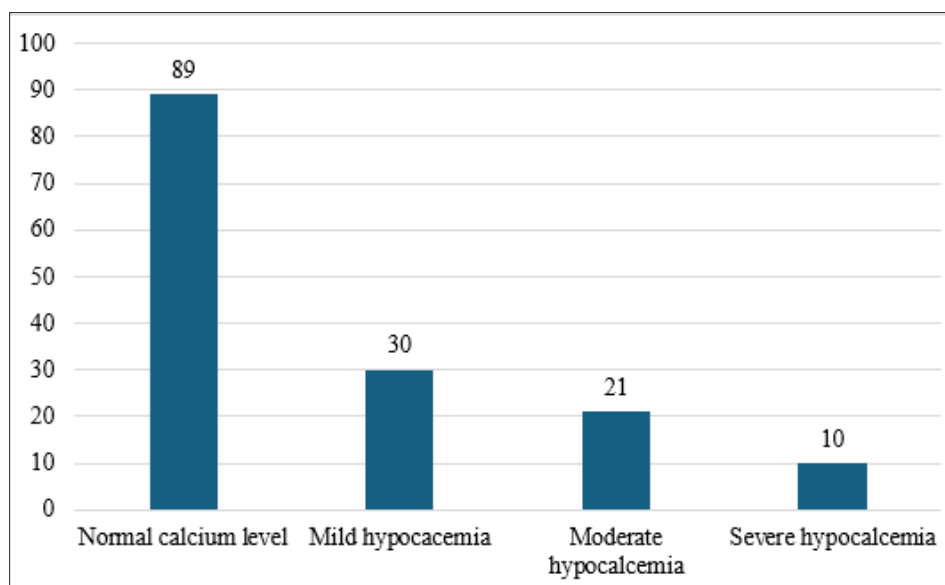


Figure 1. Grading of hypocalcemia among all dengue patients (n=150)

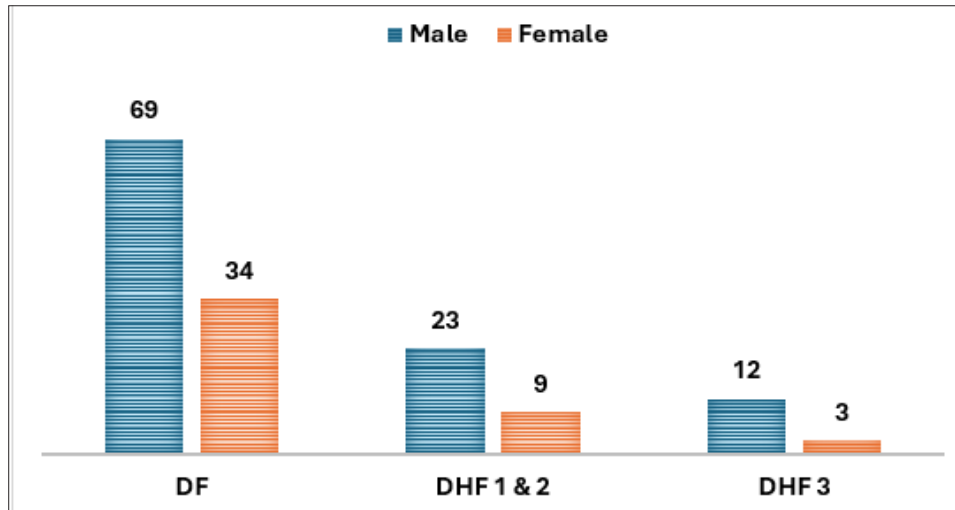


Figure 2. Distribution of Dengue classified against Gender (n=150)

Likewise, among all the 46 females, 34 (73.90%) of them had dengue fever, 9 (19.60%) of them had dengue hemorrhagic fever 1 and 2. Only 3 (6.50%) of them had dengue shock syndrome. Among the 104 males, 69 (66.30%) of them had dengue fever, 23 (22.10%) of them had dengue hemorrhagic fever 1 and 2 and 12 (11.50%) of them had dengue shock syndrome (Figure 2).

In the above Figure 2, though both grades DHF I and DHF II share the same hemodynamically stable profile without overt shock, grouping them isolates non-shock DHF as a distinct intermediate cohort between uncomplicated Dengue Fever (DF) and Dengue Shock Syndrome (DSS). And for avoiding the sparse data bias and preserving statistical power with stabilizing maximum likelihood estimation, the DHF I and DHF II were presented combinedly in subsequent analysis.

