Role of Serum Calcium Levels as a Biomarker for Dengue Severity and Prognosis: Insights from A Cross-Sectional Analytic Study

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ABSTRACT

Introduction: Dengue fever, a widespread viral infection, poses a significant global health threat with diverse clinical outcomes. Early identification of severe cases is vital for effective intervention and reducing mortality. This study investigates the potential of serum calcium levels as a biomarker for dengue severity, aiming to enhance clinical management strategies.

Method: This cross-sectional study spanning six months investigated serum calcium levels as a biomarker for dengue severity and prognosis. Dengue-positive indoor patients over 18 years were included while those with co-morbidities or endocrine conditions were excluded. Data from Medicine wards/MICU patients were categorized into non-severe and severe dengue groups based on WHO classification.

Result: The average Calcium level in non-severe Dengue (n=13), was higher (8.68 ± 0.2) compared to severe dengue (n=60) patients (7.25 ± 0.6). Hypocalcemia correlated with elevated hematocrit levels and reduced platelet counts, suggesting a relationship between calcium status and hematological parameters. Warning signs such as plasma leakage and bleeding were also linked to lower calcium levels ($p < 0.05$). Deceased patients demonstrated notably lower calcium levels (6.1 ±0.7) compared to survivors (7.57 ±0.7).

Conclusion: Serum calcium levels show promise as a valuable biomarker for predicting dengue severity. These findings highlight the importance of further research to validate and implement calcium levels as a predictive tool in dengue fever.

INTRODUCTION

Dengue fever, caused by the mosquito-borne dengue virus, remains a significant global public health concern, particularly in tropical and subtropical regions. With an estimated 390 million dengue infections occurring annually, the disease poses a substantial burden on healthcare systems and communities worldwide (World Health Organization, 2020).[1]

Dengue fever is prevalent in over 100 countries. Infection with the dengue virus may be asymptomatic or cause a wide range of clinical symptoms, from nonspecific fever to a severe life-threatening hemorrhagic/shock syndrome with multiple organ failure and death. Early detection of dengue fever, followed by fast and adequate treatment, is critical for limiting morbidity and death. [2]

One crucial aspect in managing dengue infections is the identification of reliable biomarkers that can aid in the early diagnosis, prognosis, and monitoring of the disease progression.[3]

Moreover, identifying a reliable and accessible biomarker for dengue severity could have significant clinical implications. It could enable healthcare providers to stratify patients early in the course of the disease, facilitating timely and targeted interventions for those at higher risk of developing severe complications.[4]

While the majority of dengue cases manifest as mild, self-limiting febrile illnesses, a subset of patients progresses to severe forms of the disease, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), characterized by vascular leakage, thrombocytopenia, and organ dysfunction. Early identification of individuals at risk of severe disease is crucial for timely intervention and improved clinical outcomes.[5]

The exploration of the association between serum calcium levels and the severity of dengue virus infection holds great promise in advancing our understanding of the disease. [6,7] Calcium, an essential ion involved in various cellular processes, including immune response modulation, has been implicated in the pathophysiology of several infectious diseases.

Alterations in serum calcium levels have been implicated in the pathogenesis of several infectious diseases, prompting investigations into its potential as a biomarker for dengue severity.[6]

In this context, the present cross-sectional analytic study aims to elucidate the relationship between serum calcium levels and the severity of dengue virus infection, ultimately contributing to a more comprehensive understanding of the disease and potentially improving clinical management strategies.

METHODOLOGY

The study employed a cross-sectional analytic design with a duration spanning six months to investigate the role of serum calcium levels as a biomarker for dengue severity and prognosis.

The inclusion criteria encompassed individuals aged over 18 years, irrespective of gender, who were indoor patients testing positive for dengue through NS1 or IgM assays. Exclusion criteria comprised patients who declined participation, those with co-morbid illnesses such as chronic renal disease, heart failure, or liver disease, individuals with recognized endocrine conditions leading to hypocalcemia (e.g., hypoparathyroidism), and those diagnosed with pancreatitis.