Of the 150 patients, 68 patients developed no warning signs, 6 patients had abdominal pain, 8 of them were lethargic/restless, 55 of them had edema and 3 had hepatomegaly (Figure 3).

All the dengue fever cases were considered as non-severe group and dengue haemorrhagic 1 and 2 with dengue shock syndrome patients were notified as severe group. The mean value of all continuous parameters was compared in both the groups. There was no significant difference ($p > 0.05$) found in between the two groups with regards to their age, BMI, hematocrit level, haemoglobin level and TLC count. The multivariate logistic regression shows the risk factors predicting the severity of dengue in our study is depicted below in Table 2.

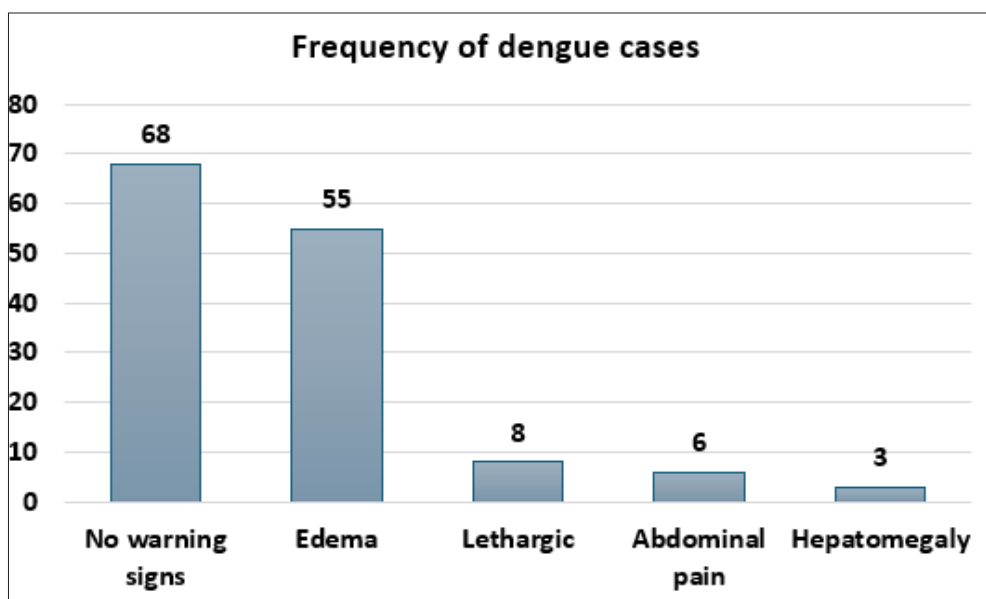


Figure 3. Distribution of Warning signs of the dengue patients (n=150)

Table 2: Multivariate logistic regression showing risk factors influencing severity of Dengue

Risk factors	Odds Ratio	95% Confidence Interval	P-value
Age	0.99	0.94-1.05	0.94
Gender (Male)	0.19	0.03-1.24	0.08
High BMI	1.10	0.64-1.89	0.71
Presence of Warning sign	0.81	0.41-1.61	0.34
Presence of Comorbidities	0.91	0.32-2.56	0.54
Hypocalcemia	3.57	1.74-7.35	0.0001*
High hematocrit	1.02	0.91-1.13	0.69
Low haemoglobin	0.94	0.48-1.84	0.86
High TLC	0.99	0.77-1.26	0.93
Low platelet	0.98	0.97-0.99	0.01*
Presence of Transaminitis	1.94	0.38-9.95	0.42

The mean serum calcium level and platelet count are decreasing significantly as the severity of dengue increases ($p < 0.05$). Further simplifying the platelet count, it was observed among the 103 cases of Dengue fever, the mean platelet count was 1,67,630 and among the 32 patients of dengue haemorrhagic fever 1 and 2, the mean platelet count was 75,470. Likewise, out of the 15 cases of Dengue shock syndrome, the mean platelet count was 90,930. A significant difference has been obtained between the mean platelet counts of Dengue fever, Dengue haemorrhagic fever 1 and 2, and Dengue shock syndrome. A total of 48 patients presented with transaminitis, 102 patients had no transaminitis, i.e. elevated hepatic enzymes which is an established Feature in dengue.

In this study, the prevalence of normocalcemia in dengue fever was 89 (59.33%). Around 30 (20%) patients had mild hypocalcemia, 21 (14%) had moderate hypocalcemia and 10 (6.66%) cases had severe hypocalcemia. Again, out of all 89 normocalcemic patients 71 (79.8%) cases of

normocalcemia had Dengue fever, 15 (16.90%) cases had Dengue haemorrhagic fever 1 and 2, and only 3 (3.40%) cases had dengue shock syndrome. Likewise, among mild hypocalcemic patients 60% cases had dengue fever, 26.70% cases had DHF 1&2, and 13.30% cases had Dengue shock syndrome. Similarly, among all moderate hypocalcemic patients 57.10% cases had dengue fever, 14.30% had Dengue haemorrhagic fever 1&2 and 28.60% cases had dengue shock syndrome. Among all severe hypocalcemic patients, 20% cases had dengue fever, 60% developed dengue haemorrhagic fever 1 and 2 and 20% cases landed in Dengue shock syndrome. A strong significance has been established between the groups with p -value < 0.05 (Table 4).

Out of all patients 103 cases of Dengue fever had a mean calcium of 8.937, 32 cases of Dengue haemorrhagic fever 1&2 had a mean calcium of 8.35, and 15 cases of Dengue shock syndrome had a mean calcium of 7.987. it is noted that as the severity of dengue increased, the total calcium levels declined (Table 5).

Table 4: Severity of dengue with hypocalcemia

	GROUP					P-value
Calcium level		DF	DHF1	DHF2	DHF3	0.0001 (FISCHER EXACT TEST)
	Normal	71 (79.80%)	8 (8.98%)	7 (7.86%)	3 (3.37%)	
	Mild	18 (60%)	1 (3.33%)	7 (23.33%)	4 (13.33%)	
	Moderate	12 (57.14%)	0 (0.00%)	3 (14.28%)	6 (28.57%)	
	Severe	2 (20%)	4 (40%)	2 (20%)	2 (20%)	

Table 5: Association between dengue severity and mean Calcium

Dengue Severity	Mean Calcium	SD	95% Confidence Interval		Significance (ANOVA)
			Lower Bound	Upper Bound	
DF	8.93	0.83	8.773	9.101	0.0001
DHF1&2	8.35	0.81	8.056	8.644	
DHF3	7.98	0.48	7.719	8.255	

Discussion

Dengue fever, a rapidly spreading vector-borne viral infection, continues to be a major global public health concern, particularly in tropical and subtropical regions of South-East Asia, the Western Pacific, and South and Central America[5]. The incidence of dengue has escalated significantly since 1960, with South Asian countries experiencing frequent outbreaks[6]. If left untreated, dengue infection carries a high morbidity and fatality rate[7].

The severity of dengue infection is categorized into four groups: undifferentiated fever, dengue with warning signs, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)[8]. Diagnosis and management typically rely on clinical findings and serological laboratory tests. While various clinical and laboratory parameters have been explored to predict disease severity, such as age, gender, hepatomegaly, abdominal pain, lethargy, malnourishment, other infections, ascites, pleural effusion, leucopenia, thrombocytopenia, hemo concentration, elevated SGOT/SGPT, and gallbladder wall thickening, these are not always routinely employed in general hospitals[9].

A growing body of research has linked low serum calcium levels with poor prognosis in dengue fever[10]. Possible mechanisms contributing to hypocalcemia in severe dengue include antibody-mediated enhancement utilizing the complement system and calcium for resistance, dysfunction of the endothelial glycocalyx leading to increased platelet activation requiring calcium, and plasma capillary leakage accumulating fluid in the third space[11].

This cross-sectional observational study, conducted at IMS and SUM Hospital in Bhubaneswar, Odisha, aimed to assess serum calcium levels in dengue patients and correlate them with disease severity. The study included 150 patients diagnosed with dengue via NS1 or Dengue IgM, with a mean age of 39.1 $\pm$ 12.44 years and an approximate male-to-female ratio of 2:1. Patients were categorized into DF, DHF 1, DHF 2, DHF 3, and DHF 4, and further classified based on serum calcium levels into mild, moderate, and severe hypocalcemia.