The sample size was determined by including all dengue-positive patients admitted to the Medicine wards/MICU of the tertiary care hospital during the specified six-month period, meeting the defined inclusion and exclusion criteria. Patients were categorized into two groups based on the

For research analysis, dengue patients were groped as non-severe and severe dengue cases. [1]

Non-severe Dengue: patients testing positive for dengue through NS1 or IgM assays without warning signs.

Severe Dengue: patients testing positive for dengue through NS1 or IgM assays with warning signs such as (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing haematocrit with decreasing platelets); and severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure).
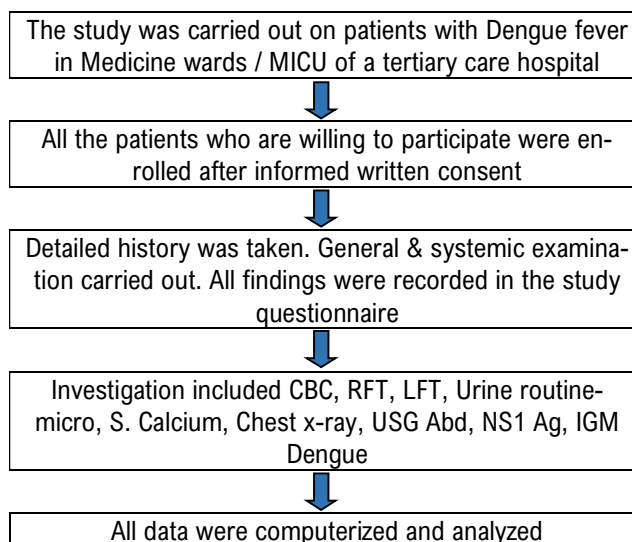
Data collection encompassed socio-demographic profiles, clinical presentations at admission, and medical histories. The information was systematically recorded and managed using an Excel spreadsheet.

For data analysis, IBM-SPSS Version 25.0 was employed. Mean and standard deviation calculations were applied for quantitative data, and an unpaired t-test was utilized to compare means between groups.

Qualitative data were presented in frequency and percentage-wise tables, with the Chi-square test employed to assess correlations between groups.

A significance level of a 95% confidence interval and $p < 0.05$ was considered statistically significant, enhancing the robustness of the study's findings and contributing valuable insights to the understanding of dengue severity and prognosis.

Study Flow Chart

The study was carried out on patients with Dengue fever in Medicine wards / MICU of a tertiary care hospital

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All the patients who are willing to participate were enrolled after informed written consent

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Detailed history was taken. General & systemic examination carried out. All findings were recorded in the study questionnaire

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Investigation included CBC, RFT, LFT, Urine routine-micro, S. Calcium, Chest x-ray, USG Abd, NS1 Ag, IGM Dengue

↓

All data were computerized and analyzed

RESULT

The table 1 shows the calcium level of dengue patients according to severity of the disease. The average Calcium level in non-severe Dengue (n=13), was higher (8.68 ±0.2) compared to severe dengue (n=60) patients (7.25

±0.6). The p-value, less than 0.01, further supports this significant distinction, suggesting that lower Calcium levels are associated with the severity of Dengue.

The table-2 shows that patients with hypocalcaemia have significantly higher haematocrit and lower platelet count compared to patients with normal calcium levels.

For patients with Hypocalcaemia, the average Haematocrit level is 38.8 (±3.9), whereas for those with Normal Ca+2, it is notably lower at 27.7 (±2.8). This substantial difference is statistically significant, indicated by a p-value of less than 0.001, suggesting that Haematocrit levels are associated with Calcium status.

Similarly, in terms of Platelet count, individuals with Hypocalcaemia have an average count of 99.4 (±27), whereas those with Normal Ca+2 have a significantly higher average count of 163 (±15.2). This difference is also statistically significant, with a p-value of less than 0.001.

Table 1: Calcium levels in patients with dengue fever

Dengue	Calcium level*	p value
Severe (n=60)	7.25 ± 0.6	<0.01*
Non-severe (n=13)	8.68 ± 0.2	

t value -8.13; *Values are in mean ± SD

Table 2: Blood parameters (Haematocrit, Platelets) in patients with and without hypocalcaemia.

variables	Hypocalcaemia (n=61)*	Normal Ca^{+2} (n=12)*	p value
Haematocrit	38.8 ± 3.9	27.7 ± 2.8	<0.001*
Platelet count	99.4 ± 27	163 ± 15.2	<0.001*

*Values are in mean ± SD

Table 3: Calcium level and warning signs among dengue patients.

Warning Sign	Calcium level*	t value	p value
Plasma Leakage			
Present (n=25)	6.78 ± 0.5	-7.33	<0.05*
Absent (n=48)	7.87 ± 0.6		
Bleeding			
Present (n=31)	6.79 ± 0.5	-10.06	<0.05*
Absent (n=42)	8.02 ± 0.5		

*Values are in mean ± SD

Table 4: Calcium level with different outcomes among dengue patients.

Outcome	Calcium Level*	p value
Discharge (n=57)	7.57 ± 0.7	0.001*
LAMA (n=12)	7.64 ± 1.03	
Died (n=4)	6.1 ± 0.7	

*Values are in mean ± SD

The table-3 compares two groups based on the presence or absence of warning signs, specifically "Plasma Leakage" and "Bleeding," in relation to their respective Calcium levels. For individuals with Plasma Leakage present (n=25), the average Calcium level is 6.78 (±0.5), while in the absence of Plasma Leakage (n=48), the average Calcium level rises to 7.87 (±0.6). Similarly, for Bleeding, those with the warning sign present (n=31) have an average Calcium level of 6.79 (±0.5), whereas in the absence of Bleeding (n=42), the average Calcium level is higher at 8.02 (±0.5). The associated p-values for both Plasma Leakage and Bleeding are less than 0.05, which signifies that the observed differences are statistically significant.

The table-4 shows the mean calcium level for patients with different outcomes. In the "Discharge" group (n=57), the average Calcium level is 7.57 (±0.7), indicating a moderate level with a relatively low standard deviation. For the "LAMA" group (n=12), the average Calcium level is slightly higher at 7.64 (±1.03), signifying a bit more variability in Calcium levels within this group. In contrast, the "Died" group (n=4) has a notably lower average Calcium level of 6.1 (±0.7), indicating a significant deviation from the other outcomes.

DISCUSSION

In present study, the mean calcium concentration in individuals with non-severe Dengue (n=13) was elevated at 8.68 ±0.2, in contrast to those with severe Dengue (n=60) who exhibited a lower level of 7.25 ±0.6. The p-value, which is less than 0.01, strengthens this notable difference, implying an association between decreased calcium levels and the severity of Dengue.

A study by Kumar G. et al. corroborates the observed trend in this research, reporting similar differences in serum calcium levels between non-severe and severe dengue cases. Their study, conducted in New Delhi demonstrated an average calcium level of 8.85, 8.27 and 7.95 respectively in Dengue Fever, Dengue Haemorrhagic Fever and Dengue Shock Syndrome. The consistency of findings across different populations strengthens the argument that lower calcium levels are consistently associated with dengue severity.[6]

According to Kesavan et al. research there is no statistically significant difference (p=0.580) in mean serum calcium levels between patients negative for dengue (8.98 meq/l) and those with dengue lacking warning signs (9.27 meq/l). However, a notably high statistical significance (p=0.0005) exists in serum calcium levels when comparing dengue cases without warning signs (9.27 meq/l) and those with warning signs/severe dengue (7.95 meq/l and 7.60 meq/l, respectively). Consequently, the incidence of hypocalcemia is more prevalent in dengue cases displaying warning signs and severe dengue infection.[8]

In the current study of For dengue patients experiencing Hypocalcaemia, the mean Hematocrit level stands at 38.8 (±3.9), whereas it is notably lower at 27.7 (±2.8) for

those with Normal Ca+2. This marked distinction is statistically significant, as evidenced by a p-value below 0.001, indicating an association between Hematocrit levels and Calcium status. Similarly, concerning Platelet count, those with Hypocalcaemia exhibit an average count of 99.4 (±27), while individuals with Normal Ca+2 have a significantly higher mean count of 163 (±15.2). This observed difference is also statistically significant, supported by a p-value below 0.001.