Our findings demonstrate a significant negative correlation between the severity of dengue infection and serum calcium levels, with a correlation coefficient of -0.892 by applying Pearson's correlation. The mean serum calcium levels were observed to decrease with increasing dengue severity: 8.93 mg/dL in DF, 8.35 mg/dL in DHF 1 & 2, and 7.98 mg/dL in DHF 3 patients. This aligns with previous studies showing that mean serum calcium levels were within the normal range in non-severe dengue patients and that hypocalcemia is an important biochemical derangement correlated with dengue severity[12].

The study found that 89 patients (59.33%) had normal calcium levels, 30 (20%) had mild hypocalcemia, 21 (14%) had moderate hypocalcemia, and 10 (6.66%) had severe

hypocalcemia. Notably, 79.8% of normocalcemic patients had Dengue Fever, while 60% of patients with severe hypocalcemia developed DHF 1 and 2, and 20% experienced Dengue Shock Syndrome. This strong significance between calcium levels and dengue severity was supported by a p-value <0.05. The mean platelet count was severely decreased in DHF I and II but moderately low in Dengue shock syndrome. The hallmark of severe dengue is systemic vascular permeability which leads to fluid leaking out of blood vessels which leads to shock. While platelet counts often drop significantly during dengue, severe leakage can occur even when platelets are only moderately low[13].

Despite the strong association found, it is important to acknowledge the limitations of this cross-sectional study. Further prospective cohort studies are needed to definitively establish low serum free calcium as a risk factor for dengue complications. The diagnosis of dengue using different tests with varying sensitivities and specificities, and the collection of blood samples on different days, could have influenced the results.

Currently, serum calcium measurement is not a routine practice in dengue infection management. However, the consistent findings of hypocalcemia in severe dengue across various studies, including ours, suggest that blood calcium levels could serve as a potential biomarker for identifying patients at risk of severe dengue[14-16]. Limited pilot studies have indicated that oral calcium carbonate supplementation, sometimes with vitamin D3, may improve clinical outcomes and increase platelet counts in dengue patients[17]. Nonetheless, the lack of randomized controlled trials means that oral or intravenous calcium therapy is not yet routinely included in published guidelines.

Further research is crucial to understand the exact mechanisms underlying hypocalcemia in severe dengue, including potential leakage into third spaces, disturbances in cellular transport, or changes in calcium-regulating hormones[18]. Investigating the role of calcium level changes in sympathetic dysfunction in DHF is also warranted. The present study underscores the need for more extensive research with larger populations to solidify the association between hypocalcemia and dengue severity, and to explore the potential benefits of calcium supplementation in improving patient outcomes. Considering the reduced morbidity observed in patients who received calcium supplementation in this study, using calcium levels as a prognostic factor and potentially incorporating calcium for enhanced outcomes in dengue management warrants further investigation. Factors such as the patient's pre-infection state, socioeconomic status, nutritional habits, and other electrolyte and micronutrient deficiencies should also be considered in future research.

Conclusion

This ever-interesting search of electrolyte abnormalities in dengue fever has taken a step ahead to prognosticate or

catch severity early with detection of hypocalcemia. Several studies strongly associate hypocalcemia in dengue fever. (19–22) In our study, the reduced morbidity in patients who received calcium supplementation strongly urges to use the calcium level as a prognostic factor in determining dengue severity, and use of calcium for enhanced outcome. However, the pre-infection state of the patient, socioeconomic strata, nutritional habits and multiple factors in the coagulation cascade and other electrolyte and micronutrient deficiencies also seem to co-ordinate the severity and hence cannot be ignored. To sum up, calcium supplementation can be considered a boon among patients of Dengue fever. Similarly, measurement of calcium levels can be of early help in determining the severity of Dengue fever.

Limitation

Further studies with larger population are required to strengthen the association between the severity of dengue fever in hypocalcemia and its correlation with the outcome.

Declarations

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Author Contributions: Dr. Subhash Chandra Dash and Dr. Sandeep Kumar Ratha: Methodology, Resources, Supervision, Validation, Critical revision of manuscript. Dr. Aiswarya Joshi, Dr. Sandeep Kumar Ratha and Dr. Sumita Sharma: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft preparation, Writing – review & editing. All authors read and approved the final manuscript.

Availability of Data and Materials: The datasets generated and/or analysed during the current study are not publicly available due to institutional data protection policies but are available from the corresponding author on reasonable request, subject to approval by the Institutional Ethics Committee.

Conflict of Interest: The authors declare that they have no conflict of interest.

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