As per Meta-analysis by Mariz et al. among the severe dengue group, the mean levels of both serum total and ionized calcium in patients were reduced. The data revealed a sensitivity of 74% (95% CI = 0.58, 0.84) and specificity of 75% (95% CI = 0.67, 0.81), with a positive predictive value of 67% and a negative predictive value of 90.7%.[9]

A similar relation was seen with another morbidity where, The results from the ROC curve analysis demonstrated that the predictive capability, when combining serum calcium level with PLT count, was deemed satisfactory (AUC 0.730, P < 0.001, 95% CI 0.681-0.780). Within this cohort exhibiting PTOM, asymptomatic hypocalcemia and thrombocytosis were observed to occur more frequently. This suggests that the serological assessment of calcium levels and PLT count could serve as valuable biomarkers for screening patients suspected of PTOM.[10]

In the current study where Plasma Leakage is present (n=25), the mean Calcium level is 6.78 (±0.5), whereas in its absence (n=48), the mean Calcium level increases to 7.87 (±0.6).

Similarly, for patients with Bleeding, those with the warning sign present (n=31) exhibit a mean Calcium level of 6.79 (±0.5), while in the absence of Bleeding (n=42), the mean Calcium level is higher at 8.02 (±0.5). The associated p-values for both Plasma Leakage and Bleeding are below 0.05, indicating that the observed differences are statistically significant.

This finding is supported by Kesavan et al. result where the mean serum calcium levels were significantly lower in cases with severe dengue infection and dengue fever with warning signs than in patients with dengue fever lacking warning signs. These findings suggest that serum calcium levels have the potential to serve as a valuable biomarker for predicting the severity of dengue infection and as a prognostic indicator.[8]Disturbances in calcium homeostasis are likely linked to the myocardial dysfunction and cardiac arrhythmias observed in dengue, as indicated by in vitro studies. Additionally, calcium has been identified as playing a role in platelet aggregation, as highlighted in research conducted by Shivanthan et al.[11]

In Manjunath's study, the mean Ca2+ level (in mmol/l) was 1.2009 (±0.09) among controls and 1.0911 (±0.10) in dengue cases, with a statistically significant difference (p=0.0001). Specifically, the mean Ca2+ level in dengue cases without warning signs was 1.0950 mmol/l, in

those with warning signs it was 1.1088 mmol/l, and in severe dengue, it was 1.0559 mmol/l. Notably, the mean Ca2+ level in severe dengue was significantly lower compared to dengue cases with warning signs (p=0.04). The study identified hypocalcemia in 56% of dengue cases, whereas only 14% of controls exhibited this condition. Alarmingly, seven children with severe dengue who succumbed to the illness had hypocalcemia. [12]

In present study, the mean Calcium level among discharged patients was 7.57 (±0.7), indicating a moderate level with a relatively small standard deviation. Conversely, among the patients who "Died" exhibited a significantly lower mean Calcium level of 6.1 (±0.7), indicating a substantial deviation from the other outcomes.

As per Shivanthan et al. Hypocalcemia is seen in cases with severe dengue and has been purported to be associated with increased mortality.[11]

According to the Uddin et al. study finding there is evidence suggesting that hypocalcemia may be more prominent in more severe manifestations of dengue[13], though it's important to note that reduced calcium levels have not demonstrated a correlation with mortality in a study done by Bunnag et al.[14]

CONCLUSION

The presented data indicates a clear association between Calcium levels and various aspects of Dengue severity and outcomes. Lower Calcium levels are observed in individuals with severe Dengue compared to non-severe cases, suggesting it may serve as a potential indicator of disease severity. Additionally, Hypocalcaemia is linked with higher Haematocrit levels and lower Platelet counts, implying a relationship between Calcium status and haematological parameters in Dengue patients. The presence of warning signs like Plasma Leakage and Bleeding is associated with lower Calcium levels, underscoring the importance of monitoring Calcium levels in such cases. Lastly, individuals who unfortunately passed away from Dengue exhibited significantly lower Calcium levels compared to those who were discharged, highlighting a potential role in predicting Dengue outcomes. These findings suggest that calcium levels may be a useful biomarker for predicting the severity of dengue fever. Additionally, calcium supplementation may be a potential treatment for dengue fever.

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